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Abstracts
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Ethical requirements

Experiments on animals or animal tissue

For work conducted in the UK all procedures must conform with current UK legislation. For work conducted elsewhere all procedures must accord with current national guidelines or, in their absence, with current local guidelines.

Experiments on humans or human tissue

All procedures must accord with the ethical standards of the relevant national, institutional or other body responsible for human research and experimentation, and with the principles of the World Medical Association’s Declaration of Helsinki.
Remote control of hypothalamic circuits controlling food intake and metabolism

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The discovery of leptin has led to the elucidation of a robust physiologic system that maintains fat stores at a relatively constant level. Leptin is a peptide hormone secreted by adipose tissue in proportion to its mass. This hormone circulates in blood and acts on the hypothalamus to regulate food intake and energy expenditure. When fat mass falls, plasma leptin levels fall stimulating appetite and suppressing energy expenditure until fat mass is restored. When fat mass increases, leptin levels increase, suppressing appetite until weight is lost. By such a mechanism total energy stores are stably maintained within a relatively narrow range.

Recessive mutations in the leptin gene are associated with massive obesity in mice and some humans. Treatment with recombinant leptin markedly reduces food intake and body weight. The low leptin levels in patients with leptin mutations are also associated with multiple abnormalities including infertility, diabetes and immune abnormalities all of which are corrected by leptin treatment. These findings have established important links between energy stores and many other physiologic systems and led to the use of leptin as a treatment for an increasing number of other human conditions including a subset of obesity, some forms of diabetes including lipoatrophy and hypothalamic amenorrhea, the cessation of menstruation seen in extremely thin women. Identification of a physiologic system that controls energy balance establishes a biologic basis for obesity.

Leptin has recently been approved for the treatment of lipoatrophy, a severe form of diabetes associated with reduced fat mass and low endogenous leptin levels. These and other data have suggested that leptin can reduce glucose and lower insulin independent of effects on food intake. Further evidence suggests that leptin’s effects on glucose metabolism are mediated by the hypothalamus. Recent studies have focused on the role of specific populations of glucose sensing neurons in the ventromedial and lateral hypothalamus. These studies have employed a novel technology for modulating neural activity using radio waves or magnets. The results of these studies have highlighted the critical role of the CNS to control blood glucose and the levels of pancreatic hormones.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

"Where is the knowledge we have lost in information?" – feedback, flipping and physiology

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Providing students with the positive, life-long benefits of a research-led and enquiry-based education is the worthy and oft-stated ambition of most universities. Such an education should be informed by the new subject knowledge being discovered and disseminated at an unprecedented rate. This new knowledge must be incorporated appropriately into an often, already full curriculum and in a way that places emphasis upon the critical nature of scientific enquiry but still delivers fundamental information. It will also be expected that any education provider should deliver against the proposed TEF agenda of improving student experience, continuation rates and employability.

It could be assumed that teaching within an institution that also delivers original research outputs may be all that is required to achieve the ambition described above. But, in an ever more challenging higher education environment how can we know that and how are we assured that our students are equipped with the knowledge and appropriate problem solving skills required by their future employers? Is it, for example, sufficient to believe that the introduction of grade point averages will placate those employers who believe grade inflation has diminished the value of a University degree or do we have a greater obligation to our students and their future colleagues? Additionally, the potential impact of TEF success upon institutional funding means we may be in danger of gaming the process and favouring teaching for the test with the very real danger that we might, inadvertently or otherwise, be driven to decrease course demands and reward recall above understanding.

Against this developing and challenging backdrop, I began, a number of years ago, to question the value of my own, traditional teaching methods. The students I taught seemed happy; my Powerpoint slides and handouts were clearer than they had ever been and my prepared jokes and anecdotes were (at least to my ears) honed to near perfection. New information was added annually although it was noticeably much harder to remove previous content. I believed I was teaching understanding. However, I had the nagging doubt that I had been, perhaps unconsciously, steered into this comfortable relationship with the students by a combination of my personal experiences, their evaluations of my modules, my peer group’s expectations and the many, ever-increasing other demands on my time. More importantly, somehow in all of this, I felt that knowledge had been forsaken for information and the students were less cognitively engaged than might be hoped for.

I therefore began experimenting with the flipped or inverted classroom approach in an attempt to utilise an active learning methodology to replace the passive approach I had lost confidence in. In this Otto Hutter lecture I will describe my experience of changing how I teach and how this energized my interest in the teaching and learning of physiology. I will describe the pitfalls and pleasures I have had along the way and provide evidence to support the success of the methodology whilst acknowledging its difficulties and limitations. Finally, whilst I accept this approach is not a panacea for all of the ills of teaching and can only ever be part of a wider, blended methodology, I hope to show why I believe that flipped and similar approaches can address many of the concerns we face with regard to learning and teaching issues in the higher education sector.

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Sex, drugs and rock and roll: Tales from preterm fetal life

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Globally around 15 million babies are born preterm each year (less than 37 weeks gestation) and prematurity is the leading cause of neonatal death. Survivors are at significantly greater risk of both short and long-term complications such as necrotising enterocolitis, renal failure, feeding difficulties and hypoglycaemia, jaundice, retinopathy, cardio-respiratory complications, brain injury and impaired brain development. Thus, preterm birth can be rightly said to be a major systems physiology challenge.

Preterm organ structure and function are, by definition, immature, and the stress of adapting to an environment the baby is not physiologically prepared for significantly contributes to injury and illness. Adapting to preterm newborn life is further complicated by exposure to adverse events such as hypoxia and infection before birth. Thus to advance the development of treatments to reduce or prevent preterm morbidity requires that we understand the adaptive responses of both the newborn, and the fetus, under a variety of physiological and pathological conditions. While younger and low-birthweight prems are at greater risk of injury and illness, we now know that even late preterm infants and near-term infants are at greater risk of poor outcomes compared to their term counterparts. Thus there is something about living in utero which promotes normal development, and this further highlights the need to understand fetal physiology.

The focus of this talk is on the fetal responses to asphyxia and I will demonstrate that our perception of the fragile, delicate preterm newborn should not colour our understanding about how the preterm fetus may respond to challenges to its environment. I will explore how we have challenged the traditional concept that the preterm fetus must be less capable than full term fetuses in mounting a defence to hypoxia due to immaturity of chemoreflex and other adaptive responses, and that this must in part underpin vulnerability to injury. I will outline the triphasic behavioural, neural, cardiovascular and cerebrovascular compensation and decompensation responses of the preterm fetus to asphyxia. This will highlight the phenomenon of tolerance of the preterm fetus, and how this tolerance paradoxically places the preterm fetus at greater risk of injury.

I will discuss why the sex of the fetus makes a difference to neonatal survival and risk injury and present data on how male and female fetuses respond to asphyxia. I will show how this study revealed the importance of metabolism and how it raises the question of whether we should augment fetal glucose and other metabolic substrates. Hypoglycaemia is significantly associated with adverse neurodevelopmental outcomes in preterm babies, however there is potential risk in manipulating glucose. I will discuss new evidence showing that the glucose paradox, well described for the adult but not for the fetus and newborn, does occur in perinatal life. I will demonstrate that while increased glucose during asphyxia may help the fetus survive, this comes at a potentially dramatic cost to the brain. Finally, I will discuss how common insults such infection and standard antenatal therapies such as glucocorticoids can interact with the fetal adaptation to asphyxia to modulate brain injury.

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Inhibition and excitation in the cerebellar nuclei

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The cerebellum carries out two distinct roles in motor control in real time: it facilitates learned, coordinated movements and corrects errors. Signals to execute these functions must be carried by the large neurons of the cerebellar nuclei, which form the major premotor projection from the cerebellum. How these neurons fire is determined by the interaction between their intrinsic ion channels, which strongly promote action potential generation, and the constant barrage of synaptic inhibition reaching them from dozens of convergent, rapidly firing Purkinje cells, which tends to suppress firing. During cerebellar behaviors, both depolarization and inhibition are elevated by mossy fiber inputs which excite large premotor cells directly as well as Purkinje cells indirectly, raising the question of how large premotor neurons of the cerebellar nuclei integrate synaptic inhibition and excitation with their intrinsic properties to generate the appropriate signals to regulate movement. Here, we will discuss intrinsic and synaptic mechanisms in the mouse cerebellar nuclei that permit distinct modes of firing in response to different patterns of synaptic inputs as measured in vitro, as well as their relation to movements measured in awake behaving mice. The data provide evidence that not only the rate but also the temporal structure of Purkinje cell firing can influence the efficacy of synaptic excitation of large neurons, the pattern of cerebellar output, and the consequences for motor behavior.

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Carotid body chemoreflex: A driver of autonomic abnormalities in sleep-apnea

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Carotid bodies are the principal peripheral chemoreceptors for detecting changes in the arterial blood oxygen levels, and the resulting chemo reflex is a potent regulator of sympathetic tone, blood pressure, and breathing. Sleep apnea is a disease of the respiratory system affecting several million humans. Apneas occur during sleep often due to obstruction of the upper airway (obstructive sleep apnea, OSA) or due to defective respiratory rhythm generation by the central nervous system (central sleep apnea, CSA). Patients with sleep apnea exhibit several co-morbidities most notably heightened sympathetic activity and hypertension. Emerging evidence suggests that intermittent hypoxia (IH) resulting from periodic apnea stimulates the carotid body and ensuing chemo reflex mediates the increased sympathetic tone and hypertension. Rodent models of IH, simulating the O2 saturation profiles encountered during sleep apnea provided important insights into the cellular and molecular mechanisms underlying the heightened carotid body chemo reflex. My presentation focuses on how IH affects the carotid body function, and...
discusses the cellular, molecular and epigenetic mechanisms underlying exaggerated chemo reflex.

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**PL06**

**A new physiology to handle an ancient challenge: Fluoride resistance in microorganisms**

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Until about three years ago, the F\(^{-}\) ion was widely considered utterly irrelevant to membrane biology – an orphan halide that does not participate in any membrane-transport phenomena. This view was overturned by Ronald Breaker’s 2012 discovery of F-specific riboswitches in many bacterial genomes, genetic elements that control expression of novel membrane proteins that function as F\(^{-}\) exporters. These exporters are found in two separate, phylogenetically unrelated classes: energy-consuming F/\(^{+}\)\(^{-}\) antiporters of the CLC superfamily found exclusively in bacteria, and thermodynamically passive “Fluc-family” ion channels found in prokaryotes, eukaryotic microorganisms, plants, and primitive marine animals (sponges, tunicates), but not in higher animals.

The physiological purpose of F\(^{-}\) exporters, at least in microbes, is to protect these unicellular organisms from inhibition of certain key metabolic enzymes by F\(^{-}\) ion, which is ubiquitous in the aqueous biosphere, and has been so since the beginnings of evolutionary time. In a sense, F\(^{-}\) export proteins are “inorganic analogues” of multidrug resistance transporters, long known to resist toxicity of the myriad small organic toxins that pervade our environment. This lecture will discuss our current understanding – still primitive at this early stage - of F\(^{-}\) resistance physiology implemented by CLC-type and Fluc-type F\(^{-}\) exporters. The unusual chemical properties of F\(^{-}\) ion explain why a passive ion channel can operate in a microbial context as an exporter to maintain a low cytoplasmic concentration of the halide anion, and the genomic organization of Fluc channels provides insights into evolution of the inverted structural repeats now commonly seen in the crystal structures of many transporters and ion channels. Finally, a recent high-resolution structure of a bacterial Fluc-type exporter shows some completely unexpected features of this unusual ion channel.

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**PL07**

**Hypoxia and uterine contractions: Not too much but not too little**

S. Wray

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During labour contractions are of such intensity that blood vessels travelling through the uterus are compressed. The myometrium (and the fetus) experiences this intermittent hypoxia and we have shown in vivo that transient decreases of myometrial pH and ATP are associated with each contraction (1). Prolonged myometrial hypoxia and the fall in pH associated with it, can inhibit or even abolish uterine contractions. We have recently found that lactate can reduce myometrial force, pH and intracellular calcium transients (2). These local changes (myometrial capillary blood) are significantly associated with dysfunctional labour, i.e. those characterized by weak contractions, and which is a major cause of unplanned Caesarean section (3). These changes in amniotic fluid lactate are prognostic for difficult labours (4).

A conundrum arises associated with these metabolic changes that are part of normal maternal physiology and labour. How in labour, despite repeated, transient episodes of local ischaemia, hypoxia and acidity, do contractions become progressively stronger, increasing in amplitude, frequency and duration over many hours. The mechanism of this vital increase in contractility in the face of deleterious metabolic changes is not understood. We have however recently found that repetitive brief periods of hypoxia produce large and sustained increases in contractility, such as occurs in labour, in samples from pregnant but not non-pregnant uterus (5). These findings suggest that ischemic tolerance is an adaptive response of the term uterus and is initiated when contractions reduce uterine blood flow and produce repeated hypoxic stresses. The released metabolites such as adenosine stimulates contractility via increasing Ca entry and stimulating intracellular pathways especially prostaglandin, suggesting the mechanism may be related to hypoxic preconditioning, which is the most powerful mechanism known for limiting infarct size in the heart.

These findings also suggest that in women, whose labours are dysfunctional, this inherent mechanism is not working and thus therapeutic strategies to stimulate it would be of benefit.

Further work is first required to determine the mechanism of this hypoxia-induced force increase (HIFI) and also to establish if its premature activation can occur and facilitate preterm delivery.


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**PL08**

**Cardiac metabolism in disease: All fuels are equal, but some fuels are more equal than others**

L. Heather

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The heart requires more energy than any other organ in the body, to support its continual contractile activity. The vast majority of ATP is generated by mitochondrial oxidative phosphorylation, with small amounts obtained from anaerobic glycolysis. A number of fuels can be utilised by the heart to make ATP, including carbohydrates, fatty acids, amino acids and ketone bodies. Because different chemical reactions are needed to breakdown these independent fuels, this results in distinct substrates producing different amounts of ATP, and different amounts of oxygen being used to extract the energy. Thus, all fuels are equal in that they can all give you energy,
but there are differences in the energetic benefits obtained and the associated costs involved. The healthy heart obtains 60-70% of its ATP from the oxidation of long chain fatty acids, with the remainder made predominantly from carbohydrates. Being able to change between these fuels is referred to as "metabolic flexibility", and is essential to match metabolism to the physiological conditions to which the heart is exposed, ensuring the best fuel is utilised in that environment. This can be regulated acutely, for example by allosteric mechanisms via the Randle cycle, or chronically, for example by transcription factors. In diseases such as heart failure and type 2 diabetes, cardiac metabolism becomes abnormal, which has consequences for energetics, metabolic flexibility and contractile function. Understanding the underlying mechanisms behind metabolic dysfunction in these diseases can help shed light on whether targeting cardiac metabolism has the potential for therapeutic benefit.

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**PL09**

**Is high blood pressure self-protection for the brain?**

E. Hart

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Hypertension is the leading contributor to the global burden of disease and mortality. However, the mechanisms that lead to hypertension are poorly understood. It is well accepted that sympathoexcitation plays a major role in the development of human hypertension, yet, exactly what triggers elevations in sympathetic nerve activity in the first instance is unclear. This talk will centre on the concept that human hypertension may develop as a vital mechanism to maintain adequate blood flow to the brain. In particular, the talk will focus on new evidence indicating a high prevalence of cerebral anatomical abnormalities, elevated cerebral vascular resistance and regional hypoperfusion in humans with hypertension. Finally, data suggesting that brain blood flow is a major factor in triggering hypertension and that lowering blood pressure may worsen cerebral perfusion in susceptible individuals will be discussed.

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**PL10**

**Neuronal networks in brain slices and whole brains**

B. Sakmann

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Initially patch pipettes were designed to record elementary events from muscle and neurons. A recording configuration that proved to be convenient to study signaling in nerve cells embedded in their (almost) natural environment was designated as whole-cell-recording. In this Lecture I will summarize work designated to throw light on electrical signaling within a neuron, between dendrite branches, soma and nerve terminal. Possible function of this intra-neuronal electrical signaling are coincidence detection of spatially separated synaptic inputs and, on a longer time scale, the induction of mechanisms that underlie synaptic plasticity. They depend on the newly discovered active electrical properties of dendrites (backward propagating and forward propagating action potentials).

What are possible functions of dendritic active voltage dependent excitability with respect to network dynamics?

One obvious function is the interaction of an actively propagating dendritic electrical signal with locally restricted synaptic signals. On a shorter time scale it mediates coincidence detection of several synaptic inputs by a pyramidal neuron and, on a longer time scale, it induces changes in synaptic strength referred to as spike timing dependent plasticity.

These discoveries provide some of experimental advances in the field of neuroscience that can claim to provide a cellular basis for understanding a brain function as a whole. In vivo recordings from the cortex major output neurons in the intact brain, pyramidal cells have indicated that coincidence detection and spike time dependent changes in connectivity are indeed implemented in pyramids of the intact brain. They critically determine the output pattern of action potentials, that is differentially read by their target cells in cortex and subcortical nuclei, e.g. in cortical and thalamic target cells of layer 5 thick tufted pyramids.


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Post-infarct remodeling of cardiac sympathetic nerves and arrhythmia
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Millions of people suffer a myocardial infarction (MI) every year, and those who survive have increased risk of arrhythmias and sudden cardiac death. Recent clinical studies have identified sympathetic denervation as a predictor of increased arrhythmia susceptibility. We have identified two types of sympathetic denervation that occur after MI in mice: sustained denervation of the infarct and border zone, and transient denervation of peri-infarct myocardium. These are caused by distinct molecular mechanisms and have very different impacts on arrhythmia susceptibility. We found that the extracellular matrix components chondroitin sulfate proteoglycans are produced in the cardiac scar after ischemia-reperfusion injury, where they cause sustained denervation of the infarct and border zone. They prevent axon outgrowth via protein tyrosine phosphatase receptor σ (PTPσ) on sympathetic neurons. We found that the absence of PTPσ, or pharmacologic modulation of PTPσ, in BalbC mice restored sympathetic innervation to the scar and borderzone. We tested the impact of reinnervation on arrhythmia susceptibility using ECG telemetry and optical mapping with voltage and Ca²⁺ sensitive dyes. Both techniques revealed a decreased number of induced arrhythmias following reinnervation. Optical mapping carried out 14-21 days after MI revealed increased dispersion of action potential duration (APD), supersensitivity to β-adrenergic receptor stimulation, and Ca²⁺ mishandling. Sympathetic reinnervation prevented all of these changes and rendered hearts remarkably resistant to induced arrhythmias. Conversely, transient denervation of peri-infarct myocardium is triggered by activation of the p75 neurotrophin receptor (p75NTR). The signalling events that follow ligand binding to p75NTR have been described extensively in cultured sympathetic neurons, but remain less clear in vivo. Activation of p75NTR in vitro induces axon regeneration through a mechanism that requires the expression and activity of the metalloclopeptase tumor necrosis factor-α converting enzyme (TACE). We investigated the role of TACE in sympathetic axon degeneration after MI in wildtype and p75NTR−/− C57Bl6 mice. We found that TACE expression was increased in cardiac sympathetic neurons 3 days after MI in a p75NTR-dependent manner. Inhibiting TACE with Marimastat (25 mg/kg/day) prevented axon degeneration outside of the infarct. Sympathetic nerves were identified by tyrosine hydroxylase (TH) immunohistochemistry and infarcts were identified by fibrinogen immunohistochemistry. However, total infarct area was increased in marimastat-treated hearts (vehicle: 6.0±0.8% vs. marimastat: 8.8±0.7% total LV, n=5-6, p<0.05). Optical mapping was carried out 3 days after MI to determine if electrophysiological properties or calcium handling were altered by transient peri-infarct denervation. In contrast to the profound effect of sustained denervation on arrhythmia susceptibility, mapping analyses showed no difference between vehicle and marimastat treated hearts in APD, APD dispersion, Ca²⁺ transient duration, or the pacing frequency at which Ca²⁺ alternans emerged. Thus, sustained sympathetic denervation after myocardial infarction has a profound impact on arrhythmia susceptibility in the mouse heart, but transient denervation does not.

Research Symposia

Compartmentalised cAMP signalling in ventricular myocytes
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Heart Failure (HF) remains a leading cause of hospitalizations and mortality. Treatment is symptomatic and unsatisfactory for some patients (notably patients suffering from HF with preserved ejection fraction), thus urging for innovative approaches to reverse the course of ventricular dysfunction. cAMP and its effector PKA are key regulators of cardiac function and inappropriate activation of this pathway is a hallmark of HF. Current treatment targeting cAMP/PKA signalling presents with limitations: β-blockers are not effective in some patients and cAMP raising agents to treat acute HF are associated with arrhythmias and increased mortality. The reasons for the disappointing performance of these drugs are unclear, revealing our limited understanding of the role of cAMP in the pathophysiology of the heart.

In addition to its role as regulator of the chronotropic, inotropic and lusitropic response to catecholamines, cAMP affects multiple other functions including, among others, cell growth, metabolism and death. This complex functional role is achieved via modulation of ion fluxes at membranes and of myofilament sensitivity to Ca²⁺ as well as via regulation of transcription factors and a variety of enzymes and other targets. A key question remains how coordination is achieved among the complex cAMP signalling networks. In recent years we and others have demonstrated that cAMP signalling is compartmentalised. Compartmentalised signalling allows individual GPCRs to generate distinct cAMP pools that, in turn, activate defined subsets of localized PKA that are tethered in proximity to specific targets via binding to A kinase anchoring proteins (AKAPs). Phosphodiesterases (PDEs), a superfAMILY of enzymes that degrade cAMP and that includes more than 50 isoforms presenting unique regulation and subcellular localisation features, play a key role in the spatial regulation of cAMP propagation, and regulate cAMP levels within individual compartments. Thus, displacement of individual PDE isoforms from their subcellular anchor site results in local elevation of cAMP.

Compartmentalisation of cAMP signalling has important implications for cardiac physiology and pathophysiology. Yet, the size and location of distinct cAMP domains, the amplitude and kinetics of the cAMP signal within each domain, their functional role and the coordination of signalling between different domains, remain largely to be defined. Understanding the details of such organisation is the current challenge in the field. Compartmentalisation of cAMP prompts the idea that with a detailed understanding of the organization, regulation and function of individual cAMP compartments it may be possible to target individual cAMP pools, rather than global intracellular cAMP levels, in order to achieve greater therapeutic efficacy and specificity.

Real-time imaging of cAMP using fluorescence resonance energy transfer (FRET)-based reporters has enhanced our understanding of compartmentalised cAMP signalling. However, major drawbacks have been the limited resolution of the FRET probes and the difficulty to directly compare...
cAMP signals generated at different intracellular sites. We have recently generated a novel FRET-based sensor (named CUTie, for cAMP Universal Tag for imaging experiments) that detects compartmentalised cAMP with unprecedented spatial resolution. Using CUTie, we have quantitatively measured cAMP in situ in the immediate vicinity of specific multiprotein complexes involved in excitation-contraction coupling (EC). We demonstrated that the cardiac response to b-AR stimulation generates cAMP signals with distinct local amplitude and kinetics and that the size of such domains is in the nanometre range, at least one order of magnitude smaller than previously thought. We demonstrated that such nano-heterogeneity of the cAMP signal depends on differential local activity of PDEs. Importantly, we found that the local coordination of cAMP signals is necessary to maximize the adrenergic effect on contractility but, at the same time, it results in vulnerability of the myofilaments to Ca2+ sensitization. In conditions of low adrenergic input, as found in HF, the difference in the cAMP response at different sites becomes more extreme with no effective signal being generated at specific sites, which may underpin the diastolic dysfunction associated with HF. The nanoscopic dimension of cAMP signalling revealed by our recent work provides a new framework for understanding cardiac adrenergic signalling and for the development of novel approaches to therapeutic intervention.


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SA003

Is nuclear pH in cardiac myocytes regulated?
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H+ ions acutely affect cardiac excitation and contraction, but their long-term effect on gene expression is unknown. This is despite in vitro evidence for the pH-sensitivity of transcription factor (TF) binding to DNA. Major remodelling of cardiac pH regulation can occur in disease states, but little is known about the mechanisms that regulate nucleoplasm pH (pHnuc), and how these could be exploited to control gene expression. Previously (1), we have shown that pHnuc dynamics can be imaged using the DNA-binding dye Hoechst 33342. Nuclear pores allow the passage of medium-sized molecules (e.g. calcine), but H+ ions must first bind to mobile buffers in order to gain access to the nucleoplasm. Fixed H+ buffering residing in the nucleus of permeabilised cells was estimated to be very weak on the basis of the large amplitude of pHnuc transients evoked by photolytic H+-uncaging or exposure to weak acids/bases. Consequently, the majority of nuclear pH buffering is sourced from the cytoplasm in the form of mobile buffers, such as diffusible histidyl dipeptides (HDPs) present in abundance in adult cardiomyocytes, but detected at lower levels in neonatal or adult failing hearts. Low nuclear pore H+ permeability and weak nucleoplasm H+ buffering can allow pHnuc to change relative to cytoplasmic pH (pHcyt) under modest acid-base fluxes. Indeed, under certain stress conditions typically associated with hypertrophic remodelling, pHnuc can change substantially, with little change in pHcyt. For example, nuclear Ca2+ release evoked by inositol-1,4,5-trisphosphate signalling stably acidifies the nucleus. This novel pH-Ca2+ interaction is weakest in cells with low GDP content, but can be restored by long-term incubation with GDP precursors. We hypothesise that nuclear pH-Ca2+ coupling arises as a result of the diffusive exchange of Ca2+ for H+ ions through nuclear pores aboard HDPs. This mode of transport takes place because HDPs bind Ca2+ and H+ ions in a competitive manner (2). By controlling nuclear Ca2+/H+ coupling, HDP molecules are novel modulators of gene expression in cardiac development and disease.

(1) Hulikova & Swietach “ Nuclear proton dynamics and interactions with calcium signalling.” J Mol Cell Cardiol. 2015
(2) Swietach, Youm, Saegusa, Leem, Spitzer & Vaughan-Jones “Coupled Ca2+/H+ transport by cytoplasmic buffers regulates local Ca2+ and H+ ion signalling” Proc Natl Acad Sci U S A. 2013 110(22):E2064-73.

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SA004

Ca-calmodulin signaling in the heart, in health and disease
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Ca is essential in cardiac electrophysiology, contraction, energetics and nuclear transcription. Calmodulin (CaM) and Ca/CaM-dependent protein kinase (CaMKII) are also important mediators of Ca signaling in myocytes. CaMKII can phosphorylate and modulate function of Na, Ca and K channels, ryanodine receptor (RyR) and IP3 receptor channels, the phospholamban-SERCA complex and myofilaments. Some of these pathways may contribute to decreased cardiac function and enhanced propensity for arrhythmias in hypertrophy and heart failure (HF). Since CaMKII expression and activation state is increased in HF, these pathways may be important in contributing to the development and consequences of HF and may represent important therapeutic targets. CaMKII effects on cardiac Na channels and RyRs may be particularly important in HF and arrhythmias, and these acquired CaMKII-dependent effects can recapitulate genetic mutations in these channels that are associated with long QT (LQT), Brugada syndromes and Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT). In particular CaMKII can phosphorylate Nav1.5 and cause both enhanced late INa (as observed in LQT3) and also loss of Na channel availability (as observed in Nav1.5 mutants linked to Brugada and short QT syndromes) which the outcome dependent on heart rate. RyR phosphorylation by CaMKII increase diastolic sarcomplasmic reticulum (SR) Ca leak (as occurs in CPVT-linked mutations in Ryr2 and calsequestrin 2). This altered RyR gating can lead to increase delayed afterdepolarizations (DADs) and serve as a source of
triggered arrhythmias as well as cause reduced SR Ca content available for release in HF myocytes. Thus, CaMKII activation in HF and arrhythmogenic conditions can mediate acquired forms of cardiac arrhythmias and contractile dysfunction in pathologic conditions. CaM and FKBP12.6 both bind to the RyR in cardiac myocytes and can reduce RyR opening and SR Ca leak. We have directly measured the binding kinetics and functional impact of both CaM and FKBP12.6 to RyR in functioning myocytes, using fluorescence confocal imaging and fluorescence resonance energy transfer (FRET). FKBP12.6 binds with very high affinity (Kₐ ~ 1 nM) but has only modest functional effects, whereas CaM binds with Kₐ ~ 20 nM and strongly suppresses diastolic SR Ca leak and Ca spark frequency. Moreover, knock-in mice expressing a mutant RyR2 that cannot bind CaM have much higher SR Ca leak, triggered activity and arrhythmias at the whole animal level. This corresponds to human CPVTs that are linked to CaM point mutations which we have shown to bind to RyR2 with higher affinity than wild type CaM, but which also fail to quiet RyR2 like WT CaM (thus explaining the human CPVT phenotype). Pathological conditions that promote SR Ca leak (heart failure, oxidative stress and CaMKII activation) cause a shift in myocyte RyR2 conformation that has reduces CaM affinity and enhances binding of the conformation-sensitive peptide DPc10. Dantrolene and increased CaM concentration can shift this conformational state back to normal and also suppress diastolic SR Ca leak and arrhythmias. This raises the possibility of a novel RyR-related therapeutic strategy, namely new molecules that, like dantrolene, can shift the RyR conformational state from pathophysiological to physiological. Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA006

Intramyocellular lipid content during recovery from exercise in older individuals is associated with a lipogenic gene expression response

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Introduction. Older individuals have lower contribution of intramyocellular lipid (IMCL) to total fat oxidation during exercise (Chee et al 2016), which may be associated with higher IMCL content, inflammation and insulin resistance commonly observed in this population.

Aim. The aim of the present study was to examine the temporal relationship between IMCL content and the expression of genes associated with IMCL turnover, fat metabolism, insulin signalling and inflammation during recovery from an acute bout of exercise in older vs young men.

Methods. Seven healthy young males [23 ± 2 yrs, body mass 77.2 ± 2.9 kg, Body Mass Index (BMI) 23.5 ± 0.6 kg/m², fasting blood glucose (FBG) 5.1 ± 0.1 mmol/L] and 7 healthy older males [72 ± 1 yrs, body mass 79.3 ± 4.9 kg, BMI 25.9 ± 1.2 kg/m² (P<0.05), FBG 5.8 ± 0.2 mmol/L (P<0.05)] performed a single bout of resistance type exercise consisting of 20 sets of leg muscle exercises at the same relative workload (75% 1-RM). Muscle biopsy samples were obtained before and 12, 24 and 48 h after the completion of exercise and analysed for IMCL content using fluorescent microscopy, and the expression of 48 genes using RT-PCR. A controlled diet based on the subjects’ daily energy requirements was provided to each subject for the entire experimental period.

Results. The IMCL content (% area of muscle fibre analysed) was 2-fold higher (0.104 ± 0.010 vs 0.058 ± 0.010; P<0.01) at baseline in older compared with young individuals, respectively, and this difference was maintained at 12 h of post exercise recovery (P<0.05). However, the IMCL content increased at 48 h in the young subjects (P=0.09) whereas it remained unchanged in the old, such that there were no longer differences between groups at this time point (0.113 ± 0.014 vs 0.107 ± 0.024, respectively). The higher lipid content in the older individuals at rest was associated with a strong trend for lower (1.8-fold) expression of adipose triglyceride lipase (ATGL) mRNA (P=0.058), which remained reduced at 48 h post exercise in older compared with young individuals. There was also higher expression of genes involved in fatty acid synthesis, namely fatty acyl-CoA synthase and PPARγ at 12 h post exercise (1.5-fold; P<0.01) and 24 h (1.7-fold; P<0.05) in the former group, respectively. In addition significant differential responses to exercise were observed between the two age groups for a number of genes and transcription factors indicative of an exaggerated inflammatory response (COX2, IL6, IkBalpha, CREB1), insulin signalling (PI3KR1, Akt2), carbohydrate metabolism (HK2, LDH, ChREBP, PDK4), and impaired...
fat metabolism (LPL, Acetyl-CoA acetyltransferase, Succinyl CoA ligase) in older compared with young individuals.

Conclusions. Acute resistance type exercise leads to molecular changes in skeletal muscle favouring reduced fat utilisation, increased lipogenesis and elevated inflammation in the old, which may explain the inflexibility of IMCL turnover in those individuals.

Carolyn Chee, Chris E. Shannon, Aisling Burns, Anna L. Selby, Daniel Wilkinson, Kenneth Smith, Paul L. Greenhaff, Francis B. Stephens. The relative contribution of intramyocellular lipid to whole body fat oxidation is reduced with age, but subsarcolemmal lipid accumulation and insulin resistance are only associated with overweight individuals. Diabetes. 2016; 65: 840-50.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA007

One week of bed rest leads to substantial muscle atrophy and induces whole-body insulin resistance in the absence of skeletal muscle lipid accumulation

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Short (<10 days) periods of muscle disuse, often necessary for recovery from illness or injury, lead to various negative health consequences. The present study investigated mechanisms underlying disuse-induced insulin resistance, taking into account muscle atrophy. Ten healthy, young males (age: 23±1 y, BMI: 23.0±0.9 kg/m²) were subjected to one week of strict bed rest. Prior to and after bed rest, lean body mass (DXA) and quadriceps cross-sectional area (CSA; CT) were assessed, and VO₂peak and leg strength were determined. Whole-body insulin sensitivity was measured using a hyperinsulinaemic-euglycaemic clamp. Additionally, muscle biopsies were collected to assess muscle lipid (fraction) content and various markers of mitochondrial and vascular content. Bed rest resulted in 1.4±0.2 kg lean tissue loss and a 3.2±0.9% decline in quadriceps CSA (both P<0.01). VO₂peak and RM declined by 6.4±2.3 (P<0.05) and 6.9±1.4% (P<0.01), respectively. Bed rest induced a 29±5% decrease in whole-body insulin sensitivity (P<0.01). This was accompanied by a decline in muscle oxidative capacity, without alterations in skeletal muscle lipid content or saturation level, markers of oxidative stress, or capillary density. In conclusion, one week of bed rest substantially reduces skeletal muscle mass and lowers whole-body insulin sensitivity, without affecting mechanisms implicated in high-fat diet-induced insulin resistance.

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SA009

Atrial mechanosensors of the rat heart

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Abnormal control of the sympathetic nervous system is a feature of heart failure. Sympathetic tone changes in response to cardiovascular demand monitored by visceral receptors sensitive to haemodynamic changes and blood oxygen levels. Atrial volume receptors (AVRs) at the venous atrial junction are mechanoreceptors which convey information to the CNS about venous volume. The details of how these receptors sense volume changes and transduce that change into a neural output have yet to be described. AVRs are typically classified as either A- or B-type according to their pattern of discharge. Broadly, A-type receptors respond to atrial contraction while B-type are stimulated by atrial filling and are considered to be volume receptors. However, intermediate AB-type discharges have also been described.

The identification of mechanosensitive channels in vertebrates remains elusive. Two channel protein families in particular are candidates: the Epithelial Na Channel/Degenerin/Acid Sensing Ion Channel (ENaC/DegenerinASIC) and Transient Receptor Potential (TRP) families [1]. The g subunit of ENaC is expressed in baroreceptor nerve terminals innervating the aortic arch and carotid sinus in mice [2]. ASIC1, 2 and 3 were found in aortic baroreceptor neurones in the nodose ganglia and their terminals in the aortic arch [3].

We have shown that in rat heart the TRP channels TRPC1 and TRPV4 are expressed in sensory endings found in regions of cavo-atrial endocardium where AVRs are located. The TRPC1 and TRPV4-IR co-localises with extensive synaptophysin (SYN)-IR, a marker of synaptic-like vesicles (SLVs) common to many mechanosensory endings [4]. So far we have found no evidence of ENaC/DEG/ASIC-IR, suggesting the volume receptor may rely on different molecular components from those reported for the baroreceptor. Nevertheless the distinctive synaptophysin reactivity indicates possible commonalities with the model proposed by Bewick et al. [5] whereby the excitability of mechanosensory endings is regulated by mechanical activity which stimulates the recycling and release of a neuromodulator from SLVs.

In a parallel study an in vitro preparation of rat vagus-cavoatrium is being perfected by colleagues in Dublin. This permits direct recording and classification of atrial mechanoreceptors. This will confirm or refute the functional significance of proteins expressed in AVRs.


Glutamatergic regulation of mechanosensitivity: isolating the atypical receptor from muscle spindles

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Modulation of muscle spindle stretch-evoked firing has an atypical glutamate receptor pharmacology; L-cysteinesulfinic acid, quisqualate and kainate are potent agonists of afferent firing. However, afferent firing is not modulated by Group I, II, or III mGluRs ligands, kynurenate (broad-spectrum iGluR antagonist) or NBQX (kainate receptor antagonist). This glutamate pharmacological profile most closely matches that of the PLD-GluR described in the hippocampus by a number of groups13–5. To allow further investigation of this atypical receptor, a functionalised kainate moiety amenable to ‘click’ chemistry (ZCZ-90) has been synthesised6. Here we describe how ZCZ-90 allowed us to make progress towards the isolation of the atypical spindle glutamate receptor by using biotin (ZCZ-180) and fluorescein (ZCZ-172) conjugated forms to visualise ligand binding in spindle homogenates and whole mount tissues, and the subsequent identification of putative glutamate receptor-like proteins associated with spindles.

To obtain sufficient spindle protein for screening, a method to extract a high yield of spindle material was first developed. The column of ~100 spindles in rat deep masseter muscle was disrupted by collagenase digestion, visualised with methylene blue, then isolated by microdissection and the protein extract extracted. Far Western blotting with ZCZ-180 confirmed that spindle homogenate contained a protein capable of binding this biotinylated kainate analogue, with bands at ~120 kDa and ~70 kDa. Bands of similar mass were detected in the hippocampus, our positive control where the PLD-GluR was first described. ZCZ-172 labelling in masseter whole mounts revealed fluorescent ligand binding on annulospiral nerve terminals. This suggests the protein to which the ZCZ ligands, and thus kainate, binds is indeed expressed in annulospiral nerve endings, and is well-placed to be involved in the glutamate-modulation of the stretch response.

To further identify the atypical receptor, we screened for all known mGluRs and kainate receptors (KARs) using Western blotting of spindle homogenates and immunofluorescence of lumbrical spindle whole mounts. We identified a rapidly migrating isoform of mGluR5 but, consistent with previous studies6, mGluR5 immunofluorescence was only present on nociceptors running parallel to spindles. However, Western blotting did show spindle homogenates contained bands for GluK2, and immunofluorescence showed labelling for GluK2 on annulospiral nerve terminals, consistent in both molecular weight and labelling pattern to ZCZ-172 labelling, respectively. This suggests the GluK2 receptor subunit is indeed involved in modulating stretch-induced afferent firing.

We are now pursuing the isolation of the atypical receptor protein. A band of a similar molecular weight to that probed by ZCZ-180 in homogenate enriched for lipophilic (membrane-associated) proteins could not be identified by mass spectrometry. However, the ‘unassigned’ peptides in this band showed strong sequence homology to glutamate receptors by NCBI-based sequence alignment. Furthermore, functional clustering analysis of the proteins associated with the unassigned peptides identified PLD as a potential regulatory hub, suggesting the protein(s) in the band likely signal through PLD. These data are consistent, therefore, with the receptor being an atypical glutamate receptor coupled to PLD. We have thus developed a functionalised kainate analogue and used it to demonstrate the expression of a kainate-binding protein in muscle spindles. This seems most likely to be GluK2, despite the spindle pharmacology resembling that of mGluRs. Metabotropic actions of kainate receptors have been described elsewhere7, yet the exact functions of these remain somewhat elusive. Our current working hypothesis is that we have uncovered a functional system which uses only the metabotropic function of these iGluR subunits. Future work will focus on isolating sufficient protein for sequencing to validate whether it is the iGluR subunit which is responsible for glutamatergic modulation of this class of mechanoreceptors. Ultimately, the receptor will be fully characterised by expression in a cell line to determine protein-protein interactions and detailed pharmacological profile. Preliminary evidence presented previously to the Society suggests this receptor is expressed on baroreceptors. If confirmed, this atypical receptor may provide a novel drug target for treating hypertension.


We are grateful to Tenovus Scotland for their continued support through the Moulton-Barrett Scholarship and the award of a Small Project Grant BP041/RGB43324, and also to Eli Lilly for supporting the development of ZCZ analogues. We also thank Aberdeen Proteomics for their help with 1D gels and mass spectrometry.

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SA011

Pleiotropic function of TRPV4 ion channels in the CNS
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TRPV4 ion channels represent osmo-mechano-TRP channels with pleiotropic function and multi-locular expression. They have been found involved in pain and inflammation. Studies have focused on the role of TRPV4 in peripheral sensory neurons, but its expression and function in central nervous glial cells and neurons has also been documented. In this overview, we will review evidence of TRPV4 function in these two cell types in the CNS, and how TRPV4 function can be modulated for therapeutic benefit of neuro-psychiatric disease. Novel TRPV4-inhibitory compounds developed recently in the author’s lab will also be introduced.

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SA012

TRPV4 in the Paraventricular Nucleus of the Hypothalamus – A role for osmosensing
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The paraventricular nucleus of the hypothalamus (PVN) is a heterogenous structure within the hypothalamic area of the brain. Research has shown this nucleus has various roles such as cardiovascular control, regulation of metabolism and is involved in the stress pathway [for review see 1]. Of particular interest to our group is the potential role of the PVN in osmoregulation [2]; water deprivation leads to increased expression of c-fos in parvocellular PVN neurons and hypertonic solutions have been shown to increase activity of PVN neurons [3,4]. However, the ionic mechanisms responsible for the role of the PVN in osmoregulation have not yet been determined. More recent studies have implicated the transient receptor vanilloid channel (TRPV4) as a possible candidate for osmosensing [5].

Using a combination of in vitro techniques we have investigated a potential role for TRPV4 in osmosensing within the PVN. Cellular mechanisms were investigated by cell-attached patch-clamp recording in mouse PVN hypothalamic brain slices. Further investigation using isolated PVN neuronal cells from Wistar rats included whole-cell current clamp recordings and intracellular calcium measurements using the ratiometric dye Fura-2AM (5μM). In addition, we hypothesise that this mechanism may in part play a role in cardiovascular control in response to osmotic challenge using in vivo blood pressure recording. CD1 mice were anaesthetized with urethane-chloralose (1.4-2.2mg/kg-7-11μg/kg), administered intraperitoneally. Blood pressure was measured by arterial cannulae and compounds applied by intracerebroventricular (ICV) injection. Results are given as mean ± SEM; significances were assessed by two-way ANOVA and Student’s paired t-tests where appropriate.

We initially investigated the effect of hypertonic challenge on the activity of neurons using action currents as an indication of action potential frequency in PVN brain slice experiments. Osmotic challenge decreased action current frequency by 79±10 % (n=10; p<0.0001; from 300 mosm (control) to 270 mosm (hypotonic)). The effect of hypertonic challenge in the reduction of action current frequency was mimicked using the TRPV4 agonist 4cPDD (1 μM) and the highly selective agonist GSK1016790A (100 nM) with reductions of 36±10 % (n=6; p<0.01) and 72 ± 8 % (n=6; p<0.05) respectively. The response to hypertonic solution was significantly reduced by the TRPV4 antagonists RN1734 (5 μM) and the highly selective HCO67047 (300 nM): hypertonic challenge: 70±14 % reduction vs. hypertonic with RN1734: 45±15 % reduction (n=6; p<0.05), and a 10±13 % reduction (n=6; p<0.01) with HCO67047 in action current frequency. These results provide further evidence TRPV4 has a role for osmosensing within neurons of the PVN. Interestingly, TRPV4 did not seem to have a role in temperature sensing in PVN neurones.

In order to confirm this is a direct effect upon PVN neurones themselves, cell-attached electrophysiology was used to record single channel activity of PVN neurones in CD1 mouse brain slices. A population of ion channels was identified with a mean slope unitary conductance of 57 ± 7 pS and reversal potential of -5±3 mV (n=8); indicative of a non-selective cation channel. This channel activity was seen in 50 % of patches (8/16), with a mean open probability (P) of 0.1±0.0 at ~40 mV. P, increased by 48±9 % (n=4) upon addition of the TRPV4 agonist 4cPDD. Furthermore, during whole-cell recordings from isolated Wistar rat PVN neurones the addition of 4cPDD and GSK1016790A resulted in depolarisations of 11±2 mV (n=4; p<0.05) and 12±5 mV (n=5; p<0.05) respectively. In addition, during Ca2+ recordings using Fura-2AM an increase in intracellular Ca2+ concentration was seen with both hypotonic solution: 77±3 nM to 198±28 nM (n=8; p<0.001) and 4cPDD: 75±2 nM to 121±14 nM (n=6; p<0.005).

In vivo studies uncovered a potential role for TRPV4 in cardiovascular control. ICV injection of hypertonic saline led to a rapid decreases in blood pressure, whereas isotonic saline had no effect (-9±2 mmHg vs. -2.1 mmHg; n=6; p<0.01). These decreases in BP were abolished by the TRPV4 antagonist RN1734 (-1±1 mmHg; n=6).

These results suggest a role for TRPV4 channels in sensing central osmolality changes within the PVN. Furthermore, central osmolality changes can modulate PVN neuronal activity, which may subsequently have effects upon cardiovascular control.


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Functional interaction between TRPV4 and anoctamin 1 causes water efflux in choroid plexus epithelial cells

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Choroid plexus is involved in the maintenance of the brain’s environment in the lateral, the third and the forth ventricles. This tissue consists of a continuous monolayer structure made of epithelial cells, leptomeninges and fenestrated capillaries. Choroid plexus epithelial cells (CPECs) are separated into apical and basolateral sides by tight junctions. The apical membrane faces the cerebrospinal fluid (CSF) in the ventricles whereas the basolateral membrane is on the side of the fenestrated capillaries. This structure is very important for formation of the blood-CSF barrier, and the most important function of CPECs is CSF production. The generation of CSF is dependent upon electrolyte transport from the basolateral to the apical membranes of CPECs and involves many ion transport proteins. On the other hand, TRPV4, a calcium-permeable channel, is highly expressed in the apical membrane of CPECs in the brain while its physiological function is poorly understood. We observed outwardly rectifying chloride currents induced by intracellular calcium increases in isolated murine CPECs although the functional expression of calcium-activated chloride channels (CaCCs) had not been reported. Therefore, we hypothesized that TRPV4 activation might affect CaCC activity. We found co-expression of anoctamin 1 (ANO1), one of CaCCs, with TRPV4 in CPECs. Therefore, physical and functional interaction between TRPV4 and ANO1 was examined in HEK293T cells and CPECs (1). Chloride currents induced by a TRPV4 activator GSK1016790A (GSK) were markedly increased in an extracellular calcium-dependent manner in HEK293T cells expressing TRPV4 with ANO1, but not with ANO4, ANO6 or ANO10, the mRNAs of which were expressed in the choroid plexus. We also found physical interaction between TRPV4 and ANO1 in both HEK293T cells and choroid plexus. We observed that ANO1 was activated at a warm temperature (37 degree C) in HEK293T cells and that the heat-evoked chloride currents were markedly enhanced after GSK application in CPECs. Simultaneous stimulation by warmth and hyposmotic induced chloride current activation in wild-type, but not in TRPV4-deficient CPECs. Cell volume changes were induced by ANO1-mediated chloride currents in parallel with membrane potential changes, and the cell volume was significantly decreased at negative membrane potentials by TRPV4-induced ANO1 activation. In native cells, cation efflux should accompany chloride movement and could help to maintain the deep membrane potentials that in turn accelerate chloride efflux. Thus, physical and functional interactions between TRPV4 and ANO1 can modulate water transport in the choroid plexus. The functional interaction between TRPV4 and ANO1 was also observed in the exocrine glands with water efflux, and the functional interaction between TRP channels with high calcium permeability and ANO1 is widely observed in other cell types such as sensory neurons.


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Dietary interventions for fetal growth restriction

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One of the common factors apparent in several animal models of prenatal stress is impairment of uteroplacental vascular function. This can lead to a reduction in the delivery of oxygen and nutrients to the developing fetus, resulting in reduced fetal growth and subsequently long-term programming of disease. Whilst we still do not fully understand the mechanisms underpinning impaired uteroplacental function in compromised pregnancies, interventions aimed at enhancing nitric oxide bioavailability remain a key area of interest in obstetric research.

Dietary nitrate (abundant in green leafy vegetables and beet-root) is an important modulator of cardiovascular function in non-pregnant humans and animals. Treatment with dietary nitrate, providing an exogenous source of nitric oxide (NO) and other bioactive nitrogen oxides, can improve blood flow, vascular function and reduce damage caused by ischaemia-reperfusion injury.

We have shown that maternal dietary nitrate supplementation, via beetroot juice, improves ex vivo uterine artery function in a model of fetal growth restriction associated with vascular dysfunction, the endothelial nitric oxide synthase knockout (eNOS\(^{-/-}\)) mouse. This improvement in vascular function was associated with elevated plasma nitrate and nitrite (NO\(_x\)) concentrations. Nitrate-depleted beetroot juice, which failed to elevate plasma NO\(_x\) levels, did not alter maternal vascular function.

Our ongoing studies aim to determine the mechanisms underlying the beneficial effects of dietary nitrate supplementation on vascular function, using both animal models and human uteroplacental tissue. In parallel, we are now also investigating the effects of a short-term dietary nitrate supplementation on cardiovascular function and uteroplacental blood flow in pregnant women. Together, these studies aim to develop an acceptable and efficacious dietary intervention that can improve both maternal and fetal outcomes in compromised pregnancies.

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The mechanisms involve determining the immediate, short and long-lasting effects are still unclear, but the lack of oxygen and increased oxidative stress are apparently triggering most of the responses. For several years, the study of the responses to hypoxic insults and pharmacological targets has been the motivation of our group. This talk will describe some of the mechanisms underlying the cardiovascular responses to hypoxia in the perinatal period and potential therapeutic approaches to diminish these effects. In the current world-wide scenario, where births and life expectancy are increasing, the Developmental Origins of Health and Disease (DOHaD) mechanisms demands urgently new treatments to deal with intrauterine hypoxia.

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SA018

Role of spinal networks in the control of diaphragm and intercostal muscle activation

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Spontaneous breathing is characterized by a complex pattern of inspiratory muscle activation. The primary source of inspiratory drive to the diaphragm and intercostal muscles arises from bulbospinal projections to spinal interneurons and motoneurons. In contrast to the conventional view that motor control of inspiratory muscle activation resides largely in upper respiratory centers, several studies indicate that important control mechanisms also exist at a spinal level. Specific reflexes, mediated by spinal interneurons, provide separate auto control of diaphragm and intercostal muscle activation while other reflexes affect the interplay between the different major inspiratory muscles and coordination of their actions. For example, diaphragm activation is modulated by excitatory and inhibitory phrenic to phrenic reflexes and intercostal to phrenic reflexes while inspiratory intercostal activation is modulated by intercostal to intercostal and phrenic to intercostal reflex effects. While some reflex effects are clearly spinal in origin, there are likely supraspinal inputs, as well. Differentiating the source of some of these individual reflex effects has been difficult to discern. New models of inspiratory muscle activation via electrical stimulation techniques however may shed some light on this issue.

Recent studies in an animal model of spinal cord injury (C2 preparation) have shown that the phrenic and respiratory intercostal motoneuron pools can be activated via low intensity, high frequency spinal cord stimulation (HF-SCS) at the T2 level in a remarkably physiological manner. The diaphragm and inspiratory intercostal muscles are activated asynchronously as in normal breathing and the firing frequencies of these muscles are nearly identical to that occurring during spontaneous breathing. Since participation of the upper respiratory centers has been eliminated, this preparation provides a useful model to separately assess the influence of spinal mechanisms on inspiratory muscle activation.

During spontaneous breathing in both animals and humans, there is greater activation of both the parasternal and external intercostal muscles in the upper interspaces and greater activation of the dorsal compared to ventral fibers within a given interspace. Interestingly, this pattern is a highly efficient one as it also matches the mechanical advantage of these individual muscles between interspaces and also muscles fibers within a given interspace. During HF-SCS following C-2 section in dogs, this same pattern has been observed for both the parasternal and external intercostal muscles, in separate trials. These results indicate that the specific complex pattern of intercostal muscle activation does not depend upon input from supraspinal centers but resides at the level of the spinal cord. While there are number of possible mechanisms operative at a spinal cord level which could mediate differential intercostal activation, these results fit the hypothesis that there is an important role of spinal interneurons in the control of inspiratory muscle activation.

In conclusion, the precise role of the spinal cord neural network in the control of breathing may have been underestimated and with the proper tools, the importance of spinal mechanisms can be further elucidated.


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Drives to human respiratory motoneurones: Integration at a spinal level

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Human respiratory motoneurones in the spinal cord receive drives that originate from multiple supraspinal sites, depending on the ‘task’, i.e. from the medulla for quiet breathing and the motor cortex for voluntary tasks. The integrated motoneurone output can be assessed by intramuscular single motor unit recordings. During quiet breathing, there is differential output from motoneurone pools of the human obligatory inspiratory muscles (Saboisky et al., 2007). The timing, relative to inspiratory flow, and the magnitude of motor unit activity is non-uniform. Specifically, for the parasternal intercostal muscles of the first-to-fifth interspaces, motor units in the rostral interspaces are active early in inspiration and discharge at a high rate compared to caudal interspaces (Gandevia et al., 2006). Remarkably, the degree of inspiratory motor unit output of the parasternal intercostal muscles parallels the spatial distribution of inspiratory mechanical advantage of these muscles across interspaces. Consequently, a principle of ‘neuromechanical matching’ between the neural drive and mechanics of portions of inspiratory muscles may govern recruitment of spinal respiratory motoneurones when driven from the pontomedullary region during quiet breathing. In targeted voluntary breaths, when drive originates from the motor cortex, the rostro-caudal pattern of motor unit output from the first-to-fifth parasternal intercostal is maintained (Hudson et al., 2011b). The parasternal intercostal muscles are also active in a different voluntary task of ipsilateral trunk rotation. The voluntary postural and inspiratory drives depolarise the same motoneurones and a concurrent postural task changes the inspiratory output of the parasternal intercostal motoneurones in a direction-dependent manner (Hudson et al., 2010). However, in a similar rotation task, the costal diaphragm is not active and the inspiratory output of the phrenic motor units is not altered (Hudson et al., 2011a). Taken together, these observations have important implications for the integration of voluntary and inspiratory drives to respiratory motoneurones at different levels of the spinal cord. We propose that premotoneuronal networks, perhaps in the spinal cord, sculpt descending drive to respiratory motoneurones from multiple sources (Hudson et al., 2011c). New data support this proposal. The recruitment behaviour of parasternal intercostal motor units of different interspaces was assessed in distinct inspiratory and voluntary rotation tasks. As expected, there was differential inspiratory activity in motor units from the 2nd and 4th interspaces with earlier onset of activity in the rostral interspace, relative to the onset of inspiratory flow. However, for the same motor units, there was no difference in the rotation torque at which the units were recruited during ramped ‘isometric’ rotations. With voluntary drive for the rotation task, there is divergence from the differential recruitment observed during inspiration. This suggests that parasternal intercostal motoneurone output at different spinal levels can change depending on task and that the output of respiratory motoneurones may be related to the precise mechanical advantage of the muscles for that task. A spinal mechanism that integrates and distributes the drive to different human inspiratory muscles would determine the differential pattern of activation across inspiratory muscles, preserve the neural and mechanical coupling when voluntary breaths are taken and allow for different patterns of activation in non-respiratory contractions. Studies in dogs reveal that high-frequency stimulation at the T2 spinal level mimics the physiological activation of the parasternal intercostal muscles (and other inspiratory muscles) during breathing (e.g. DiMarco & Kowalski, 2015). The differential activity between regions of the parasternal intercostal muscles is preserved in these animals, including differences between the medial and lateral portions of a muscle within an intercostal space. As the spinal cord was transected at the C1 level during stimulation, these data corroborate our findings in humans that spinal cord contains circuits that can distribute drive to spinal respiratory motoneurone pools.


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Contribution of the motor cortex to human breathing

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Automatic medullary mechanisms ensure continuity of breathing and adaptation to metabolic fluctuations, right from birth and throughout life. However, breathing is unusual among autonomic functions in that it also responds to motor commands arising from complex cortico-subcortical networks comprising motor and premotor areas (particularly the supplementary motor area). These networks are engaged during voluntary respiratory manoeuvres (such as voluntary apnoea, voluntary sniffing, or voluntary hyperventilation)(1). They are also engaged when the respiratory system is used for non-respiratory purposes such as speech (2), and induced respiratory neuroplasticity experiments using repetitive transcranial magnetic stimulation have suggested that these networks exert a tonic excitatory influence on breathing during wakefulness (3). Inspiratory loading experiments in healthy volunteers have demonstrated that respiratory-related cortical networks are activated when the respiratory system is faced with a mechanical constraint (functional magnetic resonance imaging and electroencephalographic data), leading to the hypothesis of the cortical origin of the “paradoxical” hyperventilation associated with inspiratory loading during wakefulness in humans (4). In disease, respiratory-related cortical activity has been described in patients with deficient respiratory automaticity.
Drive to the human diaphragm in health and disease

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The output from brainstem respiratory centres, neural respiratory drive (NRD), is directly proportional to the ventilation required to maintain blood gas homeostasis. Increases in the load imposed upon the respiratory muscles or a reduction in force-generating capacity, as occurs in respiratory disease, results in higher levels of NRD. The neural output of the brainstem respiratory centres cannot be measured directly in humans, but it can be assessed indirectly by quantifying the electromyogram (EMG) of the respiratory muscles, which provides a method of assessing the level and pattern of their activation. The EMG of the diaphragm (EMGdi), the major inspiratory muscle during resting tidal breathing in healthy individuals, can be recorded using multipair oesophageal electrodes positioned at the diaphragm crus. In chronic obstructive pulmonary disease (COPD), mechanical abnormalities including airflow obstruction, static and dynamic hyperinflation and intrinsic positive end expiratory pressure increase the load on the respiratory muscles. This results in high NRD in COPD, and disproportionate increases whenever airflow obstruction worsens (and hyperinflation increases) or ventilatory requirements increase. EMGdi activity is significantly higher in COPD than in healthy controls, and is closely related to COPD disease severity as defined by spirometry and hyperinflation (1). Work by our group, and others, has also demonstrated that exertional breathlessness intensity in health (2, 3), obstructive lung disease (COPD (4), cystic fibrosis (2)), and interstitial lung disease (5) is closely related to levels of EMGdi activity. In severe respiratory disease, breathlessness intensity is better related to EMGdi activity than respiratory muscle pressure generation and ventilation (2, 4), reflecting the impaired translation of NRD to mechanical output and respiratory airflow as a consequence of impaired ventilatory mechanics. These findings support the “corollary discharge” theory of breathlessness, that perception of breathlessness intensity involves conscious appraisal of increased motor drive to the principal respiratory muscles. This emphasises the importance of accurate recordings of NRD to advancing our understanding of the physiological basis of breathlessness in health and disease. These observations also have clear clinical implications. If breathlessness intensity is closely related to levels of NRD, simply asking the patient to rate breathlessness intensity could provide insights into the load on the respiratory system, disease severity and response to treatment that are not always evident from conventional measures of pulmonary function (6, 7).


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SA022

A mechanism for sick sleep: Studies in the nematode Caenorhabditis elegans

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Cardinal manifestations of sickness include anorexia (lack of eating) and sleepiness/fatigue. The mechanism of this sick sleep involves signaling by cytokines acting on central nervous system neurons, but the mechanism downstream of the cytokines is unknown. We studied the mechanism of sick sleep using the laboratory animal Caenorhabditis elegans. When C. elegans is exposed to environmental conditions that cause sickness, including bacterial toxins, extreme heat, high osmolality, or ultraviolet radiation, it will stop feeding (anorexia) and moving, and become less responsive to sensory stimulation. This sick behavior is abolished when a single interneuron called ALA (among a total of 302 neurons in the C. elegans nervous system) is removed (Hill et al, Cellular stress induces a protective sleep-like state in C. elegans, Curr. Biol. 24, p. 2399, 2014). Upon exposure to sick-inducing conditions, ALA is activated by cytokine epidermal growth factor, which results in ALA depolarization and the release of neuropeptides encoded by the gene fip-13 (Nelson et al, FMRFamide-like FLP-13 neuropeptides promote quiescence following heat stress in Caenorhabditis elegans, Curr. Biol. 24, p. 2406, 2014). FLP-13 neuropeptides belong to a neuropeptide family characterized by an amidated Arginine-Phenylalanine (RFamide) motif at their C-termini. FLP-13 neuropeptides activate a seven transmembrane domain receptor, which is coupled to the inhibitory G-protein G/j/o (Trojanowski et al, Distinct mechanisms
underlie quiescence during two Caenorhabditis elegans sleep like states, J. Neurosci. 35, p. 14571, 2015), resulting in a reduction of activity of wake promoting neurons. A peptidergic RFamide signaling mechanism functions also in sick sleep in Drosophila (Lenz et al, FMRFamide signaling promotes stress-induced sleep in Drosophila, Brain Behav. Immun. 47, 141, 2015), indicating that this mechanism is conserved across evolution. We propose that a similar mechanism functions in mammals to mediate anorexia and sleepiness during sickness.

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SA023

Mapping sleep regulatory genes onto neuronal circuits in zebrafish
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Sleep is a deeply conserved phenomenon, yet the genetic and neuronal mechanisms that regulate sleep are still being uncovered. Zebrafish are an excellent model system in which to investigate sleep, because of the capacity for cost-effective genetic and pharmacological screening and the larval brain’s optical translucency facilitates functional neuroanatomical studies. Zebrafish larvae as young as five days post fertilization display circadian-regulated periods of quiescence, during which the larvae are less sensitive to their environment. These sleep states are under homeostatic regulation, as depriving larvae of sleep subsequently leads to increased, deeper sleep states. Furthermore, zebrafish sleep is regulated by systems shared by humans, including the hypocretin/orexin system that is lost in narcoleptic patients. This presentation will highlight novel sleep regulatory networks unearthed through zebrafish drug and genetic screens and suggest a template for mapping the function of sleep genes onto discrete neuronal circuits.

This work is supported by a Starting Grant from the European Research Council and a UCL Excellence Fellowship.

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SA024

Exploration of Toll-like receptors 2 (TLR2) role in neuroinflammatory response in obstructive sleep apnoea
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Sleep is fundamental for optimal psychomotor functioning, adequate immune responses, brain plasticity, memory, and ultimately our cognitive performance and well-being. Disturbed sleep is a common denominator of several neuropsychiatric disorders, including schizophrenia and major depression. More recently, it has also been linked to early cognitive decline and Alzheimer’s dementia (AD). Although the mechanisms underlying these associations are not clear, dysregulated immune system and microglia cells have been suggested to play a role. Microglia are the resident immune cells in the brain, which continuously survey the brain parenchyma through their branched projections to detect tissue damage or microbial insult. Via their cell receptors, such as the Toll like receptors (TLR), they recognise important patterns and bind various compounds for elimination, including those whose accumulation may lead to Alzheimer’s dementia (e.g. beta amyloid). However, dysregulated microglia can also contribute to spread of toxic (pathological tau) proteins closely linked to dementia.

Our group’s current and past work suggests that dysregulated sleep during obstructive sleep apnoea (OSA), one of the most common, and widely underdiagnosed sleep disorders, leads to aberrant brain immune responses (neuroinflammation) in patients. We and others have suggested that this could be in part due to activated microglia cells and maladaptive immune responses in the brains of patients with OSA. OSA is a debilitating chronic multisystem disorder, with ever-increasing prevalence, fuelled by an ageing population and the obesity epidemic. It is characterized by brief periods of repetitive upper airway occlusion, periods of low oxygen in the blood and dysregulated, fragmented sleep. Mechanisms behind OSA-induced brain injury in patients are still largely unknown. However, importantly, OSA is also known to be linked to early cognitive decline and AD, major depression, anxiety disorders, post-traumatic stress disorder, and even schizophrenia.

Our preliminary data in animals further supports this hypothesis. More specifically, we have been able to show an increase in a specific TLR (TLR2) signal in a chronic intermittent hypoxia mouse model of OSA, suggesting adaptive activation of microglia in several regions of brain implicated in memory, mood and cognition. Other groups have described schizophrenia-like behaviours in mice lacking TLR2 receptors, further emphasising the importance of microglia in associated cognitive deficits in chronic neuropsychiatric disorders.

In this study, we have studied mice with and without TLR2 receptors (e.g. transgenic TLR2-luc- & TLR2-KO mice) for shared neuroimmuno-cognitive mechanisms activated during fragmented sleep and episodes of low oxygen. All mice in our study underwent a protocol regime that closely mimicked the OSA disease process in patients, after which comparison of their behaviour and cognition was explored in detail. In addition, comparison of their structural brain grey and white matter changes, and intrinsic functional connectivity between the parts of the brain that deal with memory, emotions and cognition provides some further crucial insights into the shared immune adaptive and maladaptive responses at play.

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SA025

Urethral brush cells: Ancient sentinels of the urinary tract
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Chemosensory cells in the mucosal surface of the respiratory tract (“brush cells”) utilize the canonical taste transduction cascade to detect potentially hazardous content and trigger local protective and aversive respiratory reflexes upon stimulation. The urogenital tract has been considered to lack this cell type.
Recently we newly identified a population of cholinergic epithelial cells in the mammalian urethra that exhibits a structural marker of brush cells (villin) and expresses bitter and umami taste receptors and downstream components of the canonical taste transduction signaling cascade (e.g., gustducin, phospholipase Cβ2 (PLCβ2), transient receptor potential cation channel melanostatin 5 (TRPM5)). These urethral brush cells respond to stimulation with bitter (denatonium), umami (monosodium glutamate) and uropathogenic Escherichia coli, and release acetylcholine to communicate with other cells. They are approached by sensory nerve fibers expressing nicotinic acetylcholine receptors, and intraurethral application of denatonium reflexively increases activity of the bladder detrusor muscle in anesthetized rats. In order to elucidate cross-species conservation of the urethral chemosensory pathway we investigated the occurrence and molecular make-up of urethral brush cells in placental mammals. We immunohistochemically screened 14 species including humans, at least one in each of the five mammalian taxonomic units primates, carnivora, perissodactyla, artiodactyla and rodentia. Urethral epithelial cells with brush cell shape were immunolabeled in all 14 mammals. These data indicate that urethral brush cells, widespread throughout the mammalian kingdom and evolved not later than about 64.5 million years ago, serve as sentinels, monitoring the chemical composition of the luminal content for potentially hazardous compounds such as bacteria, and initiating protective reflexes counteracting further ingestion. Deckmann K et al. (2014). Proc Natl Acad Sci U S A 111, 8287-8292

Deckmann K et al. (2015). Int Immunopharmacol 29, 51-56

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Metabolic Rate (RMR) [3] are closely associated with homeostatic indices such as meal size and Total Daily Energy Intake. This has prompted interest in the impact of behavioural EE, and especially the role of sedentariness, on appetite control. Alongside the question: ‘can you outrun a bad diet?’, we can ask: ‘can you out eat an active lifestyle?’ These questions demand a study of appetite control within an energy balance framework.

The schema of Mayer [4] proposed that physical activity (or total EE) is related to EI by a U-shaped function. At average to high levels of EE the appetite system appears well controlled (Zone of Regulation), but below a normal activity level (sedentary zone or Zone of Dysregulation) appetite is weakly controlled. Sedentariness is dangerous not only because it lowers EE but also because it can promote overeating. It has been hypothesised that ‘Body signals go awry in sedentary lifestyles; .. Sedentary persons may lose the innate ability to compensate for inactivity by reducing their eating’ [5]. This passive overconsumption can be expected to lead to fat gain. Using a 24-hour monitoring system it can be demonstrated that sedentary behaviour is positively associated with percent body fat and with traits of appetite dysregulation. In contrast the amount of moderate to vigorous activity is negatively related to body adipose tissue [6]. Prescribed mandatory medium term interventions that increase physical activity bring about a significant reduction in Fat Mass (FM) with a preservation (or increase) in FFM.

This objective evidence is a strong refutation of claims about the ‘myth of physical inactivity and obesity’ [7]. However there is large individual variability probably reflecting allelic and biological diversity leading to the identification of responder and non-responder phenotypes. Individuals behave differently due (in part) to the operation of a dual action of exercise on appetite mechanisms; this can take the form of an increase in hunger coupled with an increase in the strength of post-prandial satiety signalling. This satiety effect is associated with changes in Gastro-Intestinal peptide levels (ghrelin, GLP-1, PYY and CCK), and also with an increase in insulin and leptin sensitivity. These findings indicate a complex (bi-directional) relationship between EE and EI that cannot be described by one simple formula. Sedentariness is a risk factor for fat gain and for weakened satiety control. However, it should be remembered that a high Energy Density diet can raise EI above the EE from a PAL of 1.7-1.8. These findings endorse the importance of studying appetite control within an energy balance framework and point to the need to control both diet and activity.

Background: A decline in resting energy expenditure (REE) beyond that predicted from changes in body composition has been noted following dietary-induced weight loss. However, it is unknown whether a compensatory down-regulation in REE also accompanies exercise-induced weight loss, or whether this adaptive metabolic response influences energy intake (EI).

Methods: Thirty overweight and obese women (BMI = 30.6 ± 3.6kg m⁻²) completed 12 weeks of supervised aerobic exercise (EX). Body composition, metabolism, EI and metabolic-related hormones were measured at baseline, week six and post-intervention. The metabolic adaptation i.e. difference between predicted and measured REE was also calculated post-intervention (MApost), with REE predicted using a regression equation generated in an independent sample of 66 overweight and obese women (BMI = 31.0 ± 3.9kg m⁻²). Results: While mean predicted and measured REE did not differ post-intervention, 43% of participants experienced a greater than expected decline in REE (-102.9 ± 77.5 kcal·day⁻¹). MApost was associated with the change in leptin (r = 0.47; p = 0.04), and the change in resting fat (r = 0.52; p = 0.01) and carbohydrate oxidation (r = -0.44; p = 0.02). Furthermore, MApost was also associated with the change in EI following EX (r = -0.44; p = 0.01). Conclusions: Marked variability existed in the adaptive metabolic response to EX. Importantly, those who experienced a down-regulation in REE also experienced an up-regulation in EI, indicating that the adaptive metabolic response to exercise influences both physiological and behavioural components of energy balance in a co-ordinated fashion.

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**The adaptive metabolic response to exercise-induced weight loss influences both energy expenditure and energy intake**

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**Malhotra A, Noakes T and Phinney S It is time to bust the myth of physical inactivity and obesity: you cannot outrun a bad diet.** BJSM 2016

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Physical activity and exercise: Interactions with energy intake and energy balance

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Physical activity and exercise are frequently maligned in the popular press and in scientific journals in part because there is so much confusion. Whilst structured bouts of exercise are usually only a small part of total energy expenditure, overall physical activity is a demonstrably important component of energy expenditure and energy balance. Furthermore, specific forms of structured exercise have very potent effects on various physiological pathways and processes – irrespective of their (relatively) modest impact on energy expenditure and balance. The most powerful demonstration of this effect is in studies that have deliberately created an energy surplus alongside the imposition of regular exercise (e.g., overfeeding studies where there is deliberate weight gain alongside regular exercise). Broadly, these studies support the notion that it is indeed possible to ‘outrun a bad diet’ at least in the short term. These effects have even been observed in the tissues where excess energy is being stored (i.e., adipose). These findings are likely to be explained in part by the transient effects elicited by each bout of exercise – including the way in which exercise acutely affects the handling of ingested nutrients. The precise details and mechanisms have yet to be determined but there are several likely candidates, although it is quite probable that different forms of structured exercise exert their effects in subtly different ways. Interestingly, exercise might have a less powerful and/or distinct physiological role when introduced alongside an energy deficit from caloric restriction. This notwithstanding, the evidence is strong enough that public-facing messages should underscore the importance of the unique physiological and health benefits of certain forms of exercise irrespective of energy balance. This message needs to emphasize that the benefits of specific forms of exercise for various health outcomes will not automatically mean that these forms of exercise are appropriate forms of exercise for various health outcomes will not automatically mean that these forms of exercise for various health outcomes will not automatically mean that these forms of exercise for various health outcomes will not automatically mean that these forms of exercise are appropriate.

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Is energy expenditure the key regulating factor of fat metabolism?

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Background: Fat balance is primarily dependent upon the state of energy balance. There is furthermore a strong positive association between daily activity energy expenditure and 24hr dietary fat oxidation. The respective role of the terms of exercise (energy expenditure, intensity, duration and frequency) on nutrient metabolism is still poorly known. Purpose: To compare the metabolic effects of 4 days of activity microbursts (MICRO: 5 minutes of moderate intensity walking every hour for 9 hours) to an isocaloric single 45-min bout of moderate intensity walking (ONE) and a sedentary control condition (SED) in overweight adults.

Methods: In this ongoing study, eleven (8F/3M, mean±SD: age=32±7 yrs, BMI=31.0±1.9 kg/m2) subjects were studied under 3 different conditions (MICRO, ONE, SED) in random order. Each condition consisted of 3 days in a free living state followed by a 24hr stay in a whole room calorimeter to measure total energy expenditure (TEE) and substrate utilization. Energy intake was controlled and matched across days and conditions by design. Protein oxidation was estimated by urinary nitrogen excretion. The breakfast meal contained a fatty acid stable isotope (1-13C oleic acid, 20mg/kg mixed in breakfast), and 24 dietary fat oxidation was quantified based on exhaled 13CO2 in breath samples.

Results: As expected, 24hr TEE increased in both active conditions and resulted in negative energy balance (ONE: -381±140 kcal; MICRO: -414±125 kcal) compared to SED (-29±136 kcal, p<0.05 for both). Compared to the SED condition, 24hr total fat oxidation increased in ONE (69.4±26.6 g/d vs. 92.1±30.9 g/d respectively, p=0.048), but 24hr carbohydrate oxidation increased in MICRO (299.3±70.6 g/d vs. 364.9±82.3 g/d respectively, p=0.016). Protein oxidation did not differ between conditions. 24hr dietary fat oxidation tended to be greater in ONE compared to SED (36.2±12.3 vs. 30.8±4.8 % dose recovery respectively, p=0.08), but not in MICRO (31.3±6.8 % dose recovery, p=0.05). There was a strong correlation between changes in 24hr dietary fat oxidation induced by one continuous bout of exercise and changes in 24hr TEE (r2=0.68, p=0.003), but no relationship existed when the increase in 24hr TEE was induced by microbursts of exercise (r2=0.014, p=0.7).

Conclusion: While both active conditions increase energy expenditure and create an energy deficit to a similar extent, they had different effects on substrate and dietary fat oxidation. Energy expenditure may not be the key factor in the regulation of lipid metabolism, and other parameters, like the frequency and/or the duration of exercise, may play a role. These preliminary results need to be confirmed once the full study is complete.


This study is financially supported by a NIH/NIDDK 1K99DK100465-01 award and a Colorado Clinical Translational and Scientific Institute microgrant.
Activated endo-ents following adrenoceptor (AR) stimulation we selectively investigated dynamic changes in caveolar protein constituents following AR activation, suggesting this is not the only mechanism that targets proteins to caveolae. Indeed, the overlap between the cardiac caveolar proteome and cardiac palmitoyl proteome suggests that palmitoylation is a more powerful predictor of caveolar localisation than the presence of a CBM. Indeed, the overlap between the cardiac caveolar proteome and cardiac palmitoyl proteome suggests that palmitoylation is a more powerful predictor of caveolar localisation than the presence of a CBM, and hence that palmitoylation may recruit proteins to caveolae.

To investigate dynamic changes in caveolar protein constituents following adrenoceptor (AR) stimulation we selectively activated α1-, β1- and β2-AR prior to preparation of caveolae. Quantitative proteomic analysis indicates that with the notable exception of cavins 1, 2 and 4, very few proteins show altered abundance in caveolae following AR activation, suggesting signalling complexes are pre-formed to ensure a rapid and high fidelity response to adrenergic stimulation in cardiac muscle.

Protein glutathionylation (the reversible conjugation of glutathione to protein cysteines in a mixed disulfide) is emerging as a critical signalling event in the cardiovascular system due to its ability to regulate many physiological processes involved in cardiac homeostasis. The heterotrimeric G-protein alpha subunits Gαi and Gαs and caveolin 3 are all glutathionylated (measured using biotinylated glutathione ethyl ester labelling) in unstimulated ventricular myocytes at rest. Treatment of H9c2 cells or ventricular myocytes with the selective thioredoxin oxidizing agent diamide increases glutathionylation of Gαi and caveolin 3. Concurrently co-immunoprecipitation experiments indicate the physical interaction between both Gαi and Gαs and caveolin 3 is lost, however sucrose gradient fractionation indicates both G-protein α subunits still reside within caveolar membranes. This implies that G protein or caveolin 3 glutathionylation dynamically regulates G protein α subunit interaction with caveolin 3 and thus G-protein coupled generation of intracellular cAMP (and consequently contractility) in cardiac muscle.

Hence the caveolar signalling microdomain exhibits plasticity in both its composition and molecular interactions. Understanding the basis of this plasticity will further our understanding of caveolar control of cardiac function in both health and disease.

This work was supported by the British Heart Foundation and the British Heart Foundation/Wellcome Trust Centre for Cellular and Molecular Biology.

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Dynamic molecular interactions in caveolae: Proteomics and post-translational modifications

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The lipid raft concept proposes that membrane environments enriched in cholesterol and sphingolipids cluster certain proteins and form platforms to integrate cell signalling. Oligomers of caveolin 3 form caveolae within the cardiac sarcolemma. These lipid rafts concentrate many signalling molecules including G proteins, facilitating cellular signal transduction. The presence of a caveolin binding motif (CBM) is proposed to localise proteins to caveolae via a protein-protein interaction with a scaffolding domain in caveolin.

We defined the cardiac caveolar proteome using quantitative proteomics to identify proteins depleted from caveolar membranes prepared from rat ventricular myocytes using a standard discontinuous sucrose gradient after treatment of these myocytes with methyl-β-cyclodextrin (MβCD) to deplete cholesterol and disrupt caveolae. Proteins possessing a CBM were poorly enriched in cardiac caveolae, suggesting this is not the only mechanism that targets proteins to caveolae. Indeed, the overlap between the cardiac caveolar proteome and cardiac palmitoyl proteome suggests that palmitoylation is a more powerful predictor of caveolar localisation than the presence of a CBM, and hence that palmitoylation may recruit proteins to caveolae.

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Location, location, location: Diverse cellular distribution and functionality of caveolae-caveolins in regulation of cardiac myocyte physiology

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The lipid bilayer plays an important role in compartmentalization and organization of the intracellular environment and various organelles necessary for homeostatic and specialized functions. The plasma membrane is a semi-permeable barrier that provides protection from the extracellular environment and localizes membrane receptors and ion channels involved in signal transduction. Particular examples of membrane microdomains such as lipid rafts and caveolae have been studied in the context of localization of these signalling receptors and ion channels. But, very few researchers associate the lipid membrane, and its varied lipid-ordered domains, as a specialized cellular organelle important in regulating cellular metabolism and extracellular environment to cell/organelle communication. Caveolins, the structural proteins essential for caveolae formation, are present in three isoforms. Although the caveolin proteins are expressed ubiquitously, their level of expression varies among different tissues. Caveolin-1 (Cav-1) and caveolin-2 (Cav-2) are highly expressed in endothelial cells, adipocytes, and smooth muscle cells, while caveolin-3 (Cav-3) is predominantly found in striated (skeletal and cardiac) and smooth muscle. Caveolins were originally discovered as critical proteins for the formation of caveolae in cholesterol and phospholipid membrane (lipid) raft domains. Many studies have focused on the function of caveolins as signaling scaffolds within plasma membrane caveolae and lipid rafts. However, evidence in recent years indicates that caveolin proteins can have roles independent of caveolae and may functionize caveolae in the regulation of intracellular organelles such as mitochondrion (Figure 1A-D) and allow for stress adaptation by regulation of cellular activities, including lipid transport, gene expression, and mitochondrial function. Many independent actions of caveolins may be facilitated by their presence in other cellular membranes, including: exocytic and endocytic vesicle, the endoplasmic reticulum (ER), the Golgi complex, mitochondria, the nucleus, endosome, lysosomes, peroxisomes, and lipid droplets. Functions of caveolin at such cellular locations, the interaction of membrane caveolae with mitochondria, and the mechanisms involved in intracellular caveolin trafficking are important considerations in the regulation of cell biology. This talk will specifically focus on the ability of caveolins/caveolae to communicate with and modulate the function of intracellular organelles and the impact of this communication on cell and organ physiology.
Caveolae, flask-shaped invaginations of the plasma membrane, have long been implicated in endocytosis. Recent data question this link, and in the absence of specific cargoes the potential cellular function of caveolar endocytosis remains unclear (1). Moreover, recent data showed that caveolar biogenesis requires precise ratio between caveolar proteins. Thus, perturbation in this ratio, such as by overexpression of caveolin-1, leads to its ubiquitination and a consequent degradation through classical endo-lysosomal pathway (2,3).

We find that dynamics of endogenous caveolin-1-GFP or cavin-1-Cherry is very different from that of overexpressed proteins. We find that around 5% of the cellular pool of caveolae is present on dynamic endosomes, and is delivered to endosomes in a clathrin-independent manner. Furthermore, we show that caveolae are indeed likely to bud directly from the plasma membrane. Using a genetically encoded tag for electron microscopy and ratiometric light microscopy, we go on to show that bulk membrane proteins are depleted within caveolae. Although caveolae are likely to account for only a small proportion of total endocytosis, cells lacking caveolae show fundamentally altered patterns of membrane traffic when loaded with excess glycosphingolipid. Together, these observations support the hypothesis that caveolar endocytosis is specialized in maintenance of plasma membrane lipids.

This study has been supported by Marie-Curie FP7 fellowship. We would like to thank C. Mendoza-Topaz, K. Riento and S. Munro for comments on the manuscript.

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tion enclosed inside a single section. Caveolae distribution information was obtained using an FEI Quanta 250 FEG SEM equipped with a Gatan 3View system which takes an image of the block face after a slice has been cut away at a specific thickness (SBF-SEM). Serial images were collected at different magnifications ranging from 6 to 15 nm per pixel in the X-Y plane, while the cutting depth along the Z-axis was fixed at 50 nm for all the datasets. Images were segmented and rendered in IMOD [4] or Fiji [5]. 3-D reconstructions obtained with electron tomography reveal that both control and MI pigs are characterised by open caveolae, while closed caveolae are uncommon and are likely the result of the cutting plane not passing through the neck of the caveolae. Furthermore, analysis of the 3-D data revealed how cross sections of the network sarcoplasmic reticulum could be misinterpreted as small closed caveolae. Interestingly caveolae tend to fuse together creating structures reminiscent of the cubic membranes investigated by Landh [6]. The SBF-SEM data revealed that the sarcoclemma undergoes heavy remodelling in disease with prominent clusters of caveolae on the sarcolemma comparable to those seen in endothelial cells. Our electron microscopy data reveal gross remodelling of the sarcolemma within the peri-infarct cardiomyocytes in the MI pigs with caveolar clustering. Given that this region is involved in arrhythmogenesis this remodelling may represent a pro-survival response, with the caveolae acting to concentrate ion channels in order to re-equilibrate, at least locally, the ionic imbalance of the cell. Our future studies will characterise the morphological changes and distribution of the caveolae within the peri-infarct region employing 3-D electron microscopy techniques and immunogold labelling to examine if patches of caveolae are characterised by specific ion channels linked to arrhythmias, complemented with biochemical and functional studies.

Kozera L I, White E, Calaghan S. Caveolae act as membrane reserves which limit mechanosensitive (I(Cl,swell)) channel activation during swelling in the rat ventricular myocyte. PLoS One. 2009 Dec 14; 4(12)

We thank the British Heart Foundation (RG/11/2/28701; AK) for supporting this research

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Increased peripheral vascular disease risk progressively constrains perfusion adaptability in the skeletal muscle microcirculation

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To determine the impact of progressive elevations in peripheral vascular disease (PVD) risk on microvascular function, we utilized eight rat models spanning “healthy” to “high PVD risk” and used a multi-scale approach to interrogate microvascular function and outcomes. Healthy: Sprague-Dawley rats (SDR); lean Zucker rats (LZR); Mild Risk: SDR on high salt diet (HSD); SDR on high fructose diet (HFD); Moderate Risk: reduced renal mass hypertension (RRM), spontaneously hypertensive rats (SHR); High Risk: obese Zucker rats (OZR) and Dahl salt sensitive rats (DSS). Vascular reactivity and biochemical analyses demonstrated that even mild elevations in PVD risk severely attenuated nitric oxide bioavailability and caused progressive shifts in arachidonic acid metabolism increasing thromboxane A2 levels. With the introduction of hypertension, arteriolar myogenic activation and adrenergic constriction were increased. However, while functional hyperemia and fatigue resistance of in situ skeletal muscle were not impacted with mild or moderate PVD risk, blood oxygen handling suggested an increasingly heterogeneous perfusion within resting and contracting skeletal muscle. Analysis of in situ networks demonstrated an increasingly stable and heterogeneous distribution of perfusion at arteriolar bifurcations with elevated PVD risk; a phenomenon that was manifested first in the distal microcirculation, and evolved proximally with increasing risk. The increased perfusion distribution heterogeneity and loss of flexibility throughout the microvascular network, the result of the combined effects on NO bioavailability, arachidonic acid metabolism, myogenic activation, and adrenergic constriction, may represent the most accurate predictor of the skeletal muscle microvasculopathy and poor health outcomes associated with chronic elevations in PVD risk.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Flow motion dynamics of skin blood flow and oxygenation in healthy human skin

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The impact of low frequency (LF), periodic flow motion oscillations that reflect the activity of local vasodilator and constrictor mechanisms on tissue blood perfusion and oxygenation is much debated; and the spatio-temporal relationship between the two unclear. Our aim is to explore the spectral power, synchronicity and complexity of oscillatory rhythms in continuously acquired microvascular blood perfusion and oxygenation signals and to investigate their association with local flow motion and mechanisms of vasomotor control.

Microvascular blood flux (BF) and oxygenation (OXY; oxyHb, deoxyHb, total Hb and SO2) signals are recorded simultaneously, at the same site in the skin of healthy individuals, using a combined laser Doppler and white light spectroscopy probe (Moor Instruments Ltd, UK). To investigate system flexibility we have made measurements at a skin temperature of 33°C and during local thermal warming to 43°C (maximal vasodilation). Power spectral density (PSD) is evaluated within the frequency range (0.0095–1.6Hz) and PSD contribution calculated in the low frequency (LF) intervals corresponding to local endothelial (0.0095–0.02Hz), sympathetic (0.02–0.06Hz) and myogenic activity 0.06–0.15Hz and higher frequency (HF) intervals reflecting respiratory (0.15–0.4Hz) and cardiac (0.4–1.6Hz) activity. A frequency coherence function is used to describe the linear relationship between BF and OXY signals in the frequency domain.

The relationship between BF and OXY signals at 33°C was similar to that we have described previously in both the time and frequency domains [1]. During warming microvascular BF increased 15-fold (p<0.001). The increase in BF in the time domain was associated with an increase in total spectral power indicative of an increase in the amplitude of flow motion oscillations in the signal. In contrast, while microvascular oxygenation (OxyHb) increased 5-fold during warming there was little change in total spectral power.

In both BF and OXY signals the relative contribution of the LF PSD components fell during warming, in part due to an increase in the contribution from the HF cardiac band. The LF:HF ratios at 33 and 43°C, respectively, were for BF 0.5:0.5 and 0.2:0.8 and for OxyHb 0.9:0.1 and 0.5:0.5 (both p<0.001). Frequency coherence between the LF bands in the BF and OXY signals was high; exceeding 70% in the endothelial band at 33°C. It was unaffected by warming.

In order to explore changes in system flexibility the Lempel-Ziv complexity of the spectral properties of the signals was calculated as a measure of randomness of perfusion. There was a significant reduction in the intrinsic variability and complexity of the microvascular signals during thermally-induced vasodilation, with a fall in mean LZ complexity of BF and OxyHb of 25% and 49%, respectively (p=0.001).

Together these approaches demonstrate the relationship between the processes driving microvascular BF and oxygenation and the dissociation that may occur during perturbation of vascular homeostasis. They further show that in healthy individuals there is adaptability of flow motion which becomes less random during a vasodilator challenge. We conclude that simultaneous measurement of skin BF and oxygenation signals in combination with signal processing techniques offers an extended assessment of microvascular function which may eventually inform the clinical evaluation of compromised oxygen transport and tissue status.


KZK Supported by an EPSRC CASE award

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Exercise training causes heterogeneous changes in the transcriptome of arterioles within and among skeletal muscle

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Exercise training induces adaptations of skeletal muscle vasculature that appear to be beneficial in prevention and treatment of type 2 diabetes (T2D). The distribution of vascular adaptation to different types of exercise training are heterogeneous in part because skeletal muscle arteriolar trees are heterogeneous and because muscle fiber type composition and fiber recruitment patterns that produce different modes of exercise are heterogeneous. Thus, training-induced adaptations of vascular structure and vascular control in skeletal muscle are not homogeneously distributed throughout skeletal muscle or along the arteriolar tree within a muscle. Results will be summarized which indicate that exercise training induced changes in vascular gene expression differ along the arteriolar tree and by skeletal muscle fiber type composition. Using the OLETF rat model of T2D we observed that endothelium-dependent dilation (EDD) is blunted by T2D differentially in muscle with different muscle fiber type composition and exercise training restores EDD in a fiber type dependent manner 1-3 as exercise training improves EDD non-uniformly in the arterial tree of skeletal muscle 1-3. Both interval sprint (SPRINT) and endurance training (EX) increased ACH-induced EDD in the gastrocnemius FA and the RG2a but only EX improved vasodilation of the WG2a. Neither training program altered responses of the soleus FA. Insulin produced vasodilation of the RG2a in EX animals only. When ET-1 receptors were blocked with tezosentan RG2a’s from all three groups exhibited vasodilation to insulin. Similar results were seen in the WG2a. These results led us to conclude the EDD is blunted in T2D skeletal muscle arterioles in a muscle fiber type dependent manner. EX and SPRINT increased EDD in some arterioles but not all. Results also indicate that insulin signaling in arteriolar endothelium differs among types of skeletal muscle and among different branch orders in skeletal muscle of arteriolar trees 1, 3. EX also increased insulin-induced EDD non-uniformly in the arterial tree of skeletal muscle. Thus, results indicate that the blunting of EDD induced by T2D differs with muscle fiber type composition of skeletal muscle and that different exercise training programs reverse this dysfunction differently in arterioles from skeletal muscles of differing fiber type composition 2-3. Indeed, it is striking that exercise training improves EDD non-uniformly even within the arteriolar tree of a given muscle, the gastrocnemius 3. We determined transcriptional profiles for samples of arterioles/arteries from the same rats using techniques described previously 4-7. Results show that EX caused the largest number of changes in gene expression in the soleus and white gastrocnemius 2a arterioles with little to no changes in the feed arteries. In contrast, SPRINT caused substantial changes in gene expression in the feed arteries. IPA canonical pathway analysis revealed 18 pathways with significant changes in gene expression when analyzed across vessels and revealed that EX induces increased expression of some genes in all arterioles examined (Shc1, desert hedgehog protein (Dhh), adenylate cyclase 4 (Adcy4), G protein binding protein, alpha (Gnat1), and Bcl2l1) but decreased expression of ubiquitin D (Ubd) and cAMP response element modulator (Crem) across all arterioles. EX increased expression of endothelin converting enzyme (Ece1), Hsp90b, Fkbp5, and Ccdc4b in 4 of 5 arterioles. SPRINT had effects on expression of Crem, Dhh, Bcl2l1 and Ubd that were similar to EX. SPRINT also increased expression of Nfkbia, Hspa5, Tubb 2a and Tubb 2b, and Fkbp5 in all 5 arterioles and increased expression of Gnat1 in all but the soleus second order arterioles. Many contractile and/or structural protein genes were increased by SPRINT in the gastrocnemius feed artery but the same genes exhibited decreased expression in red gastrocnemius arterioles. Results suggest that it is unlikely that hemodynamic forces are the only exercise-induced signals mediating the regulation of vascular gene expression. We conclude that training-induced changes in arteriolar gene expression patterns differ by muscle fiber type composition and along the arteriolar tree. If these adaptations occur in a sufficient amount of muscle mass, exposure to hyperglycemia and hyperinsulinemia will decrease along with the risk of microvascular complications throughout the body. It is postulated that exercise sessions in programs of sufficient duration, that engage as much skeletal muscle mass as possible and recruit as many muscle fibers within each muscle as possible, will produce the greatest benefit. The added benefit of combined resistance and aerobic training programs and of high intensity exercise programs is not simply “more exercise is better”.


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Laughlin MH, Padilla J. Jenkins NT, Thorne PK, Martin JS, Rector RS, Akter, S., Davis JW. J Appl Physiol. 2015;119:583-603


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Structural changes to the vasculature have been quantified in capillaries, but the direct geometric effect higher heterogeneity and overall decreased capillary density has on tissue oxygenation has not been entirely elucidated. The compounding effect of heterogeneous blood flow distribution within tissue also may serve to impair mass transport and exchange of humoral substances with tissue. We aim to describe how basic changes to capillary geometry effect both tissue oxygenation and the uptake of blood glucose.

**Methods**

Capillary density and hemodynamic measurements (RBC supply rate, velocity, and oxygen saturation) from a previous study were used as input parameters for the oxygen transport and glucose uptake models [1]. These data were collected using in vivo intravitral video microscopy of rat extensor digitorum longus muscle.

A three-dimensional parallel capillary geometry was randomly generated based on mean volumetric vascular density from reconstructed in vivo networks. Six variants of the geometry were created that correspond with the measured densities for Mean FCD, High FCD (Mean FCD + 1 standard deviation), and Low FCD (Mean FCD – 1 standard deviation) for both lean and obese ZDF animals. Hemodynamics and saturation values from ZDF lean and obese animals were assigned to the vessels in the generated geometries and the resulting RBC supply rates were scaled to create a mean RBC SR, High RBC SR (Mean SR + 1 standard deviation) and Low RBC SR (Mean SR - 1 standard deviation) for each group. By combining these varying cases we produced 9 cases to represent the variability seen in the ZDF Lean group and 9 cases for the ZDF Obese group. A previously described finite difference model of oxygen transport [4] was used to calculate PO2 in each of the 18 cases using an isotopic volume discretization with 2 micron spacing. The transport of glucose and insulin from capillaries, and through the interstitial space to skeletal muscle fibers was determined using a novel finite element model. The cross-sectional distribution of capillaries from the Mean FCD, High FCD, and Low FCD parallel networks for Lean and Obese groups were used to define the two-dimensional capillary geometry.

**Results**

Mean volumetric capillary densities were 1.46±0.38% and 1.33±0.34% of the total bounding volume for ZDF lean and Obese groups respectively. Mean volumetric RBC SR calculated from experimental measurements and applied simulation cases were 8.40E–0.63 and 6.71±1.21 mL RBC / mL Tissue\(^1\) \times \times 1\(^2\) for ZDF Lean and Obese groups. Minimum tissue PO2 in low FCD and low SR cases were 30.3 mmHg in the lean simulation and 25.1 mmHg in the obese (Figure 1). Visualization of the combined effect of altered capillary density and SR on tissue PO2 in edge cases (High SR and High FCD; Low SR and Low FCD) for both groups is shown in Figure 1. Interstitial postprandial glucose concentration in the low FCD obese case was 7.3 mmol/L compared to 7.0 mmol/L in the high FCD lean glucose case. Glucose uptake rate was 17.4% lower in the low FCD obese case compared to the high FCD lean. Substantially higher heterogeneity of glucose concentration was observed in the low FCD obese case compared to the high FCD lean simulation (Figure 2).

Our model predicts that substantial decreases to FCD should cause little impact on mean tissue PO2 and minor increase in PO2 heterogeneity given maintenance of RBC SR. Changes to mean tissue PO2 were found to scale linearly with RBC SR as has been observed previously [2]. The compound effect of decreased FCD and RBC SR yield a 10% lower tissue PO2\(_2\) in the obese edge case compared to the lean (Figure 1); the driving volumes into hypoxia.

With respect to tissue oxygenation, the changes related to decreased FCD were modest. The glucose uptake model predicted interstitial glucose concentrations would vary due to capillary density (Figure 2). The presence of a significant gradients between the abluminal side of capillary endothelium and muscle fibres should be necessary in order to drive glucose uptake. Further work is needed to investigate the additional effect of higher metabolism and high heterogeneity of blood flow on mass transport into skeletal muscle.
Moving physiology and science education into the urban precollege classroom

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The American Physiological Society launched Physiology Understanding Week (PhUN week) as an outreach program that would build connections between scientists and trainees and K-12 students/schools by bringing “citizen scientists” into the K-12 classrooms, thus providing teachers with professional development that could sustain excellence in science education. The Society for Neuroscience (SfN) Brain Awareness Week (BAW) is similar, but more targeted at public education in general. Both models transform scientists (faculty and trainees) into advocates for, and participants in, excellence in science education, and both effectively inform the public about the benefits of biomedical research. At UAB, our PhUN week activities reach about 1,500 students and 30 teachers each year with inquiry-based educational opportunities in physiology. These have been an entrée to a larger cooperation between UAB scientists and teachers, resulting in many formal and informal programs. The school year programs include school year classroom programs that are aimed at supporting the existing science curriculum, e.g., the UAB School of Science and Math (high school), the Hands-On program classroom program (middle school) and ALAHASP for K-5 students and teachers. Summer programs position the students to be peer leaders by training them in the principles underlying the areas of science that they will encounter in the next school year. But much of the effort in both PhUN week and other UAB programs are geared to insure that teachers have excellent teacher professional development opportunities. Thus each summer we train teachers in curricular areas but perhaps more importantly provide them with opportunities to teach in the summer programs, in which they can experiment with inquiry-based education to a much greater extent than they can in the classroom during the school year. Such training is especially important for teachers in urban classrooms in which classroom management concerns often disallow them the freedom to be creative. Overall, UAB’s programs reach about 80,000 students and 3,000 teachers each year in Alabama, and now reach internationally, giving us the opportunity to test out education outreach in different settings. In each program, the emphasis is on training the students to be leaders in their curriculum and exciting the students about the promise of science careers, an element especially important for underrepresented minority students.

This work was funded in part by National Institutes of Health Science Education Partnership Award Grants RR-022745 and OD-016490 and a grants from the United States Department of Education (AMSTI-UAB) and the Alabama Commission on Higher Education (ALAHASP and BioTeach)

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

A model for course-based service learning to enhance undergraduate student engagement within the community

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Service-learning initiatives are increasingly recognized across institutions as an innovative approach to enrich learning and promote cognizance of civic responsibility among students. At Michigan State University (MSU), we utilize educational tools, such as service-learning, to inspire dynamic citizen leaders who seek to make a difference both locally and globally. In an upper division physiology laboratory course at MSU, students have the opportunity to partake in a service-learning based honors option that allows them to design and implement an outreach activity. This project fosters a platform that reinforces physiological concepts while also advocating for community engagement. Throughout the duration of the course, the assignment requires each student to select a physiological area of interest, design and build an activity, construct a tri-fold display board of the conceptual information, and write a brief protocol for their activity. The project activities are pilot tested for accuracy and clarity prior to inclusion at an event and a short (<1-page) summary is written as a guide. In most cases, the students take their activity to a small or large scale outreach event to teach the community. Honors projects are awarded grades following a pass/no pass criteria upon completion. This has been so popular and successful that we are now offering this as a semester long Service Learning in Physiology course for 1 Cr. Thus, by implementing an element of community service into our course, educational effectiveness is improved through a novel learning opportunity that enables a university to share its valuable resources and simultaneously stimulate a sense of efficacy among students.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Partnerships in public engagement

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There is an increasing expectation from Institutions, research funders and the public for researchers in engage with and discuss their research with the public; a requirement to go beyond traditional audiences and engage with “hard to reach” communities. Engagement is not a one way transfer of information but active dialogue between researchers and
the community, where they are equal partners and each
learns from the other. Effective public engagement has to be
learned; it a key transferable skill that can be utilised not only
in science but many other careers.

This presentation will outline different approaches to creating
partnerships in public engagement involving students (under-
graduate and postgraduate, researchers), different sections
of the community (Lewis et al., 2015), service learning (Lewis
et al., 2010), opportunities outside of formal curricula (Spur-
ing et al., 2014), formal and informal settings, patient-public
involvement (PPi) initiatives (Lewis et al., 2016) and the use
of social media. The benefits of each for all involved will be
discussed.

Lewis DI et al. (2015) Proc Physiol Soc 34, PC044
Lewis DI et al. (2010) Proc Physiol Soc 19, PC76
Spurring E et al. (2014) Proc Physiol Soc 31, C73

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requirements.

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**SA044**

**Preventive effects of resveratrol against Schistosoma mansoni-induced liver fibrosis in mice**

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In Schistosomiasis, hepatocyte injury and Kupffer’s cell acti-
vation can result in reactive oxygen species generation,
pro-inflammatory and profibrogenic mediators release. This
can result in stellate cells activation and consequently, liver
fibrosis. Resveratrol, a natural polyphenol, has been shown to
possess antioxidant and anti-inflammatory properties. How-
ever, studies into its protective effects against Schistosoma
mansoni-induced liver fibrosis are limited. The present study
was designed to examine the preventive effects of resvera-
trol on Schistosoma mansoni-induced liver fibrosis in mice.

Sixty male albino mice (CD1Swiss) weighted 18-22 gm were
divided into four groups of 15 mice as follows: normal resvera-
trol-untreated, normal resveratrol-treated, Schistosoma man-
soni-infected resveratrol-untreated and schistosoma man-
soni-infected resveratrol-treated. Resveratrol was injected
intraperitoneal (I.P) in a dose of 20 mg/kg body weight, twice/
week. At the end of the experimental period, blood samples
were collected to measure serum aspartate aminotransferase (AST),
alanine aminotransferase (ALT), and TNF-α. Liver tissue
was collected for malondialdehyde (MDA) measurement, his-
topathological examination and fibronectin gene expression
analysis. All procedures involving the animals were conducted
in accordance with the protocol approved by the Ethics Com-
mittee, Faculty of Medicine, Alexandria University. AST, ALT
and TNF-α, and MDA levels were significantly increased in the
infected resveratrol-untreated group compared to normal res-
veratrol-untreated group (all, P < 0.05). However, their levels
were significantly decreased in the infected resveratrol-treated
group compared to infected resveratrol-untreated group (all,
P < 0.05). In addition, fibronectin gene expression was highly
up-regulated in the infected resveratrol-untreated group
compared to normal resveratrol-untreated group (P < 0.05).
Administration of resveratrol significantly down-regulated
fibronectin in the infected resveratrol-treated group com-
pared to infected resveratrol-untreated group (P < 0.05).

Results of the study indicate that resveratrol can prevent S.mansoni-induced liver fibrosis via mechanisms involving its
anti-oxidant, anti-inflammatory and anti-fibrotic properties.

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described here conform with the Physiological Society ethical
requirements.

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**SA045**

**Model approaches to public engagement in Africa**

T.K. Karikari

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Public engagement is essential if scientists are to influence
policy, education and public discourse. However, engage-
ment activities are limited and challenging in developing
countries. A major barrier to effective outreach in these
countries is the lack of model programmes that address the
multiple, peculiar challenges faced. At this symposium, I will
discuss the outcomes, feedback and extensions to approaches
being used to develop innovative solutions to public engage-
ment across Africa, especially in Ghana, Nigeria, Uganda and
Tanzania. These include: (i) an evidence-based approach to
public engagement training for undergraduate and graduate
students, (ii) training programmes to develop high school
teachers’ capacity to use alternative, low-budget resources to
help practical aspects of science in resource-limited settings,
and (iii) audience-specific engagement approaches, including
those for school children, high school students and community
groups in different settings, highlighting commonalities
and divergence among audience. Furthermore, I will discuss
how these approaches could be adapted and expanded to
different developing-world settings.

Karikari TK,Yawson NA, Quansah E. Build the future of science
communication in developing countries through systematic training

Karikari TK, Yawson NA, Quansah E. Developing science communica-
tion in Africa: undergraduate and graduate students should be trained
and actively involved in outreach activity development and implement-
ation. J Undergrad Neurosci Educ 2016; 14(2); E5-E8

Yawson NA, ... , Karikari TK. Evaluation of changes in Ghanaian stu-
dents’ attitudes to science following neuroscience outreach activi-
ties: a means to identify effective ways to inspire interest in science
careers. J Undergrad Neurosci Educ 2016;14(2); A117-A123


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Physiological Society, the Biochemical Society and the
University of Warwick.

Where applicable, the authors confirm that the experiments
described here conform with the Physiological Society ethical
requirements.

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**SA046**

**Metabolic control of renin secretion**

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The renin-angiotensin system (RAS) plays an important role in
the physiological control of body fluid and electrolyte home-
ostasis and blood pressure maintenance. The mechanisms
and importance of local, intra-renal, intra-tubular RAS components, and their direct local control by endogenous metabolic intermediates are emerging research topics in the field. During the past few years our laboratory has characterized the localization and signaling of the novel metabolic receptor GPR91 in the normal and diabetic kidney and established GPR91 as a new, direct link between high glucose and renin synthesis and release, the rate limiting steps in RAS activation especially in diabetes. GPR91 (also called SUCNR1) binds the tricarboxylic acid (TCA)-cycle intermediate succinate, which can rapidly accumulate in the local tissue environment when energy supply and demand are out of balance. In a variety of physiological and pathological conditions associated with metabolic stress, the classic mitochondrial intermediate succinate has a non-traditional signaling role via GPR91 which appears to be an important mediator or modulator of renin secretion. At the classic vascular anatomical site of renin synthesis in the juxtaglomerular apparatus (JGA), macula densa cells may sense succinate in the tubular fluid that is derived from cells of the proximal tubule or the thick ascending limb, and via luminal GPR91 receptors trigger renin release in a paracrine fashion. In addition to the JGA, renin and its precursor prorenin are also produced and released within the kidney at a new tubular site, in the renal collecting duct. Interestingly, renal GPR91 expression is the highest in the collecting duct, where succinate also appears to participate in the control of the local intra-tubular RAS via tubular cell-to-cell crosstalk. This lecture will summarize our current knowledge on the control of JGA and tubular renin release by succinate/GPR91.

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SA047

Hypercapnia and the lungs

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CO₂ is a by-product of oxidative metabolism during the citric acid cycle and is expired by the lungs into the atmosphere. Elevation of the partial pressure of CO₂ in blood (hypercapnia) occurs in patients with lung diseases including those with severe chronic obstructive pulmonary disease (COPD), bronchopulmonary dysplasia and cystic fibrosis. In mechanically ventilated patients with acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) “permissive hypercapnia” is an accepted management approach in intensive care units around the world. The approach of “permissive hypercapnia” was encouraged by several reports which proposed that patients with ALI on mechanical ventilation would benefit from high levels of pCO₂. Earlier publications utilizing animal models supported the use of hypercapnia. However, more recent studies reported that hypercapnia, even independently of acidosis and hypoxia, had deleterious effects on lung function. I will present data from recent publication on the effects of hypercapnia on the lungs and model organisms such as C. Elegans and Drosophila as well a recent study reporting that hypercapnia is a predictor of poor clinical outcomes in patients with COPD. Supported in part by HL-85534 and HL-48129.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA048

Hypoxia and cellular metabolism in tumour pathophysiology

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Cancer cells are optimized for survival and successful growth via processes that enable them to outcompete normal cells in their microenvironment. Many of these cellular adaptations are promoted by the pathophysiological hypoxia that arises in solid tumour development. Our work focuses on three essential components of tumour cell biology: (i) enhancement of nutrients/metabolites uptake, (ii) increased efficiency of nutrients utilisation via metabolic alterations and (iii) handling of metabolic waste production in a way that furthers their progression whilst hampering the survival of normal tissue. Hypoxia Inducible Factors (HIFs) act as essential drivers of these adaptations via the promotion of numerous membrane proteins including glucose transporters (GLUTs), monocarboxylate transporters (MCTs), amino-acid transporters (LAT1, xCT), and acid-base regulating carbonic anhydrases (CAs) and bicarbonate transporters (NBCs). These HIF-regulated proteins in turn interact with a host of other altered pathways in the pathophysiology of cancer. In addition to a competitive growth advantage for tumour cells, these HIF-regulated proteins are further implicated in metastasis, cancer ‘stemness’ and the immune response. We will provide evidence for a number of hypoxia-associated proteins that promote cellular energy production and the regulation of acid-base levels in tumour cells. Emphasis will be placed on recent work manipulating multiple CA isoforms and NBCs, which are at an interesting crossroads of gas physiology as they are regulated by hypoxia to contribute to the cellular handling of CO₂ and pH regulation. Our research combined with others indicates that targeting of HIF-regulated membrane proteins in tumour cells will provide promising future anti-cancer therapeutic strategies.

(Parts of this work have been discussed recently in Molecular Aspects of Medicine by Parks et al. 2016)


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA049

Hypoxia and the innate immune response

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A close two-way relationship exists between hypoxia and inflammation. As a consequence responses to bacterial infection frequently occur in the setting of both regional and systemic hypoxia. We questioned whether acute exposure to systemic hypoxia and more prolonged hypoxic preconditioning could determine the clinical outcome to infection. In both a local subcutaneous S. aureus skin infection model and a
Adipose tissue inflammation by intermittent hypoxia in obstructive sleep apnoea

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Obstructive sleep apnoea (OSA) is a highly prevalent disorder, which conveys an increased risk of cardiovascular disease and death. Furthermore, there is increasing evidence of an independent association of OSA with alterations in glucose metabolism leading to insulin resistance (IR), type 2 diabetes and metabolic syndrome. The underlying pathophysiology is poorly understood but intermittent hypoxia (IH) with repetitive short cycles of desaturation followed by rapid reoxygenation as hallmark feature of OSA is likely to play a key role. Inflammatory processes are central in this pathogenesis and there is ample evidence – arising from both cell culture and in vivo models - that IH preferentially activates nuclear factor-kappa B (NF-κB) – mediated pathways with the downstream consequence of expression of pro-inflammatory cytokines, chemokines and adhesion molecules that may contribute to cardiometabolic processes. OSA is closely linked to obesity and there is increasing evidence that adipose tissue is an important target organ of pro-inflammatory mediators in response to IH. Indeed, recent animal data suggest that IH induces morphological obesity-like pro-inflammatory changes in the adipose tissue which correlate with the severity of IR and may be a crucial link between OSA and the pathogenesis of disorders affecting glucose metabolism.

A greater understanding of the detailed mechanisms underlying IH-induced adipose tissue inflammation should lead to the identification of therapeutic targets and therefore, further translational studies involving cell, animal and human models are strongly required.

Funded by the Health Research Board of Ireland

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Relationship between sleep and plasma amyloid-beta in prodromal Alzheimer’s disease

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Evidence suggests that amyloid-beta (Abeta) deposition accompanies sleep deficits in Alzheimer’s disease (AD). However, it remains unknown whether impaired sleep and changes in plasma Abeta levels run in parallel in amnestic mild cognitive impairment (aMCI) subjects, and whether both markers are further associated with cortical thinning in canonical AD regions. To address these issues, we first evaluated whether plasma Abeta levels are related to changes in sleep physiology and/or cortical thinning in aMCI subjects. Second, we investigated if sleep deficits and/or increased Abeta levels accounted for cortical thinning in aMCI subjects.

Overnight polysomnographic (PSG) recordings, cerebral magnetic resonance imaging (MRI), and plasma Abeta levels were obtained from 21 aMCI patients and 21 healthy older (HO) subjects. A sleep technician performed scoring of sleep stages, and cortical thickness was measured by using the analysis pipeline of Freesurfer.

aMCI, but not HO subjects, showed significant relationships between disrupted slow-wave sleep (SWS) and increased plasma levels of Abeta42. We also found that shortened rapid-eye movement (REM) sleep in aMCI correlated with thinning of the posterior cingulate, precuneus, and postcentral gyrus; whereas higher levels of Abeta40 and Abeta42 accounted for grey matter (GM) loss of posterior cingulate and entorhinal cortex, respectively.

These results support the relationship between Abeta and altered sleep physiology previously observed in animal models of AD amyloidosis, and provide precise cortical correlates of these changes in older adults with aMCI.

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voxlwise correlations between sleep quality and fractional anisotropy (FA), radial diffusivity (RD) and axial diffusivity (AD) performed using Randomise. Age, gender and education level were included as covariates and the significance threshold set at p<0.05 using threshold-free cluster-enhancement.

Results: 147 participants (33%) displayed PSQI scores greater than or equal to 6 and were classified as having poor sleep quality. Compared with participants classified as good sleepers, poor sleepers displayed significantly increased RD in widespread regions, including within fronto-subcortical tracts.

Conclusions: Poor sleep quality was associated with reduced white matter integrity in community-dwelling older adults, in overlapping regions to those identified in studies of primary insomnia. Interventional studies will shed light on whether improving poor sleep can have beneficial effects on white matter microstructure during the ageing process.

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SA054

Effect of APOE ε4 genotype on objective but not subjective sleep quality and in a population of healthy older adults: results from the Brain in Motion Study

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It has been estimated that the prevalence of Alzheimer’s disease and related dementias will triple by the year 2035, unless effective interventions or treatments are found for the neurodegenerative disease. Understanding sleep as having a bidirectional relationship with Alzheimer’s disease risk and progression is a burgeoning area of investigation. Specifically, there is emerging evidence that both sleep disturbances and the APOE ε4 allele are associated with increased dementia risk. Previous research has suggested that in Alzheimer’s disease (AD), individuals carrying the APOE ε4 allele have decreased sleep quality compared to individuals without the APOE ε4 allele. This investigation aimed to determine if healthy older adults, with no cognitive impairment, with the risk allele (APOE ε4; age 65.8±5.1; mean±SD; 37.5% female) have more sleep complaints or objective sleep disruption compared to healthy older adults, with no cognitive impairment, without the risk allele (APOE ε4; age 64.9±5.0; 66.7% female). Within the larger Brain in Motion study (1), a subset of participants completed at home polysomnography (PSG) and actigraphy sleep assessment. Subjective sleep complaints were determined using the Pittsburg Sleep Quality Index (PSQI). Results from our study are directly relevant to an emerging research suggesting sleep as both a risk factor and symptom of neurodegenerative disease. This investigation found a significant relationship between presence of APOE ε4 allele and objective sleep disturbances measured by both actigraphy and PSG, but not subjective sleep complaints in a healthy population screened for dementia. Specifically, individuals with an APOE ε4 allele had significantly lower objective sleep efficiency measured by both PSG (73.8±17.0 vs 87.8±3.2; p < .05) and actigraphy (84.6±6.5 vs 89.7±3.3; p < .05). On the gold standard measure, PSG, there were also significant difference between APOE ε4 groups on wake after sleep onset (WASO) and total sleep time. Individuals with an APOE ε4 allele had significantly worse sleep profiles with less time spent asleep (460.3±25.4 vs 490.1±57.9; mean±SD; p < .05), and significantly more WASO (108.1±73.9 vs 48.5±17.5; mean±SD; p < .05). Finally, small changes in sleep architecture were observed between groups. Individuals with an APOE ε4 allele had a significantly lower percent of total sleep time spent in stage-2 NREM sleep (58.8±6.8 vs 64.2±6.4; mean±SD; p < .05). There were no differences between groups in subjective sleep quality measured by the PSQI. Additionally, there were no differences between groups on arousal or awakening indices. We provide evidence that the Alzheimer’s disease risk allele APOE ε4 may be an important determinant of disrupted sleep in otherwise cognitively and physically healthy older adults. These data suggest that the impact of APOE ε4 allele on objective sleep quality may precede subjective sleep complaints in individuals at increased risk for dementia.


This study was funded by CIHR (Canadian Institutes of Health Research) and The Brenda Stafford Foundation Chair in Alzheimer Research (BSFCAR). We would like to acknowledge the contributions of the Brain in Motion participants, staff and trainees. LLD was supported by an Alberta Innovates Health Solutions Postdoctoral Fellowship and the BSFCAR. AVT was supported by an Alzheimer Society of Canada Doctoral Award and the BSFCAR. SJG was supported by the BSFCAR. JKR was supported by funds from a Heart and Stroke Foundation of Canada Grant-in-Aid (PI=MJP, Co-Applicant-PJH). MJP holds the BSFCAR

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SA055

Manipulating the microbiome to enhance intestinal repair and resolution of inflammation

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Homeostasis of the intestinal mucosa relies on different factors including growth factors, antimicrobial peptides, oxygen radicals and proteases. Among them, we observed that the equilibrium of proteolytic activity plays a central role at
mucosal surfaces. This equilibrium can be modified both by the host and by the microbiota, which both can release proteases and protease inhibitors. This lecture will review the effects of proteolytic activity at the intestinal mucosa surface in physiology and pathophysiology. Next, the identification of mucosal proteolytic actors will be discussed. Finally, new therapeutic approaches manipulating the microbiome and its properties to release protective molecules in the context of inflamed intestinal mucosa will be reviewed. Manipulation of proteolytic activity but also of mucosal cytokines, growth factors and oxygen radicals will be evoked.

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SA056

Pro-resolving lipids attenuate adipose inflammation in obese patients

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Background: Inflammation in the adipose tissue is a key driver of obesity-related pathologies. The resolution of inflammation is actively regulated by specialized pro-resolving mediators (SPMs), such as the arachidonic acid derived eicosanoids Lipoxin A4 (LXA4) and Lipoxin B4 (LXB4). LXA4 attenuates obesity-induced adipose inflammation in mice, resulting in protection against liver and kidney disease (Börgeson et al., Cell Metabolism, 2015). The current study attempts to translate these findings from rodent to human pathophysiology.

Method: White adipose tissue explants were obtained from the greater omentum of obese (BMI 35-55), non-diabetic, bariatric surgery patients (n=4). The adipose tissue was incubated ex vivo with vehicle, LXA4 (1 nM) or LXB4 (1 nM) for 6 hours at 37 degrees celsius. Supernatant cytokines were determined using ELISA, and tissue leukocytes were isolated and characterized by flow cytometry. Patients were recruited in accordance with the Helsinki Declaration; ClinicalTrials.gov #NCT02322073. ANOVA with Bonferroni correction was used to assess statistical significance.

Results: In adipose tissue explants, obtained from obese patients, lipoxin treatment increased the percentage of CD206+ M2 Mφs (LXA4 +66%, LXB4 +57%), although M1 Mφs CD11c expression remained unaltered. Furthermore, LXA4 reduced T-cell CD69 expression, which may reflect a less activated phenotype, although lymphocyte CD4+ and CD8+ remained unaltered. Finally, LXA4 significantly reduced adipose TNF-α levels (p<0.05), which is a key functional response in promoting metabolic health. Ongoing experiments further delineate the molecular mechanisms involved in the lipoxin-mediated attenuation of adipose inflammation.

Conclusion: This “proof of concept” study indicates that lipoxins are able to attenuate adipose tissue inflammation in obese humans. Given the critical role of adipose inflammation in obesity-induced pathophysiology, our results may suggest a therapeutic potential of lipoxins in attenuating obesity-related disease.

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SA057

Elucidation of thirteen series resolvins that regulate host responses in infections

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Identification of mechanisms that regulate host responses during self-resolving inflammation are of wide interest. This is because therapeutics that activate the host response to clear infections will provide new leads to combat infections without weakening the immune response. Herein, we identified two new families of host-protective molecules in in mice during infections and in human tissues following both sterile and infectious challenge. Investigations of their physical properties demonstrated that they carried n-3 docosapentaenoic acid backbones, conjugated triene and diene-double bond systems, and an alcohol at carbon-13, thus they were termed 13-series resolvins (RvT). RvT demonstrated potent protective actions in Escherichia coli infections with ng/mouse activities. These molecules stimulated bacterial phagocytosis at pm-nM potencies, regulated inflammasome components with human pyroptosis in vitro and mice in vivo, as well as increased mouse survival up to 60% during lethal infections. The production of these bioactive molecules during neutrophil-endothelial cell interactions was initiated by endothelial cyclooxygenase-2 (COX-2) that gave 13R-hydroxy-docosapentenoic acid. The biosynthesis of this intermediate was increased with atorvastatin via S-nitrosylation of COX-2. The host protective actions of atorvastatin and RvT’s proved additive in murine E.coli infections where they accelerated resolution of inflammation and increased >60% survival. RvTs and atorvastatin each engaged both overlapping and characteristic host protective pathways, including the upregulation of the anti-inflammatory eicosanoid prostaglandin I2, as well as the down regulation of the inflammation-initiating eicosanoids PGE2 and TXB2. Together these results identify novel host protective molecules produced during both sterile inflammation and in bacterial infections, namely 13-series resolvins from n-3 docosapentaenoic acid. These molecules orchestrate key innate host protective responses in infections. In addition, these endogenous pathways can serve as statin-markers and may provide novel opportunities for limiting some of the unwanted responses to statins.
Resolution of acute inflammation – lipids, macrophages and adaptive immunity

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I am interested in the cells, soluble mediators and receptors that collectively help switch inflammation off, so-called inflammatory resolution. My overall hypothesis is that understanding how acute inflammation resolves will provide insight into the aetiology of chronic inflammatory diseases. In addition, identifying mediators and receptors essential for resolution will help develop drugs that will drive ongoing inflammation down a pro-resolution pathway. However, recent work suggests that resolution of acute inflammation is not the end of immune responses to infection/injury but, that through cells of the mononuclear phagocyte system expressing arachidonic acid, docosahexaenoic acid and eicosapentaenoic acid metabolising enzymes resolution acts as a bridge between innate and adaptive immunity. We believe that defects in these pathways may contribute to the aetiology of, for instance, “inflammaging” and that rectifying these defects may improve vaccine efficacy in the elderly. To understand these processes better as they pertain to humans and human ageing, we have developed models of acute, self-limiting inflammation in healthy volunteers. As a result of these studies we are mapping pathways of inflammation with emphasis on mononuclear phagocytes and lipid mediators and are finding clear differences between young and older individuals with regard to pro-resolution processes. In my presentation, I will present these new data and speculate on their potential contribution to the aetiology of chronic inflammation and the role ageing plays in this process.

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SA059

Cardiovascular alterations during exercise in adults with type 2 diabetes

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Adults with type 2 diabetes mellitus (T2D) consistently show reduced peak pulmonary oxygen uptake (VO₂) responses during maximal incremental exercise (Green et al, 2015). These lower peak VO₂ responses are associated with reduced peak cardiac output and greater fractional oxygen extraction levels, suggesting a reduced ability to deliver oxygen to exercising tissues. In addition, young and middle-aged adults with T2D of short duration (mean time since diagnosis = ~3-5 years) manifest slower rates of oxygen uptake (VO₂ kinetics) at the onset of moderate submaximal exercise, but this effect is absent in older (> 60 years) individuals. This is likely due to a larger diabetes-induced exertional hypertension response during submaximal exercise in middle-aged compared with older people, which accompanied with normal cardiac output responses, results in blunted systemic vascular conductance responses among the former (O’Connor et al, 2015). Furthermore, peak blood flow during graded exercise is reduced and the dynamic response of blood flow during submaximal exercise is slowed in isolated lower limbs in younger and middle-aged adults with T2D (Kiely et al, 2014). These vascular defects most likely contribute to the exertional hypertension, impaired peak and dynamic responses of VO₂ and exercise intolerance. As a consequence of this exercise intolerance, adults with T2D perceive even light to moderate exercise as more difficult than healthy peers, and this might contribute to their relatively lower levels of physical activity and increased risk of cardiovascular outcomes and all-cause mortality in later life.


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SA060

Is left ventricular function already impaired in the adolescent with diabetes?

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Diabetes is associated with reduced aerobic capacity and impaired left ventricular function. Large cohort studies show that people with diabetes are also more likely to develop ischaemic heart disease and heart failure. It is assumed that morphological and autonomic changes resulting from chronic hyperglycaemia and/or hyperinsulinaemia contribute to diabetic cardiac dysfunction. It is less clear whether adolescents, whose diabetes duration is relatively shorter, also have reduced cardiac reserve and impaired left ventricular function. This presentation will describe the left ventricular response to acute exercise in adolescents with diabetes and how exercise training affects the adolescent diabetic heart.

National Heart Foundation (New Zealand). New Zealand Society for the study of Diabetes

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Phospholipid scramblases collapse the plasma membrane lipid asymmetry, externalizing phosphatidylserine to trigger blood coagulation and mark apoptotic cells. Several groups showed that some TMEM16 homologues are Ca²⁺-dependent ion channels and/or phospholipid scramblases. The recent crystal structure of nhTMEM16 showed a possible location for the lipid pathway as each monomer exposes a hydrophilic groove (~10 Å) to the membrane core. It was thus proposed that that during scrambling the lipid headgroups interact with the groove while the acyl chains remain in contact with the membrane’s core and that ions and lipids share a common pathway. To test these hypotheses we investigated how mutants located in the groove affect scrambling and ion transport and how increasing the headgroup size of the scrambled lipids affects transport. We identified several residues lining the groove that are important for scrambling but have minimal effects on ion transport, suggesting that the two functions are not tightly coupled. Interestingly, the identified residues primarily affect scrambling in the absence of Ca²⁺ with lesser effects in high Ca²⁺, suggesting that the lipid-pathway interactions are Ca²⁺-dependent. We generated NBD-labelled phospholipids with headgroups conjugated to PEG molecules as large as 5 kDa, with diameters up to 40 Å. We found that in the presence of Ca²⁺ all tested lipids are scrambled, despite having headgroups several fold larger than the groove. Similarly, lipid transport in the absence of Ca²⁺ retains similar size selectivity to that in the presence of saturating Ca²⁺, suggesting that the two states have similar size. Taken together our results suggest that the lipid pathway is narrower than in the Ca²⁺ bound state and that in a membrane environment the cavity could be substantially wider than seen in the structure.

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The TRPM2 channel integrates multiple input signals, but is not a channel-enzyme

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Transient Receptor Potential Melastatin 2 (TRPM2) channels are Ca2+-permeable nonselective cation channels that open under conditions of oxidative stress and play important roles in immune cell activation, insulin secretion, and postischemic cell death. They are activated by ADP ribose (ADPR) binding to the carboxy-terminal cytosolic NUDT9-homology (NUDT9H) domain, and modulated in intact cells by a variety of signaling molecules, including Ca2+, various adenine nucleotides, and H2O2. Furthermore, the NUDT9H domain was reported to hydrolyze ADPR, classifying TRPM2 as a channel-enzyme. We have studied TRPM2 channels in cell-free inside-out patches, under rapid direct superfusion of the cytosolic channel surface. Rundown of TRPM2 channels following patch excision could be prevented by a pore substitution. Combining electrophysiology with biochemical purification of test compounds ruled out direct effects by the majority of proposed modulators, and identified only three essential direct activators: ADPR, Ca2+, and phosphatidylinositol-bisphosphate (PIP2), simultaneous presence of all three of which is required for channel gating. So far, ADPR-2'-phosphate is the only identified natural ADPR substitute capable of channel activation. All other proposed modulators must act indirectly, by altering the local concentrations of the three primary agonists in intact cells. The binding sites for activating Ca2+ are intracellular, but in close proximity of the cytosolic pore entrance. PIP2 is bound with high affinity, but complete PIP2 depletion inactivates the channels. NUDT9H catalytic site mutations do not affect channel gating, and a non-hydrolyzable ADPR analog supports near-normal channel activity, demonstrating that ADPR hydrolysis is not linked to channel gating. Soluble chimeric models of the isolated NUDT9H domain are capable of ligand binding, but display no ADPase activity, suggesting that TRPM2 is not a channel-enzyme.

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The role of skeletal muscle contractile duration in the science of inactivity physiology

M.T. Hamilton

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Dr. Marc Hamilton, Director and Professor of the Texas Obesity Research Center, will describe the development of the concepts and directions for the emerging field of 'inactivity physiology': the science that led to the concept that because of the specific ways skeletal muscle and the rest of the body respond to widely varying doses of muscular contractile activity, then it logically follows that "too much sitting is not the same as too little exercise". This relatively new research discipline has been rapidly growing since his seminal work introducing this concept a decade ago. There have now been over 1000 research papers by peers investigating this concept because of its profound public health implications. Since he first proposed the inactivity physiology paradigm a decade ago, there has been a global movement of experts taking notice of the time people sit. The effects of sedentary time on health and disease are generally independent of traditional exercise. In contrast to the influence of brief amounts of MVPA (the 2.5 hrs/week recommendation), many aspects of good health appear to be more related to a much greater duration (42 hrs/week is the average time of total upright muscular activity). Therefore, inactivity physiology is about understanding the effects of muscular activity that is generally performed at a lower perceived effort of physical activity distributed throughout dozens of hours each week, and spread over almost the whole waking day. Current studies are probing the association of inactivity with molecular and physiological responses related to risk factors for metabolic syndrome, coronary artery diseases, type 2 diabetes, obesity, some cancers, deep venous thrombosis, and mortality. Our early work identified the most potent known molecular mechanism known to regulate lipoprotein lipase activity in skeletal muscle. In fact, non-fatiguing and intermittent light activity throughout each hour of the day has better effects on lipoprotein lipase and multiple other key processes than does exercise training. Another example is a novel inactivity-responsive gene that potentially may prevent novel inactivity. Experimental and epidemiological studies have recently provided evidence that the healthy effects of low-intensity physical activity are independent of the type of moderate activity historically recommended (but practiced by <10% of the population). This has generated much enthusiasm among public health experts because of the potential for providing more effective behavioral solutions to the millions of people who cannot (or will not) do traditional exercise.


Increased life expectancy does not necessarily increase health expenditure, but ill-health in the final part of life certainly does, and on average adults in the UK spend the last decade of life in ill-health. Ageing is associated with a progressive decline in muscle metabolic health and functional capacity, leading to a loss of independence. This presentation will describe the changes in muscle mass, strength, fibre composition, power, oxygen utilisation and insulin sensitivity that occur with ageing in humans, and will identify mechanisms that potentially explain these negative changes. In particular, given ageing is associated with increased sedentary behavior, consideration will be given to whether the biological features attributed to muscle “ageing” per se may in fact be a consequence of previous and/or current levels of physical inactivity. Indeed, data will be presented showing that many of the purported negative effects of muscle ageing can be relatively quickly manifested in young people simply by exposure to inactivity. Current knowledge gaps include the relative contribution that physical inactivity plays in the development of many of the features associated with poor muscle metabolic health in older age, including muscle centric mechanisms linking physical inactivity and/or sedentary time to impaired metabolic health. Similarly, data demonstrating positive effects of government recommended physical activity guidelines (or indeed any other physical in/activity interventions relevant to preservation of health) on muscle specific health, the decline in which is strongly associated with functional deterioration in older adults, are largely non-existent. It is imperative therefore that research examining interactions between ageing, physical activity and muscle metabolic health is prioritised so that it can that inform on the “normal” muscle ageing process and on strategies for improving health span and well-being.

Population Ageing in the UK, its Constituent Countries and the European Union (2012)

Effects of breaking up sedentary time on postprandial metabolic responses

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There is a large body of observational data showing strong associations between time spent engaged in sedentary behaviour and a number of adverse health outcomes, including mortality, cardiovascular disease, type 2 diabetes and obesity. These often persist after adjustment for time spent in moderate-to-vigorous physical activity, although our recent data suggests that association between sedentary time and adverse health outcomes may be attenuated in those with high levels of physical activity and/or high functional capacity. There is also observational evidence to suggest that individuals who break up sedentary time more frequently have a more favourable cardio-metabolic risk profile – particularly with respect to adiposity variables – than those who habitually engage in prolonged periods of uninterrupted sedentary time, independent of total time spent sedentary. Recent data from lab-based studies have shown that breaking up periods of prolonged sitting with short walking breaks, elicits reductions in postprandial glucose, insulin and triglyceride responses. Studies evaluating the effects of breaking up sitting with static standing on these postprandial blood responses have had more mixed results, with reductions in postprandial glucose and insulin responses observed in older women with impaired glucose regulation, but no clear changes in younger normoglycaemic adults. Our recent data suggests that increasing the frequency of breaks in sedentary time, independent of total time spent sitting, increases energy expenditure and fat oxidation, which provides an explanation for the association between frequency of sedentary breaks and adiposity observed in the epidemiological data.

Cystic fibrosis, CFTR and hypoxic responses of the lung

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Mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) cause cystic fibrosis (CF), a fatal disease associated with recurring lung infections due to

In the UK, the percentage of people aged 65 years and over increased from 15 – 17% (1.7 million) between 1985 and 2010. Furthermore, it is projected that by 2035 people aged >65 years will make up 23% of the UK population, with those aged >85 years accounting for 5% of the total population (1). Increased life expectancy does not necessarily increase health expenditure, but ill-health in the final part of life certainly does, and on average adults in the UK spend the last decade of life in ill-health. Ageing is associated with a progressive decline in muscle metabolic health and functional capacity, leading to a loss of independence. This presentation will describe the changes in muscle mass, strength, fibre composition, power, oxygen utilisation and insulin sensitivity that occur with ageing in humans, and will identify mechanisms that potentially explain these negative changes. In particular, given ageing is associated with increased sedentary behavior, consideration will be given to whether the biological features attributed to muscle “ageing” per se may in fact be a consequence of previous and/or current levels of physical inactivity. Indeed, data will be presented showing that many of the purported negative effects of muscle ageing can be relatively quickly manifested in young people simply by exposure to inactivity. Current knowledge gaps include the relative contribution that physical inactivity plays in the development of many of the features associated with poor muscle metabolic health in older age, including muscle centric mechanisms linking physical inactivity and/or sedentary time to impaired metabolic health. Similarly, data demonstrating positive effects of government recommended physical activity guidelines (or indeed any other physical in/activity interventions relevant to preservation of health) on muscle specific health, the decline in which is strongly associated with functional deterioration in older adults, are largely non-existent. It is imperative therefore that research examining interactions between ageing, physical activity and muscle metabolic health is prioritised so that it can that inform on the “normal” muscle ageing process and on strategies for improving health span and well-being.

Population Ageing in the UK, its Constituent Countries and the European Union (2012)

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Physical inactivity and age-related declines in muscle metabolic health

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In the UK, the percentage of people aged 65 years and over increased from 15 – 17% (1.7 million) between 1985 and 2010. Furthermore, it is projected that by 2035 people aged
ineffective clearance of the airways. As CF patients frequently suffer from ventilation-perfusion (V\textsubscript{A}/Q) mismatch, we speculated whether hypoxic pulmonary vasoconstriction (HPV), the physiological mechanism protecting from V\textsubscript{A}/Q inequalities, may be abrogated in this condition. Thus, functional CFTR may be critical for HPV. While the effects of CFTR in airway epithelial cells have been extensively studied, the functional role of CFTR in pulmonary arterial smooth muscle cells has thus far been largely obscure. In this study we characterized the role of CFTR in the pulmonary vasculature in acute hypoxia and linked our findings to the sphingolipid system, a known central mediator of HPV.

In pulmonary artery smooth muscle cells (PASMCs), we studied the effects of CFTR deficiency or CFTR inhibition on two mechanisms known to be essential for the induction of HPV, (i) hypoxia-induced Ca\textsuperscript{2+} influx and (ii) hypoxia-induced translocation of transient receptor potential canonical 6 (TRPC6) to caveolae. In coimmunoprecipitation experiments in PASMCs, we further probed for direct protein-protein interaction between CFTR and TRPC6 in response induced by nSMase and S1P. downstream signaling of nSMase and/or S1P was largely reduced by nSMase inhibition. Exogenous applied S1P receptor 2 (S1P\textsubscript{2}) or S1P4 as well as the corresponding wild type mice were assessed. The effects of exogenous applied neutral sphingomyelinase (nSMase) on the pulmonary arterial pressure were analyzed. Downstream signaling of nSMase and/or S1P was characterized.

CFTR inhibition and/or deficiency reduced HPV ex vivo and led to aggravated hypoxemia following partial airway occlusion in vivo. Accordingly, CFTR inhibition diminished hypoxia-evoked Ca\textsuperscript{2+} signaling and TRPC6 translocation in PASMCs. Moreover, hypoxia led to a direct protein-protein interaction between CFTR and TRPC6, which was blocked by CFTR inhibition. Both, HPV and hypoxia-induced TRPC6 translocation were largely reduced by nSMase inhibition. Exogenous applied nSMase, however, induced TRPC6 translocation and mimicked the pulmonary vasopressor response evoked by hypoxia in a CFTR-dependent manner. Hypoxia-as well as nSMase-induced pulmonary vasoconstriction required S1P signaling as revealed by SphK inhibition and dual inhibition of S1P\textsubscript{2} and S1P\textsubscript{4}. Analogously, HPV was reduced in lungs of SphK-deficient mice as compared to wild type mice. Finally, the synergistic vasopressor response induced by nSMase and S1P was dependent on TRPC6, phospholipase C and rho kinase.

Our data suggest a key role for CFTR in the induction of HPV. Thus, CFTR malfunction due to CFTR gene mutations may be causative for V\textsubscript{A}/Q mismatch in CF patients.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

**SA070**

**Does exercise increase or decrease pain? Central mechanisms underlying these two phenomena**

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Regular physical activity (exercise) can reduce pain in people with chronic musculoskeletal pain; whereas, unaccustomed exercise can exacerbate pain. This apparent dichotomy in pain response to physical activity is poorly understood, making exercise prescription for individuals with pain challenging. Dr. Sluka will present ongoing research examining the neurobiological mechanisms underlying the increased pain with an acute bout of exercise, and compare these to the mechanisms that decrease pain with regular physical activity using animal models of pain. All experiments were conducted after approval by the institutional review board, and in accordance with National Institutes of Health guidelines. We will show that there is a balance between inhibitory and excitatory pathways in brainstem sites that modulate pain so that regular physical activity increases overall inhibition to prevent the onset of chronic musculoskeletal pain. To induce chronic musculoskeletal pain, we show that repeated acid injections, or acid injections combined with fatigue, result in long-lasting widespread hyperalgesia. Regular physical activity, induced in mice with wheel running, prevents the hyperalgesia in these
animal models of pain. We show increases in phosphorylation of the NR1 subunit of the NMDA receptor and the serotonin transporter (SERT) in the caudal raphe nuclei after induction of chronic musculoskeletal pain. Increasing NR1 expression in the RVM by viral delivery of cDNA to NR1 induces muscle hyperalgesia, while decreasing expression by viral delivery of an miRNA to NR1 prevents development of hyperalgesia. We further show increased phosphorylation of the NR1 subunit NMDA receptor, decreases in serotonin and increases in the serotonin transporter in the RVM, and increases in phosphorylation of the transcription factor CREB, in the amygdala, cingulate and insular cortices. Regular physical prevents these increases in p-NR1, SERT, and p-CREB in the RVM and cortex, and increases serotonin and 5-HT receptors in RVM, and the analgesia produced by regular physical activity is prevented by blockade of opioid receptors in the RVM and periaqueductal gray. Modulation of opioid expression in physically active mice, knockouts or pharmacological blockade, prevents the activity induced decreases in SERT but not p-NR1. Thus, a complicated network involving the cortex and the brainstem that involves a balance between multiple inhibitory and excitatory systems mediates the hyperalgesia induced by an acute bout of exercise in physically inactive mice, and the ability of regular physical activity to prevent chronic pain.


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SA071

The changing role of supraspinal sites and endogenous pain control systems in early life: Life-long consequences for health and well-being

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Pain in infancy is a distinct clinical concern and is managed sub-optimally. The study of pain processing during early life has shown that in mammals, nociceptive withdrawal thresholds are lower, and response magnitudes are greater and longer lasting compared to adults. The central nervous system is highly plastic during postnatal development, and maturation of nociception requires activity-dependent processes to attain an adult-like state (see Hathway, 2014). Normal adult processing of noxious sensory inputs requires a constant balance between synaptic excitation and inhibition within the “Pain Pathway”. Descending neuromodulatory pathways play a key role in modulating spinally mediated nociceptive reflexes. The periaqueductal grey (PAG) and nuclei within the rostroventral medial medulla (RVM) are pivotal within this loop for the top-down control of spinal dorsal horn (DH) pain processing (see Hathway, 2014). Both the PAG and RVM integrate pain-related activity from forebrain structures and bi-directionally modulate DH excitability. Opioidergic activity within the descending pathway is one of the major neurotransmitter systems responsible for endogenous pain control. Our work and others have previously shown that significant postnatal refinement occur in the opioidergic signalling system (Hathway et al., 2009, 2012; Kwok et al., 2014). This immaturity of CNS processing of pain does not just reflect the altered properties of efferent structures from the mid-brain and brainstem but also extends to structures such as the primary somatosensory cortex which receive ascending input from the DH via the thalamus (Devonshire, Greenspon and Hathway 2015). In this talk I will discuss altered pain processing within CNS structures during postnatal development and present evidence that shows that early life pain experience alters the normal development of these structures. I will also present new data that maps the functional maturation of the endocannabinoid systems within the PAG, RVM and DH from a molecular to whole animal physiological level.

All animal use procedures were performed in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986 and followed the guidelines of the International Association for the Study of Pain. Using Sprague-Dawley rats of both genders, we detected endocannabinoids (eCBs) expression alters the normal development of these structures. I will also present new data that maps the functional maturation of the endocannabinoid systems within the PAG, RVM and DH from a molecular to whole animal physiological level.

Tissue levels of the eCBs (anandamide (AEA), 2-acylglycerol (2-AG), oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) were assessed. Significant age-related alterations in the levels of all eCBs were detected in all three structures studied. These changes in lipid levels were also accompanied by alterations in transcript levels of components of the eCB system. Within the PAG levels of NAPE-PLD (a synthetic enzyme for AEA), significantly increased with age, whilst levels of the CB1 receptor decreased. Within the DH levels of mRNA for this enzyme were elevated at P21 compared to both P10 and adult. In the RVM GPR55 mRNA levels were significantly elevated at P21 compared to both P10 and adults. Immunohistochemical analysis identified clear alterations in the expression pattern and immunofluorescent intensity of CB1, CB2, NAPE-PLD and DAGLα throughout the PAG, RVM, DH axis. As well as alterations in opioidergic systems in the postnatal period there are concurrent changes in eCB pathways that
play a role in the normal maturation of pain detection and responding. The impact of alterations in top-down pain processing in early life and the effect of early life injury to alter these processes has considerable implications for life-long health and well-being.

Hathway GJ et al. (2009) J. Physiol. 587(Pt 12):2927-2935
Devonshire et al. (2015) Neuroscience 305:343-350

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA072

**DNIC and descending monoaminergic transmission: An investigation into the inhibitory function of serotonin**

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Endogenous descending inhibitory and facilitatory control pathways, which originate in brain stem and higher brain areas and project to and terminate in the dorsal horn of the spinal cord, can alter spinal outputs and pain levels. Changes in descending pathways can lead to chronic pain syndromes. The pre-clinical investigation of such changes can be translated and utilized in the clinic such that successful targeted pharmacological treatment outcomes are more quickly recognized and prescribed to maximize patient benefit. This is no more apparent than when considering the phenomenon of diffuse noxious inhibitory controls (DNIC) and the patient equivalent, conditioned pain modulation (CPM).

DNIC are a unique form of endogenous descending inhibitory control acting on spinal and trigeminal wide dynamic range neurons. One pain inhibits another. Previously we have shown that DNIC are present in normal animals, lost after neuropathy but are still present in sham-operated animals that show no persistent pain phenotype. It is known historically that in neuropathic animals there is a loss of descending inhibitory noradrenergic controls alongside a gain of 5-HT3 receptor-mediated facilitations. We investigated the pharmacological basis of DNIC and whether it could be restored after neuropathy because the antagonists yohimbine and atipamezole attenuated this descending inhibition. DNIC was restored in spinal nerve ligated (SNL) rats by blocking 5HT-3 receptor-mediated facilitations with the antagonist ondansetron, or by enhancing noradrenaline modulation through the use of reboxetine (noradrenaline reuptake inhibitor, NRI) or tapentadol (dual function as a μ opioid receptor agonist, MOR, and NRI).

After neuropathy (in terms of descending facilitations) block of facilitatory 5HT3 receptors allows DNIC to be induced, suggesting that excitations can swamp inhibitions. The proposed underlying noradrenergic mechanisms explain the relationship between CPM and the use of tapentadol and duloxetine (a serotonin-NRI) in patients. Presently we are investigating the effects of spinal citalopram (a serotonin reuptake inhibitor, SRI) on the expression of DNIC in SNL animals. Interestingly, despite a larger available pool of serotonin in these animals, DNIC are now revealed, but are abolished following dual application of spinal citalopram and 5HT-7 receptor agonist SB-269970, or spinal citalopram and atipamezole. This preliminary data suggests that in SNL animals, in the presence of a deluge of serotonin in the spinal cord, inhibitory 5HT7 receptors are activated such that DNIC are now expressed. Further, the data points towards a tonic inhibitory noradrenergic component in SNL animals that must be present for the DNIC-revealing actions of citalopram.

Ultimately, balancing excitations and inhibitions with drugs acting on monoamine systems may be of benefit not only in restoring normal descending inhibitory balance but also conceivably in prevention of persistent post-surgical and other pains because CPM is a predictor.


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SA073

**Midbrain control of sensory and motor systems**

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The ability to interact with challenging environments requires co-ordination of sensory and motor systems that drive appropriate defence behaviours. The ventrolateral midbrain periaqueductal grey (vPAG) lies at the heart of the defence- arousal system and its integrity is paramount to the expression of defence behaviours (1). To date, attention has focused on the sensory consequences of vPAG activation, as part of co-ordinated passive coping strategies triggered by inescapable stressors (2). Work from this laboratory has shown that chemical activation of descending pathways from the vPAG powerfully modulates responses of spinal neurones to cutaneous mechanical and thermal (heat and cold) stimulation, *in vivo* (3-5). Of relevance to survival, in naïve animals generalised activation of the PAG selectively inhibits spinal nociceptive processing, as compared to non-noxious cutaneous transmission. This pattern of response is thought to support defence behaviour by filtering out nociceptive input that could distract an animal from carrying out behaviours necessary for survival and leaves intact transmission of non-noxious information that provides precise information with the capacity to direct motor activity and promote survival.

Despite the fundamental importance of motor behaviours evoked from the vPAG, very little is known about their underlying neural pathways and mechanisms. As a first step, this significant gap in understanding was addressed by (i) reports that activation of the vPAG enhances α-motoneuron excitability which, it is proposed, underlies fear-evoked freezing behaviour (6-7) and (ii) the demonstration that these effects are dependent, at least in part, on the integrity of the cerebellum (6); the largest sensorimotor structure in the brain.
Furthermore, vlPAG-modulation of sensory transmission in motor circuits was evidenced by inhibition of peripheral nerve-evoked cerebellar cortical field potentials, which from individual PAG stimulation sites, was accompanied by facilitation of spinal motor outflow (as assessed by α-motoneuron excitability; (7)). These studies provided initial insights into the pathways and mechanisms that mediate co-ordination by the vlPAG of sensory and motor processing in survival networks. Our demonstration that survival networks include interactions between the vlPAG and the cerebellum led us to investigate the effects of descending control from the vlPAG on a pre-cerebellar sensory pathway originating in the spinal cord; the spino-olivary tract. Consistent with our previous studies of descending control of spinal nociception, activation of the vlPAG selectively inhibited the cutaneous nociceptor- (as compared with non-nociceptor) evoked responses of antidromically identified spino-olivary neurones. However, a novel and important finding was the facilitation of proprioceptive responses of spino-olivary neurones (7). This raises the possibility that cerebellar input from spinal circuits signaling limb position and movement can be enhanced, thus refining sensory inputs that direct motor control. Such an effect is entirely consistent with a role for the vlPAG in co-ordinating motor behaviours in defence situations.

Taken together, our studies provide strong evidence that the vlPAG can orchestrate differential changes in ascending somatosensory-motor projections and spinal motor systems simultaneously. We suggest that this differential gating of nociceptive cutaneous and proprioceptive information to the cerebellum by the vlPAG, together with the enhancement of motor outflow may promote a condition in which the animal is ready (enhanced proprioceptive input and increased muscle activity) and able to escape (less likely to be perturbed by noxious sensory information), thus assisting survival.


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Temporal partitioning of cardiac metabolism by the cardiomyocyte circadian clock

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Over the course of a normal 24 hours, the environment of, and the demands placed upon, cells/organs/organisms fluctuate dramatically. Organisms have therefore evolved circadian clocks, intracellular molecular mechanisms that allow individual cells to perceive the time of day. In doing so, circadian clocks confer the selective advantage of anticipation, enabling both rapid and appropriate responses to environmental stimuli/stresses upon their onset. Research in my laboratory has focused primarily on identifying the roles of the circadian clock within the heart. Over the past 15 years we have accumulated evidence supporting the concept that the cardiomyocyte circadian clock temporally partitions cardiac processes, including transcription, ion homeostasis, signal transduction, and metabolism. In the latter case, we have revealed that the cardiomyocyte circadian clock directly regulates myocardial glucose, fatty acid, and protein metabolism, promoting oxidative metabolism during the active phase (thereby providing ATP in anticipation of increased contractility) and protein turnover during the sleep phase (thereby promoting growth and repair in anticipation of the subsequent active period).

In addition, we have found that the cardiomyocyte circadian clock modulates responsiveness of the heart to physiologic stimuli (e.g., insulin) and pathologic stresses (e.g., ischemia/reperfusion) in a time-of-day-dependent manner, thus synchronizing the heart with its environment.

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Time-of-day variation in vascular function

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There is a strong time-of-day variation in vasoconstriction in response to sympathetic stimulation that may contribute to the time-of-day variation in blood pressure, which is characterised by a dip in blood pressure during the resting-period when sympathetic activity is low. Vasoconstriction is known to be tightly regulated by nitric-oxide signalling from the endothelial cells, so we have looked at the effect of the time-of-day on the ability of endothelial nitric oxide synthase (eNOS) signalling to modulate of vascular contractility. We used wire-myography to measure contractile force from 2-4 mm ring segments of the superior mesenteric artery and surrounding arteries in acute perfused mesenteric vessels that were either Wistar rats active-period (day) or resting-period (night). Data showed that mesenteric resistance vessels exhibit a time-of-day variation in their contractile-response to α1-adrenoreceptor (ADR) and muscarinic activation, characterised by a reduced vasoconstriction in response to an increased concentration of phenylephrine and enhanced vasodilation in response to acetylcholine during the active- versus the resting-period. This reduced vasoconstriction in response to phenylephrine is also present in response to...
high-K contractions, suggesting a mechanism independent of the signalling pathway involved in α1-ADR activation. This time-of-day difference in response to phenylephrine and ace-
tylcholine is absent in the presence of L-Nitro-Arginine Methyl Esters (L-NAME), and the variation in contraction in response to high-K requires an intact endothelium. We found a large increase in eNOS expression (mRNA and protein) during the active- versus resting-period vessels, which may reflect the presence of a functioning peripheral circadian clock in mesenteric resistance vessels.

The peak in eNOS-signalling during the active-period and its dampening effect on vasoconstriction in response to α1-ADR activation would reduce any sympathetic-driven rise in peripheral resistance, which, combined with the reduction in the positive inotropic response of the ventricles to sympathetic stimulation (Collins & Rodrigo, 2010), would limit the rise in blood pressure with a beneficial impact on stroke and hypertensive heart disease. This time-of-day variation in endothelium-derived nitric oxide signalling may also have far reaching physiological consequences over and above regulation of blood pressure. For example; elevated nitrite levels in the blood have been linked to cardioprotection from ischaemic conditioning and nitric oxide in response to eNOS activation by sheer stress has an anti-platelet aggregation action. Thus an increase in eNOS activity during the active period may play a protective role against cardiovascular pathologies.


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SA076

Circadian biology and consequences of disturbing rhythms for the cardiovascular system

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In nearly every species on earth, life pulses with a day/night or circadian rhythm. In humans, these daily rhythms are driven by a genetically inherited biological clock mechanism that is present in all our cells. This mechanism regulates how we physiologically adapt to light/dark, activity/rest, and wake/sleep. However, it also influences far more than daily habits. Cardiovascular disease, obesity, diabetes and cancer have all been linked to disruption in the circadian mechanism. When the mechanism is disturbed, things go awry. In recent years there has been a flurry of research investigating how living cells keep time, and what these circadian rhythms mean for health and disease. Our research has revealed two new promising circadian strategies that benefit the treatment of cardiovascular disease. 1) Day vs. night timing of drug administration (chronotherapy) is an effective strategy that uses circadian biology to benefit treatment of heart disease. The rationale is that timing of therapy matches biologic need, which varies across 24h day/night. 2) Maintaining sleep and circadian rhythms in intensive care units is a promising non-pharmacological approach to improve outcomes after heart attacks. Circadian rhythms research leads directly to clinical strategies that can benefit the treatment of disease.


SA077

Molecular basis of circadian rhythmicity in renal physiology and pathophysiology

M. Gumz1,2

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Accumulating evidence suggests that the molecular circadian clock acts as a master regulator of gene expression in the kidney. The core clock genes, including Bmal1, Clock, and Cryptochrome 2, all exhibit circadian expression in the mouse kidney (Figure). Global transcriptomic approaches have revealed the important finding that there are thousands of genes in the kidney subject to regulation by the molecular clock [1][2]. One example is that the circadian clock protein Per1 transcriptionally regulates the alpha subunit of the epithelial sodium channel (αENaC)[3]. Candidate gene approaches have also yielded information regarding regulation of renal sodium transport genes by the molecular clock [4]. To date, the evidence linking the molecular kidney clock to rhythmic renal function provides strong support for the concept that circadian control of gene expression underlies rhythms in physiological function. In this presentation, an overview of the molecular and physiological evidence for the kidney clock and the implications for the regulation of renal physiology and pathophysiology will be presented.


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NMDA receptor mutations, early onset epileptic encephalopathy, and personalized medicine

S. Traynelis and H. Yuan

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NMDA receptors are ligand-gated cation-selective ion channels that mediate a slow, Ca\(^{2+}\)-permeable component of trans-mission at excitatory synapses in the central nervous system. The glutamate receptors are tetrameric assemblies comprised of two GluN1 and two GluN2 subunits. GluN1 subunits are encoded by a single gene (GRIN1), which undergoes alternative splicing. The GluN2 subunits are encoded by a family of four genes (GRIN2A, GRIN2B, GRIN2C, GRIN2D) that are differentially expressed both anatomically and developmentally. Over the past decades, rare variants in a number of genes encoding various ion channels have been linked to neurological disease, sometimes referred to as channelopathies. More recently, a large number of de novo and inherited GRIN mutations have been identified in patients with neurological conditions (Yuan et al., 2015). These variants are absent from the healthy population, and are most commonly associated with seizure disorders. Among the GRIN genes, GRIN2A harbors the most rare variants (<1%) and de novo mutations that are associated with neurological disease, with the most common phenotype being patients suffering from seizure disorders. For this reason, GRIN2A has been proposed to be a locus for childhood epilepsy (Carvill et al., 2013; Lemke et al., 2013; Lesca et al., 2013). Despite the identification of hundreds of missense NMDA receptor mutations and rare variants, there are still less than a dozen de novo mutations for which functional data are available describing the effects of the amino acid exchange on receptor response properties. However, without understanding how these variants impact receptor function, it is difficult if not impossible to assess their role in terms of the neurological conditions they are associated with. We have sought to advance our understanding of the role of these mutations in neurological disease by obtaining functional data on all known variants as well as new cases identified by our collaborators. These studies have revealed some patient populations with de novo mutations in the ion channel pore and associated linkers that show similar enhancement of function, raising the possibility that these patients may be amenable to therapeutic treatment. Functional data will be presented showing the effects of different mutations in the ion channel pore and the adjacent linkers on NMDA receptor response time course and channel properties. In addition, the idea that mutations that enhance NMDA receptor function could be neurotoxic will also be discussed. A better understanding of the functional properties of these rare variants and mutations will help to elucidate the role these mutations play in the neurological conditions they are associated with, as well as advance our understanding of how the NMDA receptor works.


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also theoretically a predictable choice for reversing the excess excitability seen in neurons in epileptic tissue (5). However, other Kv channels may be more obvious choices based on pure biophysics. Many more channels, including endogenous, exogenous and synthetic channels also represent candidates for developing mechanistically-based gene therapies (6,7).


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SA080

A hyperkplexia mutant that affects glycine receptor deactivation

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Glycinergic transmission in the spinal cord and brainstem is vital for maintaining skeletal muscle function by inhibiting neuronal excitation. Dysfunctional glycinergic inhibitory transmission underlies the debilitating neurological condition known as hyperkplexia or startle disease. This is characterised by exaggerated startle reflexes, muscle hypertonia and apnoea and its treatment often requires the potentiation, by the use of positive allosteric modulators, of GABA-mediated inhibitory signaling. Here we have investigated the asparagine to lysine (N46K) missense mutation in the glycine receptor (GlyR) α1 subunit gene (GRLA1), which is found in the ethynitrosourea (ENU) induced murine mutant, Nmf11. The mutation causes a reduced body size, evoked muscle tremor, muscle stiffness, seizures and morbidity usually by postnatal day 21 (Traka et al., 2006).

Although N46 lies in close proximity to the glycine binding site, it does not form part of a recognised binding loop or signal transduction pathway. Using whole-cell patch-clamp electrophysiology revealed that introducing the N46K mutation into recombinant GlyR α1 homomeric receptors, expressed in human embryonic kidney (HEK) cells, reduced the agonist potencies of glycine, β-alanine and taurine by 9-, 6- and 3-fold respectively, without significantly affecting their relative maximum responses. In addition, the potency of the competitive GlyR antagonist strychnine was reduced by 15-fold, suggesting that N46K is likely to be affecting binding to the orthosteric binding site rather than agonist efficacy. Replacing N46 with a variety of hydrophobic, charged or polar residues revealed that the amide moiety of asparagine was crucial for GlyR activation. Additionally, from structural modelling studies, co-mutating N61, located on a neighbouring β loop to N46, rescued the wild-type (WT) phenotype depending on the charge of the amino acid side-chain, suggesting that these two residues (N46 and N61) might interact.

Single-channel recording of homomeric glycine receptor activity identified that mean burst length for the N46K mutant (3.5 ± 0.46 ms) was reduced when compared with WT receptors (10.4 ± 1.3 ms). Using rapid piezo-driven agonist applications further revealed faster glycine 90 – 10% deactivation/desensitisation times for the N46K mutant (26.1 ± 4.4 ms; n=9) compared to the WT receptor (98.2 ± 10.9 ms; n=10; p<0.05). Values are means ± S.E.M., compared by unpaired t-test. Overall, our data are consistent with N46 ensuring correct alignment of the α1-α1 subunit interface by interaction with juxtaposed residues to preserve the structural integrity of the glycine binding site, which affects the egress of bound glycine from the orthosteric site. This represents a new mechanism by which GlyR dysfunction can induce startle disease.


This work was supported by the MRC and The Leverhulme Trust.

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SA081

Mutations in GluN2 NMDAR subunits associated with neurodevelopmental disorders

K. Marwick, P. Skehel, G.E. Hardingham and D.J. Wylle

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The NMDA receptor is a subtype of ionotropic glutamate receptor that displays voltage-dependent block by Mg2+ and a high permeability to Ca2+. These receptors play important roles in synaptogenesis and synaptic plasticity. Recently, individuals with a range of neurodevelopmental disorders have been found to carry heterozygous missense and gene disrupting mutations in NMDAR genes. Mutations arising de novo are of particular interest as they are more likely than inherited mutations to be associated with a highly deleterious phenotype.

We noted a number of de novo mutations affecting the M2 pore region of NMDAR subunits found in individuals with childhood onset epilepsies and intellectual disability1,2. Hypothesising that these mutations underlie their carrier’s severe neurode-
Using a recently developed technique. Our findings show that properties and on triheteromeric NMDAR receptors generated regulations of the mutation's effect on NMDAR single channel properties, consistent with a role in disease causation.


Funding source: Wellcome Trust Clinical PhD fellowship awarded to KM. Conflicts of interest: none declared.

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SA082 Role of parafacial neurons in generating active expiration J.L. Feldman and R.T. Huckstepp Neurobiology, UCLA, Los Angeles, CA, USA

We hypothesize that breathing in mammals results from interactions between two oscillators: the preBötzinger Complex (preBötC) is the kernel for inspiration, while the retrotrapezoid nucleus/parafacial respiratory group (RTN/pFRG) is vital for active expiration and CO₂ chemoreception. We functionally dissected the RTN/pFRG by transfecting different populations of neighboring neurons with allatostatin or HM4D (DREADD) Gα/o-coupled receptors, and then analyzed the effect of their hyperpolarization on breathing in adult rats [1]. We identified two functionally separate parafacial nuclei: ventral (pFV) and lateral (pFL). We conclude that the pFV provides a generic excitatory drive to breathe, even at rest, whereas the pFL is a conditional oscillator quiet at rest that, when activated, e.g., during exercise, drives active expiration. We then looked at the interactions between the preBötC and pFV, by independently altering their excitability in adult rats [2]. Hyperpolarizing preBötC neurons decreased inspiratory activity and initiated active expiration, before ultimately progressing to apnea, i.e., cessation of both inspiration and active expiration. Depolarizing pFV neurons produced active expiration at rest, but not when inspiratory activity was suppressed by hyperpolarizing preBötC neurons. We conclude that in adult rats active expiration is driven by the pFL but requires an additional form of network excitation, i.e., ongoing rhythmic preBötC activity sufficient to drive inspiratory motor output or increased chemosensory drive. The organization of this coupled oscillator system, which is essential for life, may have implications for other neural networks that contain multiple rhythm/pattern generators.


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SA083 Proton detection and breathing regulation by the retrotrapezoid nucleus P.G. Guyenet and D.A. Bayliss Pharmacology, University of Virginia, Charlottesville, VA, USA

I will briefly present ten pieces of evidence which supports the following conclusions:

1) All RTN neurons (~ 800 in mice, ~2000 in rats) express Vglut2, Phox2b and neuromedin B; 50% express galanin. RTN neurons innervate selectively the lower brainstem regions that contain the respiratory pattern generator.

2) Many RTN neurons express cFos in rodents exposed to hypercapnia but not hypoxia.

3) In anesthetized rats, RTN unit activity increases by 0.5 Hz for every 0.01 change in arterial pH; this response persists after pharmacological blockade of the respiratory pattern generator.

4) Optogenetic activation of RTN in conscious rodents increases inspiratory frequency and amplitude, elicits active expiration and controls post-inspiratory air flow. If Vglut2 is deleted from RTN neurons, their optogenetic activation no longer stimulates breathing.

5) Optogenetic inhibition of RTN in unanesthetized rats reduces breathing in direct proportion to arterial pH; notably, RTN inhibition has virtually no effect when arterial pH exceeds 7.5.

6) Optogenetic inhibition of RTN produces a massive reduction of breathing frequency and amplitude during quiet waking or non-REM sleep in rats but has no effect on breathing frequency during REM sleep.

7) In vitro, RTN is activated by [H+] (slices) and the effect persists after cell isolation.

8) The pH response in vitro requires expression of a proton-activated GPCR (GPR4) and a proton-modulated potassium channel (TASK-2). These proteins are undetectable in astrocytes and the rest of the lower brainstem respiratory network.

9) The central respiratory chemoreflex is greatly reduced in global knock-out mice. Reintroducing GPR4 specifically into RTN neurons restores a normal chemoreflex in GPR4 KO mice.
Role of astrocytes and purinergic signaling in the chemical drive to breathe

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The retrotrapezoid nucleus (RTN) is a region critical for respiratory chemoreception. This region contains a subset of neurons that are intrinsically sensitive to changes in CO2/H+ and communicate this information to other respiratory centers to regulate depth and frequency of breathing. Interestingly, RTN astrocytes also appear to be specialized to support the activity of chemosensitive neurons by providing a CO2/H+-dependent mechanism. At the whole animal level, we found that Kir4.1 cKO mice show a reduced ventilatory response to CO2. It is also well known that cerebral blood flow is highly sensitive to changes in CO2/H+; an increase in CO2/H+ will cause vasodilation and increased blood flow, which in turn will facilitate removal of excess CO2/H+. Considering that CO2/H+-induced vasodilation would counter-regulate chemoreceptor activity, we hypothesize that CO2/H+-evoked ATP release from astrocytes will antagonize CO2/H+-vasodilation in the RTN, and thus prevent CO2/H+-induced washout and further enhance chemoreceptor function. Therefore the second goal of this study is to determine whether purinergic signaling in the RTN provides specialized control of vascular tone in a manner that directly enhances activity of chemosensitive neurons. Pharmacological evidence suggests that the mechanism by which RTN astrocytes sense CO2/H+ involves inhibition of Kir4.1-5.1 channels, therefore, to definitively test the role of Kir4.1 and astrocyte in respiratory control, we made an astrocyte specific inducible Kir4.1 knockout mouse model (Kir4.1 CKO). We found at the cellular level that astrocytes in slices from Kir4.1 CKO mice no longer express a CO2/H+-sensitive current or modulate chemosensitive neurons by a purinergic-dependent mechanism. At the whole animal level, we found that Kir4.1 CKO mice show a reduced ventilatory response to CO2. It is also well known that cerebral blood flow is highly sensitive to changes in CO2/H+; an increase in CO2/H+ will cause vasodilation and increased blood flow, which in turn will facilitate removal of excess CO2/H+. Considering that CO2/H+-induced vasodilation would counter-regulate chemoreceptor activity, we hypothesize that CO2/H+-evoked ATP release from astrocytes will antagonize CO2/H+-vasodilation in the RTN, and thus prevent CO2/H+-induced washout and further enhance chemoreceptor function. Therefore the second goal of this study is to determine whether purinergic signaling in the RTN provides specialized control of vascular tone in a manner that contributes to the drive to breathe. We show in vitro and in anesthetized rats that purinergic signaling in the RTN maintains vascular tone during high CO2/H+, and disruption of this mechanism by P2 receptor blockade with PPADS (100 µM) decreased the ventilatory response to CO2. Together, these results expand our understanding of how RTN astrocytes control breathing by showing that i) CO2/H+-evoked ATP release contributes to chemoreception by preventing CO2/H+-induced vasodilation; and ii) Kir4.1 is an important component of astrocyte chemoreception.

Estrogen actions in the brain and the basis for differential actions in men and women

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Estrogens have profound effects in the brains of all mammalian species studied, including humans. Classical investigations in the female of the species focused on central regulation of ovulation and reproductive behaviours, but mounting evidence demonstrates potent estrogenic influences on diverse brain functions, including learning, memory, mood and neurodegenerative processes. Consequently, recent years have seen an explosion in the scientific literature documenting the neurotrophic, neuroprotective and psychoprotective actions of estrogens, indicating important therapeutic potential for brain-active estrogenic compounds. The majority of these studies have been performed in females, where the ovaries are the main source of circulating estrogens. However, it is now recognised that that estrogens and estrogen receptors also play important roles in the physiological control of many tissues and organs, including the brain, of the male of the species. This understanding came with the realization that estrogens can be synthesized locally from steroid precursors, including circulating testosterone, by aromatase enzymes, which are found in neurons and glial cells of the brain. Furthermore, estrogen receptors are widely distributed in the brains of both sexes. It is, therefore, tempting to speculate that estrogen-based therapy holds potential for the treat-
ment of brain disorders that affect both males and females. Recent experimental investigations in males would, however, caution against extrapolation of research findings in one sex to another, because estrogens can have different, even opposite effects, as well as similar effects, in males compared with females, depending on the situation. For example, our own and other studies using rodent models of Parkinson’s disease (PD), demonstrate that ovarian factors, specifically estradiol, can protect females against the loss of dopamine in the striatum, which is pathognomonic of PD. This supports the view that the notable sex differences seen in both clinical and experimental PD, where females fare better than males, may be attributable to the female physiological levels of circulating estradiol. In contrast, in male rats we have demonstrated that physiological levels of estradiol are not protective, and may even exacerbate the loss of striatal dopamine in experimental PD. Sex dimorphisms have also been demonstrated for estrogen’s ability to influence synaptic plasticity, neurotransmission and cognition. Current evidence points to fundamental sex differences in the organization of brain circuitry in early development as a major factor that underpins sexually dimorphic responses of the brain, and the actions of estradiol in particular. It is now widely recognised that there are notable sex differences in the incidence and manifestations of virtually all brain disorders, including neurodegenerative diseases (PD, Alzheimer’s disease), addictive behaviours, anxiety and depression. A better understanding of the basis of sex differences in brain physiology and hormonal actions is, therefore, vital if we are to unravel the nature and origins of sex-specific pathological conditions and to optimise treatments for both women and men.


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Estrogen and the CF gender-gap

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Cystic fibrosis (CF) is the most frequent inherited disease in Caucasian populations and is due to a defect in the expression or activity of an anion channel encoded by the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Many studies have reported a difference in survival between males and females with CF, termed the “CF gender gap”, showing a decreased median survival of 10 years for women. The precise physiological and molecular mechanisms underlying the CF gender gap are not fully known and although it appears that a gender gap exists before girls reach puberty, recent studies have shown the prominent role of the most potent form of estrogen, 17β-estradiol (E2), in exacerbating lung function in CF females. Early cohort studies showed an association between female gender and an earlier median age of chronic lung infection with mucoid Pseudomonas aeruginosa (Psa.) (2). There also exist a stronger association between the age at first Pseudomonas aeruginosa infection and the severity of lung disease in females with CF compared to males (3). These studies suggest that E2 could have an effect on immunity and that has since been confirmed in numerous studies. Injecting CF mice with E2 before infection with Psa, increased the number of white blood cells in broncho-alveolar lavage and also increased the inflammatory infiltrate and mRNA of various inflammatory mediators. Chotirmall et al. have studied different aspects of the involvement of E2 in the modulation of infection and immunity. They have shown that the female hormone upregulates SLPI, an anti-protease with anti-inflammatory properties, leading to the inhibition of NK-κB, potentially compromising a protective inflammatory process. This group has also shown that E2 induced the transformation of Psa. from a non-mucoid to a mucoid form by inducing the production of alginate. This study also reported a correlation between the levels of plasma E2 and the severity of lung exacerbations in CF females (4). Estrogen can also regulate fluid and ion transport. Indeed, it has been shown in other organs that E2 can inhibit K+ channels involved in the regulation of epithelial repair, therefore, E2 could negatively regulate epithelial wound healing in CF lung, an organ strongly damaged by the cycles of recurring infections and inflammation processes. Moreover, papers from 1998 and 2011 reported that E2 increases Na+ absorption and ENaC activity in alveolar cells (5,6). Our study from 2013 confirmed the existence of the same effect in bronchial epithelial cells and showed that E2 impaired the airway surface liquid (ASL) dynamics by decreasing ASL height through an increase in ENaC and the Na+/K+ATPase activities. The hyperabsorption of Na+ leads to an increase in Na+ and water absorption through the bronchial epithelium. We demonstrated that the rapid effect of estrogen on ASL height and ENaC involved an extra-nuclear Estrogen Receptor α with similar responses generated by a non-nuclear estrogen dendrimer conjugate that allowed us to discriminate between genomic and non-genomic effects of E2 (7).

Finally, as survival increases, CF patients are more at risk to develop non-pulmonary CF-related diseases. Some recent studies showed, for example, that in patients with a severe genotype, there are more CF women with CF Related Diabetes than men and diabetes is linked to a decreased survival in CF females. It has also been demonstrated that female CF mice had reduced bone formation rate than their male counterparts. There is now strong evidence from epidemiology, molecular endocrinology and physiological studies that raised plasma levels of estrogen can exacerbate lung pathophysiology and function in CF females as well as affecting other organs. The molecular basis for the CF gender gap involves a compromised innate immune response, combined with impaired airway surface liquid dynamics arising from Na+ hyper-absorption and dehydration of the periciliary layer. In addition, estrogen acts directly on Psa. conversion to a virulent mucoid phenotype and biofilm production, completing a vicious cycle of compromised mucociliary clearance, infection and inflammation. Rosenfeld M, Davis R, FitzSimmons S, Pepe M, Ramsey B. Gender gap in cystic fibrosis mortality. Am J Epidemiol. 1997 May 1;145(9):794-803.


Sex differences in renal function
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Several clinical and experimental studies have demonstrated that there are clear sex differences in renal function and disease. Men have a greater risk of developing non-diabetic chronic kidney disease (CKD) and progressing to end-stage renal disease compared to women. However, this sexual dimorphism is not observed in patients with diabetic nephropathy. Experimental studies have shown a role for sex hormones, primarily androgens and estrogens, in the regulation of renal physiology and pathology. While it is generally thought that the beneficial effects of estrogens contribute to the relative protection of women against renal disease, it is also possible that the presence of androgens increases the risk of men to develop CKD.

Important modulators of renal function that have been shown to be influenced by sex include the renin-angiotensin system, the sympathetic nervous system, the endothelin system and nitric oxide, and the effects of sex hormones on these systems may be involved in the sex differences observed in progression of CKD. Understanding sex differences in renal function and progression of renal disease is critical for optimal treatment of CKD and understanding differences in the response to renal therapies and improving treatment options for both men and women with renal disease.

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Sex differences in hypertension: Immune mechanisms underlying resilience and susceptibility
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Introduction: Our previous studies demonstrated that T cells modulate the development of angiotensin II (Ang II)-stimulated hypertension in a sex-specific manner; male T cells augment the magnitude of hypertension while female T cells do not. After adoptive transfer of male T cells into male recombination-activating gene-1 null mice (Rag-1-/-M), the mean arterial pressure and the frequency of the prohypertensive CD3+ interleukin-17+ T cell subset was 20 mm Hg and 2.25X higher, respectively, than after adoptive transfer of female T cells. Approach: To determine if this sex-specific effect of T cells is due to intrinsic sex chromosome (XX vs XY) differences between male and female T cells or to sex differences in exposure to gonadal hormones, Rag-1-/-M mice were exposed to 800 rads (8 Gy) using a 137 Cs irradiator. Seven days prior to irradiation, Baytril (0.17 mg/mL) was added to the sterile drinking water of the recipient Rag-1-/-M mice and the mice were maintained on this antibiotic supplemented water for an additional two weeks after irradiation to prevent infections. Four to six hours after irradiation, the mice received (via retro-orbital injection) 5 x 10^7 (in 0.15 mL phosphate buffered saline) unfractionated bone marrow (BM) cells isolated from the femur and tibia of wild type male or female (WT-M or WT-F) mice. Radiotransmitters were implanted four weeks later and after a stable baseline was established, an osmotic minipump containing Ang II (490 ng/ml/min) was inserted followed by continuous measurement of mean arterial pressure for two weeks by telemetry. Results: We found no difference in the magnitude of hypertension induced by Ang II infusion between BM^gRag1^/-M (143±4.1 mm Hg; n=6) and BM^hRag1^/-M (149±4.7 mm Hg; n=6) mice or in the frequency of the CD3+ interleukin 17+ T cell subset [BM^gRag1^/-M, 2.36 ± 0.21% vs. BM^hRag1^/-M, 2.06 ± 0.51%; n=6/group]. Conclusions: Sex-specific T cell modulation of blood pressure is due to gonadal hormone effects on T cell expansion including the prohypertensive CD3+, interleukin 17+ T cell subset, rather than intrinsic sex chromosome differences between male and female T cells.

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Antibiotic- and diet-induced effects on the gut microbiota-brain axis
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Accumulating evidence indicates that disruption of the gut microbial community (dysbiosis) impairs mental health. Causality in gut microbiota-brain relationships has been probed by the use of germ-free mice and antibiotic-induced gut dysbiosis. However, both models have limitations, as the blood-brain barrier is impaired and brain ultrastructure and neurochemistry are altered in germ-free mice, while antibiotics may directly interfere with brain function. To address the concerns related to antibiotic-induced gut dysbiosis, the effect of intragastric treatment of adult mice with multiple antibiotics on gut microbial community structure, metabolite profile in the colon, circulating metabolites, expression of neuronal signalling molecules in distinct brain areas and cognitive behaviour was investigated. 16S rDNA sequencing confirmed antibiotic-induced microbial community disruption, and metabolomics disclosed that gut dysbiosis was accompanied by depletion of bacteria-derived metabolites in the colon and alterations of lipid species and converted microbe-derived molecules in the plasma. Novel object recognition, but not spatial, memory was impaired in antibiotic-treated mice. This cognitive decline was associated with brain region-specific changes in the expression of brain-derived neurotrophic factor, N-methyl-D-aspartate receptor subunit 2B, serotonin transporter and neuropeptide Y system. Pharmacokinetic analyses ruled out that these molecular and behavioural alter-
The intestinal tract is a multifaceted environment where commensal bacteria both influence and are affected by the intestinal epithelium and underlying gut immune cells and the enteric nervous system. Recent studies have implicated gut microbes in having a role in brain development as well as in cognitive function and stress and anxiety-related behaviours. Gut microbes can influence the gut-microbial-brain axis via a number of pathways, including through the central nervous system, via the innate and adaptive immune system, and by modulating the hypothalamic-pituitary-adrenal axis (HPA). A major form of communication between gut microbes and the host comes from the production of a large number of metabolites and neuroactive compounds through microbial metabolic activity. For example, complex carbohydrates can be fermented in the colon by bacteria to produce short-chain fatty acids including butyrate, acetate, and propionate; these compounds are essential for gut health and they can also enter the blood and act in the brain through specific receptors. Certain microbes can generate neurotransmitters and neuro-modulators, such as gamma-aminobutyric acid (GABA), nor-epinephrine, dopamine, acetylcholine, and serotonin. While ~90% of human serotonin is synthesized in enteric enterochromaffin cells in the gut and some strains of commensal bacteria produce serotonin, this neurotransmitter cannot cross the blood-brain barrier. However, its precursor, tryptophan can enter the brain. Tryptophan is an essential amino acid that is obtained from the diet. Tryptophan is metabolized by tryptophan hydroxylase to serotonin in the brain and the intestinal tract, or alternatively by indoleamine 2,3-dioxygenase to form kynurenine. Alterations in metabolic pathways related to tryptophan conversion can be induced by diet and result in alterations in tryptophan/serotonin ratios in the brain. The types of metabolic products that are produced by microbes are dependent upon the substrate available; thus, altering diet can have significant effects on gut microbial production of specific metabolites. Western-style diets that are high in fat and sugar and low in dietary fiber have been shown to have significant effects on stress- and anxiety-related behaviours, as well as altering cognitive function. These types of diets are associated with specific alterations in gut microbes and microbial function, including increases in Proteobacteria and reductions in production of short chain fatty acids. Another interesting finding is that the type of diet consumed by the host can significantly alter the ability of probiotics to modulate host physiological function, suggesting that production of certain metabolites may be critical for probiotic efficacy. Overall, it is clear that diet and commensal microbes interact at several different levels to alter host physiology through both direct and indirect mechanisms.

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mice infected with EPEC as neonates, and sham-infected controls, with behavior analyzed using software (Noldus EthoVision XT). Intestinal physiology (short circuit current [Isc] and conductance [G]) was characterized by Ussing chambers, and the composition of the microbiota was determined by qPCR using well-characterized primer sets. Data is provided as the mean ± SE and p values calculated by Student T-test. Adult mice neonatally infected with EPEC showed impaired cognition (Exploration ratio [%]: 75.2±4.9 Sham vs. 62.3±2.6 EPEC; n=12-13, p<0.05), without evidence of anxiety-like behavior (Time spent in light box [s]: 295±27 Sham vs. 275±29 EPEC; n=13-15) compared to sham-infected controls. Intestinal physiology was altered, with increased secretory state (Isc [µA/cm²]: 38.4±5.3 Sham vs. 57.8±4.4 EPEC; n=4-6; p<0.05) and permeability (G [mS/cm²]: 3.2±0.3 Sham vs. 4.4±0.4 EPEC; n=4-6; p<0.05) as well as long-lasting intestinal dysbiosis (including decreased levels [% of Eubacteria] of Lactobacillus and increased levels of Enterobacteriaceae and Bacteroides; n=6-8, p<0.05). In conclusion, our data demonstrates that neonatal bacterial infection leads to alterations of the developing MGB axis that persist into adulthood.

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SA093

Early life clinical dose penicillin exposure induces long-term effects on gut microbiota, brain inflammation and behavior which are partially restored by beneficial microbe administration

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Antibiotics (AB), and more particularly penicillins, are the most frequently dispensed drugs in pediatric patients (1) and there is currently increasing concern that AB exposure early in life may have long-term consequences for health. Epidemiological studies have revealed that early life AB exposure increases the risk of diseases that may persist into adulthood such as allergies, inflammatory bowel diseases and obesity (2). Experimental models have shown that the critical period for antibiotic-driven shift in gut microbiota to alter immune and metabolic responses occurs between birth and weaning (3,4). The effects of AB on brain and behavior have been demonstrated in previous studies (5–7) by using high doses of cocktails of mainly broad-spectrum antibiotics administered to adolescent or adult rodents. In this study, we have investigated the long-term effects on gut microbiota, brain and behavior of a clinically relevant dose of oral penicillin given early in life, to both male and female Balb/c mice. Pregnant dams received penicillin V in drinking water 1 week before delivery and until weaning. Penicillin is absorbed by the gastro-intestinal tract, crosses the placenta and is found in the breast milk. The pups therefore received penicillin in utero and during the first 3 weeks of life while nursing. At weaning, pups were separated from their mothers and received regular drinking water. At 6-weeks old, the offspring (n = 72) were subjected to a battery of behavioral tests and gut (ileum, colon) and brain (hippocampus, frontal cortex) tissues were collected after the last test and processed for qPCR and western-blot analysis. Feces were collected at 3-weeks old and 6-weeks old. We found that early life AB exposure had lasting effects on gut microbiota composition, modified the tight junctions of the blood-brain barrier, induced inflammation in the frontal cortex and was associated with changes in brain neurochemistry (Crhr2, Bdnf, Avpr1b). Also, AB-treated mice exhibited decreased anxiety-like behavior, reduced social behavior and preference for social novelty as well as an unexpected aggressive behavior. Supplementation with Lactobacillus rhamnosus JB-1TM during AB treatment restored certain biological and behavioral parameters. This study revealed that clinical dose penicillin given early in life to mice had long-term effects on behavior, brain inflammation and gut microbiota, and may raise concerns about the long-term behavioral consequences of AB therapy.


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SA094

Acidic extracellular pH reduces contractile function of human collecting lymphatic vessels

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One overlooked aspect of the cardiovascular system’s reaction to acid-base disturbances is how lymphatic vessels respond to a metabolic challenge. Blood vessels are well known to
vasodilate in acidic conditions through various cellular mechanisms. Whether extracellular pH similarly influences lymphatic vessels is particularly relevant given that the lymph they transport can originate from tumour environments and areas of infection and inflammation where interstitial pCO₂, [H⁺], and [HCO₃⁻] can significantly deviate from systemic levels. We aimed to establish whether extracellular acidification reduces the contractile activity of human collecting lymphatic vessels. Human lymphatic vessels sourced from surgical patients (obtained after informed consent from each patient prior to surgery) were investigated. The use of human collecting vessels was approved by the ethical committee for the Danish Regional Health Authority and the study was conducted in accordance with the standards set by the Helsinki Declaration. Segments of thoracic duct (diameter) or intestinal lymphatics (diameter) were mounted in wire or pressure myographs for isometric force or isobaric diameter recording. The extracellular pH of the physiological salt solution immersing the vessels was maintained at different levels by altering the composition of CO₂ and HCO₃⁻ in the solutions; control levels (pH 7.4, 22 mM HCO₃⁻, 5% CO₂) were compared with varying grades of metabolic acidosis (pH 7.1 and 6.8 obtained by lowering (HCO₃⁻)) and respiratory acidoses (pH 7.1 obtained by increasing pCO₂). Spontaneous and agonist-induced contractile behaviour (in response to noradrenaline and serotonin) were investigated in parallel under all four conditions. Paired comparison between pH 7.4 conditions and acidosis demonstrated that lowering extracellular pH inhibited phasic contractile activity of the thoracic duct whereas agonist-stimulated contractility was generally more robust. The isometrically-assessed contractility was attenuated when pH was lowered: at pH 6.8, all spontaneous phasic activity ceased, noradrenaline- and serotonin-stimulated tonic constriction was lowered by ~50% and agonist-stimulated phasic activity was highly abrogated. Agonist-induced tonic contractile responses were minimally affected during moderate acidosis (metabolic and respiratory acidosis pH 7.1) whereas phasic contractile activity was substantially lowered. In the absence of agonists, pressurized thoracic duct segments displayed myogenic tone at transmural pressures of 7 and 20 mmHg. At both pressure levels, metabolic acidosis pH 6.8 significantly lowered myogenic tone (spontaneous and noradrenaline-stimulated) and phasic activity. We propose that phasic contractile activity of lymphatic vessel – which is considered the primary mechanisms for lymph propulsion – is attenuated by pathophysiologically realistic degrees of acidosis.

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SA096

Regulation of substrate metabolism by mitochondrial matrix thioesterases

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Mitochondrial β-oxidation is crucial for maintaining cellular health when lipid supply to the cell is high. Yet, there is evidence that links organ pathology to mitochondrial fatty acid oxidation, per se. The associated pathologies range from insulin resistance and poor recovery from ischemia-reperfusion episodes in various organs, to the development of renal fibrosis. Often, studies into the mechanistic bases of such pathologies operate under a paradigm of “insufficient” or “excessive” FAO as a causative factor. However, “classic” studies, the literature on genetic β-oxidation disorders and the recent use of sophisticated proteomics platforms to detect post-translational modifications all suggest that the abundance of fatty acyl-CoA can be regulated within the mitochondrial matrix. In terms of mechanisms, pyruvate dehydrogenase kinases, enabling entry into the Krebs cycle of β-oxidation-derived acetyl-CoA, were identified in the earlier literature. Several years then passed before interest was re-invigorated with genetic mouse studies on the mitochondrial sirtuins (protein deacylases) and on carnitine-o-acyltransferase (CrtA7; converts short-chain acyl-CoAs into carnitine esters). Thus how β-oxidation, particularly acyl- and acetyl-CoA abundance, is regulated is again emerging as an important mechanism to mitigate potentially deleterious effects of high lipid supply. Yet, the full complement of matrix mechanisms that undertake this regulation, and whether and how they interact, are major questions. We have identified a new mechanism that regulates acyl-CoA abundance in the mitochondrial matrix: Acot2. The existence in the matrix of acyl-CoA thioesterases (Acot) that hydrolyze acyl-CoA esters into an acyl chain and free CoA has been known for many years, and these Acots have been cloned. However, their biological role, especially in highly aerobic tissues such as the skeletal muscle, has remained

SA095

Metabolic flux as a mediator of fatty liver disease

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In addition to respiration, liver mitochondria possess high capacity anaplerotic and cataplerotic pathways that supply substrates for gluconeogenesis and other biosynthetic activities. Fluxes through these biosynthetic pathways are increased in insulin resistant liver and, hence, through the mitochondrial pathways that support them. The energetics associated with poorly controlled gluconeogenesis, for example, place a concomitant pressure on hepatic energy demand. During nonalcoholic fatty liver disease (NAFLD) this hepatic workload may impinge on compromised mitochondria, incite oxidative stress and inflammatory processes that reinforce insulin resistance. In this lecture, I will present in vivo stable isotope tracer data and explore how nutritional state, cell signaling and metabolic mechanisms regulate the anabolic workload of the liver. NAFLD results in the loss of normal metabolic responsiveness of hepatic oxidative flux, causing reduced flux in fed mice but elevated anaplerotic and oxidative flux in the fasted state. Conditional loss of hepatic insulin action also caused an upregulation of oxidative flux, while activation of mTORC1 recapitulated the effects of NAFLD by eliminating the normal metabolic flexibility of the TCA cycle. Preventing the upregulation of anaplerotic/catablerotic and oxidative flux in obese mice prevented oxidative stress and inflammation in liver despite not reducing liver fat. Thus, metabolic flux through certain mitochondrial pathways in liver may contribute to the pathological effects of NAFLD.

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unexplored. Moreover, given the existence of CrAT, a role for Acots in β-oxidation regulation might seem redundant. Yet our initial studies using a new mouse model of the matrix enzyme, Acot2, clearly support the hypothesis that Acot2 regulates entry of long-chain fatty acyl-CoA into β-oxidation, or siphons it off from β-oxidation. The major substrates for Acot2 are C14:0-, C16:0- and C16:1-CoA. In this way, Acot2 could function as a control point for β-oxidation, a concept that would expand how we think about β-oxidation regulation. The associated phenotypes in the Acot2 depleted mice consuming a standard chow diet generally point to a beneficial metabolic role for Acot2 in muscle and liver. This suggests that a matrix-resident mechanism that limits long-chain fatty acyl-CoA abundance and/or ensures CoA availability in the mitochondrial matrix is protective.

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SA097

Peroxisomes in skeletal muscle protect against mitochondrial dysfunction and insulin resistance

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Excess intramuscular lipids are thought to play a causal role in the pathogenesis of insulin resistance; thus, strategies aimed at reducing this lipid burden offer therapeutic potential. Peroxisomes provide an intriguing option to meet this goal as they have the ability to process a broad spectrum of lipid species. As such, the purpose of this study was to determine if skeletal muscle peroxisomes protect against lipid-induced insulin resistance. To test this hypothesis, we developed a muscle-specific peroxisome-deficient mouse model by deleting Pex5 in skeletal muscle (Pex5m-/-) and weaned them onto a moderate fat (25%) diet to which they had ad libitum access for 20 weeks. Pex5m-/- mice had similar body weight and composition when compared to Pex5fl/fl littermate controls, but exhibited impaired glucose tolerance. This impairment in whole body glucose homeostasis was associated with a 25-30% decrease in mitochondrial function in Pex5m-/- mice, suggesting that peroxisomes help protect against mitochondrial dysfunction in skeletal muscle. To further test this potential link between peroxisomes and mitochondria in skeletal muscle we analyzed peroxisomal adaptations in a murine model where mitochondrial lipid overload.

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SA098

Molecular bases of function and drug modulation of α7 nicotinic receptors: Implications for drug discovery

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The homopentameric α7 nicotinic receptor (nAChR) is one of the most abundant nAChRs in the nervous system and it is also expressed in many non-neuronal cells. It is involved in a range of neurological, psychiatric and inflammatory disorders and is emerging as a novel drug target. We have combined site-directed mutagenesis and cell expression with single-channel kinetic analysis to delineate molecular mechanisms and structures underlying α7 activation and drug modulation. Enhancement of α7 function by positive allosteric modulators (PAMs) is a promising therapeutic strategy to improve cognitive deficits. PAMs have been classified only on the basis of their macroscopic effects as type I, which only enhance agonist-induced currents, and type II, which also decrease desensitization. To decipher the molecular basis underlying these distinct activities, we explored the effects of representative members of each type on single-channel currents. In the absence of PAMs, single-α7 channels appear as very brief and isolated opening events. Both types of PAMs enhance open-channel lifetime and produce episodes of successive openings, thus indicating that the two affect α7 kinetics. Both PAM types require different structural determinants for their allosteric action and show different sensitivity to temperature, suggesting different mechanisms of potentiation. Due to its homeric nature, α7 contains five identical agonist binding sites. We developed a strategy that allowed us to determine the number of ACh occupied sites required for activation from the amplitude of each individual single-channel opening. The results revealed that ACh occupancy of only one of α7 five agonist binding sites allows activation and that open-channel lifetime of a single-occupied receptor is indistinguishable from that of receptors containing five intact binding sites. The unique ability to elicit a full biological response with a single-occupied site adapts α7 to volume transmission, a prevalent mechanism of ACh-mediated
signaling in the nervous system and non-neuronal cells. α7 is also present in immune cells and it is emerging as an important drug target for inflammation. We found that Natural Killer Cells express functional α7 nAChRs that trigger calcium mobilization. Activation of α7 decreases NF-kB levels and nuclear mobilization, leading to marked anti-inflammatory effects which are evidenced by decreased cell-mediated cytotoxicity and IFN-γ production. Thus, α7 in these cells may constitute a novel target for regulation of the immune response. Deciphering the molecular basis underlying α7 responses has implications for the design of novel and more specific therapeutic compounds.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Pentameric ligand-gated ion channels functioning at the atomic resolution
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Pentameric channel-receptors, including nicotinic acetylcholine, glycine and GABAA receptors, play a key role in fast excitatory and inhibitory transmission in the nervous system and are the target of numerous therapeutic and addictive drugs. They carry several neurotransmitter binding sites which govern the opening of a transmembrane ion channel. Extensively expressed in animals, they were found in several bacteria, especially the homolog from the cyanobacteria <i>Gloeobacter violaceus</i> (GLIC) which functions as a proton-gated ion channel. The simplified architecture of this archaic homologue, as well as its prokaryotic origin, allowed solving its X-ray structure in two closed and one open conformation. Those static structures suggest that channel opening occurs through symmetrical quaternary twist and "blooming" motions, together with tertiary deformation, according to a global transition that couples channel opening with reorganization of the binding pockets for neurotransmitters and allosteric effectors. To investigate the dynamics of the proton, we further engineered multiple fluorescent reporters, each incorporating a bimane and a tryptophan/tyrosine, whose close contact causes fluorescence quenching. We show that proton application causes a global compaction of the extracellular subunit interface, coupled to an outward motion of the M2-M3 loop near the channel gate, and that these movements are highly conserved in lipid vesicles and detergent micelles. Real-time recordings show that most structural reorganizations are completed within 2ms, much faster than channel opening. Our work thus identifies and structurally characterizes a new pre-active intermediate state in the transition pathway towards activation. Altogether, these combined structural and functional data give insights into the allosteric mechanisms operating in these integral membrane proteins, and pave the way for the rational design of new classes of allosteric modulators.


Towards studying ligand binding and channel gating in nicotinic acetylcholine receptors using confocal patch-clamp fluorometry
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Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels mediating neurotransmission in the peripheral and central nervous system as well as at the neuromuscular junction. Much is known about the gating behavior of these channel proteins, however, the reciprocal relationship between activation state and agonist binding is still elusive. It has been shown that the confocal patch-clamp fluorometry (cPCF) (Biskup et al., 2007) is a suitable technique to study activation-state dependent agonist binding by combining the electrophysiological patch-clamp technique and confocal microscopy employing fluorescently tagged agonist molecules. Herein, we adapted this technique for whole-cell recordings with HEK293 cells to study adult muscle-type nAChRs. First, we synthesized a fluorescently tagged acetylcholine derivative by linking the dye Cy3 to the acetylcholine moiety via a short polyethylene glycol linker. The resulting derivative Cy3-ACh was found to be a highly efficient \( I_{\text{max,Cy3-ACh}}/ I_{\text{max,ACh}} = 0.97 \pm 0.01 \) (n=3; values are means ± S.E.M.) and highly potent agonist. The apparent affinity, \( E_{50} \), and the Hill coefficient, \( H \), both derived by fitting the Hill equation to averaged relative peak currents \( I/I_{\text{max}} \) (n=3 to 8), were 0.99 µM and 1.8, respectively. Fast concentration jumps were realized by a piezo-driven double-barreled application pipette. Because desensitization of cells attached to the chamber bottom was slowed in comparison to the desensitization of cells lifted up after whole-cell configuration was established (means ± S.E.M.: 186±21.3 ms; n=5 vs. 85.4±12.8 ms; n=5; p<0.05 t-test), we concluded that solution exchange is faster and more defined in lifted cells. Confocal fluorescence imaging was realized with a confocal laser scanning microscope (LSM710, Zeiss, Germany). Cy3-ACh was excited with a 543 nm HeNe laser line. To define the position of the lifted cell for confocal imaging, the background was stained with a reference dye, Dy647, excited with a 633 nm HeNe laser line. We confirmed that Dy647 did neither bind to the nAChRs nor to the plasma membrane. Regarding unspecific fluorescence signals due to the patch-pipette, we found unpolished borosilicate glass preferable compared to quartz glass: After application of 10 µM Cy3-ACh, unspecific fluorescence of quartz pipettes was more than 3 times higher compared to borosilicate pipettes (4.9±1.3 a.u. in quartz; n=4 vs. 1.3±0.3 a.u. in borosilicate; n=3; p<0.01 t-test). After application of Cy3-ACh to nAChRs expressing HEK293 cells a clear membrane staining was visible, which was not found in non-expressing control cells. The binding was reversible and the agonist could be washed off completely after 13 seconds. Repeated application of Cy3-ACh to the same cell resulted in similar fluorescence intensities. Herein, we showed that cPCF can be applied to directly relate agonist binding to different activation states of nAChRs. It can be concluded that confocal PCF is a flexible approach to study ion channels and ionotropic receptors not only in excised membrane patches but also in whole mammalian culture cells, expanding the scope of application significantly.

Investigating the role of arginine residues in agonist recognition with unnatural amino acids

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The pentameric ligand-gated ion channel (pLGIC) family can be loosely divided into excitatory cation-selective channels and inhibitory anion-selective channels. Agonists bind to the interface of principal and complementary subunits, where excitatory and inhibitory agonists alike rely on an amine moiety being coordinated by an aromatic box within the principal face. In contrast to the excitatory pLGIC agonists acetylcholine and serotonin, however, the inhibitory pLGIC agonists GABA, glutamate and glycine also possess a carboxylate moiety. Recognition of this carboxylate is facilitated by a positively charged arginine residue that is highly conserved in Loop D of inhibitory pLGICs and whose mutation to alanine greatly impairs agonist recognition. Scant, if any, evidence exists as to why arginine is preferred over other amino acids, at this position, and we therefore sought to establish if the positive charge of this side chain or other electrostatic interactions are required for recognition of the agonist carboxylate. As conventional mutagenesis is insufficient for dissecting the requirements of agonist recognition on chemical properties of the arginine side chain, we replaced this Loop D arginine with isosterics, isoelectric analogues in glutamate-gated chloride channels (GluCls) and glycine receptors (GlyRs) expressed in Xenopus laevis oocytes. Expression of these mutants was achieved by co-injecting oocytes with mutant channel cRNA containing the amber stop codon and analogues ligated to tRNA containing the appropriate anticodon. Agonist-gated currents were then measured with two-electrode voltage clamp. In GluCls, the presence of uncharged arginine analogues at the Loop D position was far less detrimental to glutamate sensitivity than substitution with conventional amino acids such as lysine and alanine. This suggests that although the positive charge of arginine may make some contribution to the recognition of carboxylate agonists, it must be other electrostatic interactions that make arginine so well-suited to this role in agonist recognition.

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SA102

The nuclear receptor FXR decreases murine enteroendocrine L cell response to gut microbiota metabolites, the short chain fatty acids

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Background and Aims: Diabetes mellitus involves many metabolic disorders including a decrease in incretin effect. One of the incretin hormones, Glucagon-Like Peptide-1 (GLP-1) is produced and secreted by enteroendocrine L cells which represent 1% of the intestinal epithelial cells. We have recently shown that bile acid nuclear receptor Farnesoid X Receptor (FXR) activation in enteroendocrine L cells decreases glucose-induced ChREBP-dependant proglucagon gene transcription. By inhibiting glycolysis pathway, FXR also decreases glucose-induced GLP-1 secretion (Tabelsi et al., 2015). The aim of this study is to investigate the role of FXR in the L cell response to other GLP-1 secretagogues and especially to short chain fatty acids (SCFA). SCFA, acetate, propionate and butyrate are metabolites produced by the gut microbiota by fermentation of non digestible polysaccharides (Tremaroli & Bäckhed, 2012). Indeed, in addition to their contribution of 5 to 10% of the daily energetic resources, SCFA are also signalling molecules as they bind to the transmembrane receptor GPR43/FFAR2, thereby promoting GLP-1 secretion by L cells (Psichas et al., 2015 ; Wichmann et al., 2013 ; Tolhurst et al., 2012).

Materials and Methods: FXR was activated in vitro in the murine cell line GLUTag and in vivo in C57Bl6/j mice by the synthetic agonist GW4064. GLP-1 secretion tests (ELISA) in response to butyrate, one of the SCFA, were performed in vitro in GLUTag cells and ex vivo on murine colonic explants. GPR43 mRNA levels were evaluated by qPCR in GLUTag cells incubated with GW4064, in colon from mice treated with GW4064, KO FXR mice and treated 3 weeks with colesvelam, a bile acid sequestrant, which display a drastic down regulation of FXR transcriptional activity.

Results: FXR activation decreases GLP-1 secretion in response to butyrate both in vitro in GLUTag cells and ex vivo in mouse colonic explants from C57Bl6/j mice treated 5 days by the synthetic FXR agonist GW4064. In parallel, FXR activation in vitro and in vivo decreases GPR43 mRNA levels. As a mirror effect, FXR KO mice and colesvelam treated mice exhibit an increased GPR43 mRNA level in colon.

Conclusion: FXR activation decreases GPR43 expression and L cell capacity to respond to SCFA in terms of GLP-1 secretion. Disregulation of FXR in intestine seems to be a good way to increase L cell capacity to respond to glucose and to gut microbiota metabolites and thus to improves metabolic control.


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Neuromodulation of the carotid bodies to treat diabetes

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Dysmetabolic features like insulin resistance, dyslipidemia and obesity are characterized by autonomic dysregulation. Recently, a new line of research linking autonomic dysfunction and metabolic diseases has emerged with the report that the carotid bodies (CBs) are involved in the development of insulin resistance (1,2). The CBs are arterial chemoreceptors that sense changes in arterial blood O2, CO2, and pH levels. Apart from the control of ventilation, the CB has been confirmed to sense glucose, being implicated in the control of energy homeostasis. We have recently described that CB activity is increased in rodent models of insulin resistance. Additionally, we have shown that selective bilateral resection in these parameters throughout time.

To test the impact of continuous reversible electrical block of the CB on insulin action and glucose homeostasis, we have implanted electrode cuffs at the CB after 14 weeks of high-frequency stimulation during 9 weeks and metabolic parameters have been evaluated. The reversibility of blocking was investigated during 5 weeks after stop high frequency stimulation. At the end of the experimental protocol all the animals have been sacrificed by an overdose of pentobarbitone.

We demonstrated that CSN denervation re-established insulin sensitivity, normoglycemia and normoinsulinemia, glucose tolerance, autonomic function and normalized mean arterial pressure in animal models of prediabetes and early type 2 diabetes. Additionally, high frequency stimulation electrical block of the CSN, increased insulin action and restored glucose tolerance in HFHSu rats during the 9 weeks of electrical block. The effect of electrical blocking on the CSN was reversible, since 5 weeks after stop blocking the animals developed again insulin resistance and glucose intolerance.

We conclude that modulation of CB positively impacts on insulin action and glucose tolerance and that electrical modulation of CSN may represent a novel therapeutic approach for diabetes.


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Carotid body ablation abrogates hypertension induced by intermittent hypoxia mimicking sleep apnoea

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Chronic intermittent hypoxia (CIH), the main feature of obstructive sleep apnoea syndrome (OSA), enhances carotid body (CB) chemosensory activity, produces autonomic dysfunction characterized by higher sympathetic outflow, induces cardiac arrhythmogenesis and promote hypertension. We tested whether autonomic alterations, high prevalence of cardiac arrhythmias and the progression of hypertension induced by CIH are critically dependent on functional CB afferent chemoreflex drive. All experiments were approved by the Bioethical Committee of the Universidad Autónoma de Chile and the P. Universidad Católica de Chile, Santiago, Chile.

We studied the changes in cardiac autonomic imbalance, cardiac baroreflex gain and arrhythmia score during the experiments. Finally, we measured extracellular matrix (ECM) remodelling in cardiac atrial tissue. Compared to Sham rats, CIH rats displayed hypertension (109.8±2.3 mmHg, CIH vs. Sham, respectively, P<0.01), heart rate variability shifts towards higher sympathetic tone (Low Freq HRV, 60.6±3.4 n.u. vs. 49.6±3.3 n.u., CIH vs. Sham, respectively, P<0.05), reduced baroreflex gain (4.5±0.94 bpm mmHg⁻¹ vs. 14.7±1.11 bpm mmHg⁻¹, CIH vs. Sham, respectively, P<0.01), increased arrhythmias (184.0±22.4 events/h vs. 101.8±25.7 events/h, CIH vs. Sham respectively, P<0.01) and a 1.5 fold increase in atrial collagen content. Remarkably, rats exposed to CIH but that underwent CBA exhibit a marked and significant reduction in BP (pre vs. post CBA, 135.5±2.2 vs. 124.2±2.1, P<0.01), reduced low freqHRV (pre vs. post CBA, 60.6±3.4 n.u. vs. 43.9±3.5 n.u., P<0.05) and reset the midpoint operating point of the cardiac baroreflex (pre vs. post CBA, 111.5±5.6 mmHg vs. 102.1±1.65 mmHg, P<0.05). In addition, CBA reduced the number of arrhythmias by 60% in CIH rats. Interestingly, CBA failed to restored cardiac ECM remodelling. Our results show that autonomic alterations induced by CIH are critically dependent on the CB and support a main role for the CB in the CIH-induced hypertension. In addition, our results suggest that reductions in arrhythmia incidence during CIH were related to normalization of cardiac autonomic balance and not to cardiac tissue fibrosis.

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Molecular pathways contributing to carotid body dysfunction in heart failure: Insights for therapy

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Sympathetic neural overactivity is a major contributor to the progressive decline in cardio-renal function that occurs in chronic heart failure (CHF). In addition, breathing dysregulation is often associated with CHF, which in turn likely aggravates the autonomic imbalance. Maladaptive tonic activation of the carotid body (CB) chemoreflex contributes to this sympathetic overactivity and breathing instability. CB ablation reduces chronic sympathetic outflow and improves breathing stability, renal and cardiac function, and survival in CHF animals, confirming a dysregulatory role of the CB in CHF. The tonic increase in CB afferent activity and chemoreflex drive that contributes to its maladaptive role in CHF is likely driven by a number of neuro-humoral and hemodynamic factors that are altered in CHF. There is a marked change in redox state in the CB of CHF animals (rats, rabbits) toward elevated reactive oxygen species (ROS) production. This is mediated, in part, by an upregulation of NOX2 superoxide production driven by increased angiotensin II and other possible factors also known to be elevated in CHF such as endothelin-1 and inflammatory cytokines. The elevated oxidative burden is further aggravated by downregulation of antioxidant systems such as SOD1 and SOD2 in the CB. The increased ROS inhibits hyperpolarizing K⁺ channels in CB glomus cells to enhance afferent excitability. Conversely, nitric oxide (NO) plays an important role in tempering CB glomus cells excitability by facilitating activation of voltage gated K⁺ channels, NO production in the CB is markedly suppressed in CHF due to downregulation of both nNOS and eNOS in CB cells. These changes appear to be driven by a chronic reduction in blood flow to the CB. A chronic reduction in blood flow to the CB, resembling that seen in CHF, recapitulates the molecular and functional changes in the CB that occur in CHF animals. To explore the molecular mechanisms responsible for these effects, we assessed the role of a flow-mediated transcription factor Krüppel-like Factor 2 (KLF2) in the CB of CHF animals. KLF2 plays an important role in the suppression of angiotensin converting enzyme and activation of nitric oxide synthase expression, and KLF2 is downregulated in the CB of CHF animals. Adenoviral transfection of KLF2 to the CB in CHF rabbits markedly reduces resting renal sympathetic nerve activity (RSNA) and CB chemoreflex sensitivity, and normalizes breathing stability and autonomic indices. In addition KLF2 knockdown (siRNA) in the CB of normal rabbits increases CB chemoreflex sensitivity, resting RSNA, heart rate, oscillatory breathing, cardiac autonomic imbalance, and arrhythmia incidence, similar to the sequelae of events that occur in CHF. The translational implications of these studies are discussed in relation to potential therapies for CHF.

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**PCA001**

**Vagal tone and exercise capacity**

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**Background:** Higher baroreflex sensitivity, enhanced high frequency component of heart rate variability and a fast heart rate recovery with cessation of exercise in elite athletes suggests plasticity in the central nervous mechanisms controlling the heart. This experimental study was designed to directly test the hypothesis that the strength of vagal tone determines exercise capacity. We hypothesised that vagal withdrawal or recruitment should respectively reduce or enhance exercise capacity. We targeted preganglionic neurones of the dorsal motor nucleus of the vagus nerve (DVMN) that functionally innervate the left cardiac ventricle.¹,²

**Methods:** In male Sprague-Dawley rats (380-420 g), DVMN neurones were transduced to express an inhibitory Gi-protein-coupled Drosophila allatostatin receptor (AlstR) (n=8) or control green fluorescent protein (GFP) (n=8). Application of the insect peptide ligand allatostatin (5 μl) produces rapid, selective inhibition of targeted neurones. A pharmacological study investigated the role of muscarinic and neuronal nitric oxide-mediated mechanisms using systemic treatment with atropine methyl nitrate (2 mg/kg, i.p., n=5) or selective neuronal NO synthase inhibitor 7-nitroindazole (7-NI) (30 mg/kg, i.p., n=8). For optogenetic activation, DVMN neurones were targeted to express an optogenetic construct ChIEF (n=9) or control transgene GFP (n=10) and stimulated with blue laser light (445 nm, 10 ms pulses, 15 Hz, 15 min). Exercise capacity was determined using a treadmill with a shock grid set at the minimum of 0.1 mA. Rats were preselected for their compliance after a three day recruitment protocol and randomized. The experimental protocol involved starting speeds of 20-30 cm/s over 5 min after 15 min acclimatisation. Speeds were then raised in 5 cm/s increments every 5 min until the hind limbs made grid contact four times within a 2 min period. The calculated work (Joules, J) was used as an index of exercise capacity.

**Results:** Acute inhibition of the DVMN neurones by allatostatin resulted in a dramatic reduction in exercise capacity (8±2 vs 202±27 J; p=0.0001; ANOVA). In rats given atropine and vehicle no significant differences in exercise capacity were observed (113±20 vs 112±22 J; p=0.9; t-test). Systemic administration of 7-NI was associated with a significant reduction in exercise capacity (33±19 vs 129±19 J, p=0.0002; t-test), as did 4 h of atropine treatment (63±12 vs 116±20 J, p=0.0019; t-test). Rats expressing ChIEF by the DVMN neurones displayed a significantly higher exercise capacity 4 days following optogenetic stimulation (94±11 vs 47±6 J; p=0.002; ANOVA). Improvements were similar to that observed in naive rats trained to exhaustion over the same period (105±16 vs 47±6 J in rats expressing GFP; p<0.0001; ANOVA).

**Conclusion:** These results suggest that the strength of parasympathetic tone determines exercise capacity.


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**PCA002**

**Selective optogenetic recruitment of c-fibre vagal efferents is sufficient to preserve left ventricular function in a rat model**

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**Introduction:** Vagus nerve stimulation has been shown to reduce the extent of myocardial infarction (MI) and slow the progression of left ventricular (LV) dysfunction in animal models of heart failure. The precise mechanisms underlying the beneficial effect of vagus nerve stimulation in heart failure are poorly understood. The Vagus is complex, containing both sensory and motor fibres, which transmit information to and from many viscera. It remains unclear whether the potential benefit of vagus nerve stimulation is due to the recruitment of afferent (sensory) or efferent (motor) fibres by stimuli delivered via implantable stimulators. In this study we targeted preganglionic neurones of the dorsal motor nucleus of the vagus nerve (DVMN), established to functionally innervate the heart,¹,² to express light-sensitive optogenetic proteins and determined the effect of optical stimulation of vagal C-fibre efferents on LV function in a rat model of MI-induced heart failure.

**Methods and Results:** Male Sprague-Dawley rats, DVMN neurones were targeted using viral vectors to express either the light sensitive protein - Channelrhodopsin variant ChIEF or a control transgene (eGFP). Four weeks later, animals underwent permanent left anterior descending coronary artery ligation or sham surgery. Blue light stimulation (445 nm, 10 ms pulses, 15 Hz) of the transduced neurones via a pre-implanted optode was performed under mild sedation (1% isoflurane) for 15 min every 48 hours for 4 weeks commencing 2 days after the surgery. High-resolution Doppler recordings of mitral flow and LV pressure measurements were taken to assess LV function. Development of MI-induced LV dysfunction in this model was found to be associated with marked reduction of ejection fraction (33±3 vs 50± 5 % in sham-operated animals expressing eGFP; p=0.01); E/A ratio (0.99±0.09 vs 1.20±0.10 in sham-operated animals animals expressing eGFP; p=0.04) and LV deceleration slope (3580±350 vs 2470±290 mm/s/s in sham-operated animals animals expressing eGFP; p=0.02). Optogenetic stimulation of vagal preganglionic neurones expressing ChIEF resulted in significant improvements in ejection fraction (49±3 vs 33±3 % in post-MI rats expressing eGFP; p=0.005), E/A ratio (1.2±0.1 vs 0.9±0.1; p=0.03) and deceleration slope (-2670±260 vs -3580±350 mm/s/s; p=0.04). Complementary improvements were also recorded in the maximum and minimum differentials of LV pressure.

**Conclusion:** Using optogenetics for highly selective recruitment of vagal efferent projections from the DVMN and high-resolution ultrasound for sensitive measurements of LV function, we demonstrate that vagal C-fibre efferents exert trophic effects on LV function that can be exploited to slow the progression of heart failure developing after MI.


MB PhD funding for A.M was provided by The Rosetrees trust

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**PCA003**

A study on the role of magnesium in experimental hyperthyroidism. Effects on the cardiac response to ischemia-reperfusion injury

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Hyperthyroidism is associated with various effects on the myocardium that could potentially render the heart less tolerant to ischemic stress. The present study was designed to determine the possible role of magnesium in modulating the myocardial response to ischemia-reperfusion (I/R) injury in hyperthyroid rats.

Male Wistar rats were divided into three groups: control group, hyperthyroid group: hyperthyroidism was induced by daily intraperitoneal (i.p.) injection of L-thyroxine (T4: 300 microg/kg) for 14 days and magnesium-treated hyperthyroid group: hyperthyroidism was induced by daily i.p. injections of T4 (300 microg/kg) and magnesium sulfate (50 mg/kg) for 14 days. Isolated rat hearts were perfused in a Langendorff apparatus then subjected to 30 min global ischemia followed by 30 min reperfusion. Isolated hearts were studied for basal intrinsic activity as well as their responses to ischemia-reperfusion. The activity of lactate dehydrogenase (LDH) in the coronary effluent, level of malondialdehyde (MDA) in heart tissue and cardiac weights were determined. Plasma levels of magnesium and MDA were also measured.

Hyperthyroidism was associated with reduced plasma magnesium level. Hyperthyroid rats exhibited fast contractility and diastolic relaxation. During reperfusion, hyperthyroid hearts showed depressed recovery of contractile performance, lusitropic activity and myocardial flow, greater LDH release and cardiac MDA together with development of cardiac hypertrophy and increased plasma MDA. Magnesium administration reduced postischemic myocardial dysfunction and provided functional improvement as evidenced by the enhanced recovery of contractile performance, lusitropic activity and myocardial flow, diminished LDH release, cardiac oxidative stress and plasma MDA as well as prevention of cardiac hypertrophy.

It is concluded that magnesium diminution could contribute in loss of myocardial tolerance to I/R stress in vitro and enhanced oxidative injury in hyperthyroidism. The improvement of functional recovery of postischemic hearts together with reduction of oxidative stress by magnesium supplementation suggests favorable protective effects of magnesium against myocardium I/R injury in the hyperthyroid state, that may be mediated possibly through its antioxidant action.

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**PCA004**

Cardiomyocyte STIM1 is a key regulator of Ca2+-dependent kinase activity in the mouse heart

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The ER/SR Ca2+ sensor, stromal interaction molecule 1 (STIM1) is a major regulator of store-operated Ca2+ entry (SOCE), in non-excitable cells. In the adult rodent heart STIM1 has been shown to be essential in the progression of pathological hypertrophy (2); however, the physiological role of STIM1 in the heart is not well understood. We have shown that mice lacking cardiomyocyte STIM1 (cSTIM1-KO) develop ER stress, mitochondrial abnormalities, and dilated cardiomyopathy (1). However, the specific signaling pathways regulated by STIM1 in the heart remain unknown. Therefore, we used a discovery-based kinomics approach to identify kinases that were differentially regulated by STIM1. 12-week male control and cSTIM1-KO mice were injected with saline or phenylephrine (PE, 15 mg/Kg; s.c.), that activates SOCE in cardiomyocytes. 15 min following treatment mice were sacrificed and hearts processed for kinomic analysis. 2 μg of whole cell lysates were prepared and loaded on to a Ser/Thr kinase chip. Primary analysis was performed in BioNavigator 6.0 (PamGene) and subsequent analysis using theKinexus PhosphoNET database and GeneGo MetaCore. Downstream confirmation was performed using standard immunoblotting techniques. In parallel experiments standard electrocardiogram analysis was performed +/- PE under 2% isoflurane anesthesia. Pathway analysis of the kinomic array revealed significantly lower Protein Kinase C (PKC) and PKG signaling in the hearts of the KO in comparison to control hearts at baseline. Immunoblotting confirmed that activation of Ca2+-dependent PKCα at pThr497 (1.00 ± 0.03 vs 0.75 ± 0.06, p<0.05) and one if its downstream targets MARCKS at pSer158/162 were lower in cSTIM1-KO hearts. Similar reductions in KO hearts were found for several additional kinases such as MEK1/2 at pSer217/221, AMPK at pThr172 (1.00 ± 0.04 vs 0.57 ± 0.09, p<0.05) and PDK1 at pSer241 (1.00 ± 0.06 vs 0.75 ± 0.03, p<0.05). PKC and PKG regulate cardiac contractility via the regulation of voltage-dependent ion channels. Additional analysis of the kinomic data identified several genes including those encoding the L-type Ca2+ channel, Cav1.2, the delayed rectifier potassium channels K+ channels, Kv1.2 and Kv1.6, all as potential target downstream peptide sequences of the identified kinases. In support of the potential impact of these changes on cardiac contractility, electrocardiogram analysis revealed that KO have significantly lower HR (461.7 ± 0.00 vs 428.7 ± 1.98 bpm, p<0.05) and prolonged QT interval (23.2 ± 0.00 vs 47.6 ± 0.00 ms, p<0.05) in comparison to control mice. In conclusion we have shown for the first time that activation of Ca2+-dependent kinases, such as PKC and PKG, are regulated by STIM1 in the adult mouse heart. This has important implications in understanding how STIM1 contributes to the regulation of normal cardiac physiology.


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Silencing HMGB1 expression inhibits the heart failure induced by adriamycin in AMPK via TLR4 dependent manner through MAPK signal transduction

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Introduction: Adriamycin (ADR) an anticancer drug widely used for cancer treatment has adverse effects on many tissues, like heart, kidney. However, the most negative effect its is seen in heart tissue. It is not still completely understood the mechanism of ADR-induced heart failure. ADR’s toxic effect is related to reactive oxygen species (ROS), mitochondrial membrane potential depolarization and defect of energy production (1-3). Eventually, all of those mechanisms may trigger/exacerbate to heart failure due to initiated apoptosis. High mobility group box 1 (HMGB1) is a chromatin protein highly conservative among the species. It has a connection between cell’s survival and death pathways (4). In studies, not only can HMGB1 be released under stress condition, but also under a pathologic condition in the cell. HMGB1 can trigger the apoptosis (5). Until now, there has been no any report refers the interaction between ADR and HMGB1 and AMP-activated kinase (AMPK) in the same experimental study. The aim of the study was to investigate whether ADR-induced heart failure mediates HMGB1 to initiate the apoptosis through (AMPK-α1) by TLR4 or not. Methods: In the study, H9c2 cell line has used the study. It was created four groups as a control, HMGB1 inhibition, ADR, ADR+HMGB1 inhibition. HMGB1 inhibition was performed by using specific small interfering RNA (siRNA, 10 nM). ADR was used at 2 µM concentration for 36 and 48 hours. HMGB1 inhibition and ADR were performed cotreatment. Western blot and qRT-PCR identify protein and genes expressions related to apoptosis. Apoptosis is determined by using TUNEL and FITC-IETD-FMK methods. Results: ERK1/2 gene expressions were down by ADR, although P38 gene expression was high by ADR for 36 and 48 hours treatment. AMPK had a lower expression by ADR treatment as well. ADR+HMGB1 inhibition caused to low expression of ERK1/2, AMPK but not P38 gene expression. Although ADR gave rise to decrease AMPK, P-AMPK, ERK1/2, PERK1/2, P38, JNK protein expressions, increase caspase-3 protein expression, ADR+HMGB1 inhibition led to increasing AMPK, P-AMPK, ERK1/2, PERK1/2, P38, JNK and decrease caspase-3 protein expression. The number of TUNEL positive and active caspase eight cells at ADR group was higher than control and HMGB1 inhibition groups (p<0.01). However, the number of TUNEL positive and active caspase-8 cells at ADR+HMGB1 was lower vs. ADR group (p<0.01). Conclusion: HMGB1 plays an important role in amplifying on ADR toxicity on the heart by TLR4 via MAPK signal transduction. The project was financially supported by Scientific and Technological Research Council of Turkey (114S118).

Keywords: Adriamycin, HMGB1, AMPK, TLR4, apoptosis, heart muscle cell

The TUNEL positive cell number at 36 hours incubation time. A1, A2: Control; B1,B2: HMGB1 inhibition; C1, C2: ADR group; D1,D2: ADR+HMGB1 inhibition


The project was financially supported by Scientific and Technological Research Council of Turkey (114S118).

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PCA006

Lower omega 3 index as a marker for increased propensity of hypertensive rats to malignant arrhythmias

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Background: Reports, including ours, indicate that lower omega-3 (ω-3) index accompanied by cardiac extracellular and myocardial connexin-43 remodelling and enhanced autoantibody production to the adrenergic beta-1 receptors (b1-AAB) are implicated in development of heart failure and increased incidence of lethal arrhythmias. Based on these results we aimed to explore the effect of ω-3FA supplementation on ω-3 index, b1-AAB, matrix metalloproteinases (MMP), connexin 43 and susceptibility to arrhythmias in aged male (♂) and female (♀) spontaneously hypertensive rats (SHR).

Methods: 1 year-old SHR and age-matched healthy Wistar rats (WR) fed with ω-3FA (Vesteralens, Norway, EPA+DHA 200mg/day) were compared with untreated rats. The hearts from the anaesthetized rats by Narkețan (100 mg/kg) and Xylapan (10 mg/kg) were rapidly excised into ice-cold saline to arrest heart beat and the weight of the heart and left ventricle (LV) was registered. LV tissue was taken for examination of MMP-2 activity using zymography; Cx43 expression and its cellular distribution using Western blot and immunohistochemistry. Susceptibility to electrically-induced ventricular fibrillation (VF) was tested using Langendorff-perfused heart. Gas chromatography was used for determination of red blood cells ω-3FA and ω-6FA composition. Blood serum was used for the detection of b1-AAB.

Results: Compared to healthy WR ω-3 index was lower in both ♂ and ♀ SHR. This parameter was significantly increased due to ω-3 FA intake in both sexes of SHR. ♂ and ♀ SHR also exhibited a significant increase of serum levels of b1-AAB, activity of MMP2, down-regulation and miss-localisation of Cx43. It was associated with higher incidence of VF. ω-3FA intake resulted in significant decrease of b1-AAB levels and MMP2 activity, upregulation of Cx43 and partial elimination of Cx43 miss-localisation in both ♂ and ♀ SHR. It was associated with decreased incidence of electrically-induced VF.

Conclusions: These findings suggest multiple cardio-protective effects of omega-3 intake that can contribute to decreased susceptibility of the hypertensive rat to lethal arrhythmias. This work was supported by VEGA 2/0167/15, 2/0076/16 and APVV2/0348/12 grants, SKS grant

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PCA007

Is AMPK required for acute acclimation to hypoxia?

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During hypoxia ventilatory adjustments are critical to the maintenance of oxygen (O2) delivery during sleep or ascent to altitude (1). We have recently demonstrated that the AMP-activated protein kinase (AMPK), a ubiquitously expressed metabolic sensor (2), is required for the hypoxic ventilatory response (HVR). Briefly, in mice with targeted deletion of the catalytic AMPK-α1 and -α2 subunits in tyrosine hydroxylase (TH)-expressing cells, including the O2-sensing carotid body type I cells and catecholaminergic neurons of the brainstem, the HVR was attenuated and apnoea-duration index augmented during hypoxia (3). Here we report on further investigations into the impact of AMPK deletion on the HVR. AMPK-α1-α2 double knockout (AMPK dKO, n=6) and control (AMPK-α1-α2 floxed, n=8) animals were placed in an unrestrained whole body plethysmography chamber, and changes in breathing frequency (breaths/min), tidal volume (ml/g) and minute ventilation (Mv, ml/min/g) monitored during exposure to mild (12% O2) or severe (8% O2) hypoxia for 10min; all results are reported as mean±SEM. At 8% O2, control mice exhibited acute increases in Mv (21.2±9% after 1min, relative to normoxic breathing (21% O2)) followed by respiratory depression that returned Mv to normoxic levels (±5% at 5min; ±4.8±5.5% at 10min), as reported previously (4). By contrast, throughout the period of hypoxia AMPK dKO mice exhibited severe and persistent hypventilation (Mv = -8.6±6% at 1min, -33.3±6% at 5min; -29±4.5% at 10min) relative to normoxia. A ventilatory defect in AMPK dKO mice was also observed upon exposure to mild hypoxia (control versus (vs) knockout: 42.8±8% vs 16.5±6.7% at 1min, 21.6±9.7% vs -1.4±8.4% at 5min; 20.3±11.3% vs -4.4±7.7% at 10min). During 8% O2 apnoeas (complete cessations of ventilatory effort >0.6 sec) of controls displayed a clear time-dependent reduction of frequency (min: 1: 3.4±0.7 at 2-3min; 1.4±0.4 at 5-6min; 0.6±0.1 at 8-9min), which was absent or markedly attenuated in AMPK dKO mice (4±0.7 at 2-3min; 3.5±0.7 at 5-6min; 2.9±0.7 at 8-9min). This time-dependent reduction in apnoea frequency was not observed for control or knockout mice upon exposure to mild hypoxia (12% O2), where apnoea frequency was comparable between controls and knockout mice (min: 1: 0.8±0.3 vs 1.0±0.3 at 2-3min; 0.5±0.2 vs 1.2±0.4 at 5-6min; 1±0.4 vs 0.8±0.3 at 8-9min). Most intriguingly, these measures of apnoea frequency are equivalent in magnitude to that which control mice exhibited after 10min acute acclimation to severe hypoxia (8% O2). We conclude that upon exposure to severe, but not mild, hypoxia mice engage a compensatory mechanism that reduces apnoea frequency in a manner dependent on AMPK expression. This adds further weight to our proposal (3) that modulators of AMPK activity or expression could ameliorate sleep disordered breathing associated with metabolic syndrome-related disorders or ascent to altitude.


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Adequate oxygen uptake in exercise requires low pulmonary flow heterogeneity

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Under resting conditions, the amount of atmospheric oxygen necessary to satisfy tissue needs is readily acquired, and pulmonary oxygen uptake remains adequate even in the face of increased pulmonary flow heterogeneity and/or decreased diffusing capacity. This high degree of reserve in the healthy lung is nevertheless sometimes exhausted under extreme conditions such as in critical illness. Under conditions of exercise, however, oxygen transport and utilization are contingent upon adequate lung function with relatively little reserve. The purpose of this study is to investigate the impact of pulmonary flow heterogeneity on oxygen delivery in exercise.

A theoretical model of pulmonary oxygen uptake is used to simulate varying degrees of heterogeneity as characterized by the coefficient of variation (CV) of pulmonary capillary blood flow, assuming prescribed tissue oxygen utilization. Uniform alveolar ventilation is assumed, and flow heterogeneity is therefore equivalent to ventilation-perfusion matching. Under resting conditions (245 ml O₂/min), the observed level of arterial oxygen tension is consistent with a high degree of perfusion heterogeneity (CV = 3.0). When moderate or severe exercise conditions with values of cardiac output and arteriovenous oxygen content obtained from the literature [Roca J et al. (1989) AJP 67:291] are considered, it is found that this degree of heterogeneity would lead to an inability to maintain arterial PO₂ and sustain levels of oxygen demand corresponding to experimental measurements. For conditions of moderate exercise (2750 ml O₂/min corresponding to 60% VO₂(max)) and extreme exercise (4460 ml O₂/min corresponding to VO₂(max)), and with normal values of lung diffusing capacity, the model implies that the CV has to be much lower, in the range of 0.5 to 1, to maintain adequate tissue oxygen supply and to support the observed arteriovenous saturation differences (ΔS = 0.68 and 0.75 respectively).

In conclusion, this model shows that although a substantial degree of pulmonary flow heterogeneity does not significantly impair oxygen uptake at rest, in exercise a much lower degree of heterogeneity can be tolerated in order to achieve sufficient pulmonary oxygen uptake to prevent tissue hypoxia. The principal mechanism for minimizing heterogeneity in ventilation-perfusion matching is local regulation of pulmonary blood flow incorporating mechanisms such as hypoxic pulmonary vasoconstriction (HPV), which would be active at the low venous oxygen levels found in exercise. Our results strongly suggest that effective HPV is needed to achieve adequate oxygen transport under conditions of increased oxygen demand in exercise.

Supported by NIH grant HL070657.

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Moderate intensity exercise improves heart rate variability in obese adults with type 2 diabetes

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Several populations based studies show that regular physical activity is an important component of a healthy lifestyle and lack of activity is a predictor of cardiovascular mortality. Physical inactivity is closely related to cardiovascular disease and a widening variety of other chronic diseases, including type 2 diabetes, obesity and hypertension. Many studies have suggested beneficial effects of regular exercise in preventing sudden cardiac death in healthy individuals and in patients with cardiovascular diseases. Exercise therapy has been shown to improve autonomic nervous system modulation of heart rate variability (HRV) in healthy individuals. Therefore, exercise training may improve cardiac autonomic regulation in a variety of clinical populations including obese adults with type 2 diabetes. Thus, the main aim of this study was to determine the effect of thrice-a-week, six months, moderate aerobic exercise on cardiac autonomic function as measured by HRV in obese adults with type 2 diabetes. 41 obese adults with type 2 diabetes volunteers were involved in this study. Anthropometric and metabolic variables were measured, and resting electrocardiogram (ECG) for the HRV analysis at spontaneous respiration was recorded for 5 min in supine position before and after six months of supervised aerobic training given thrice-a-week. The mean age, body mass index (BMI), and duration of diabetes of the study population were 44.1 ± 4.5 years, 30.94 ± 1.36 kg/m², and 16.3 ± 2.7 years, respectively. In time domain variables, standard deviation of all RR intervals (SDNN), the square root of the mean of the sum of the squares of differences between adjacent RR intervals (RMSSD) and percentage of consecutive RR intervals that differ by more than 50 ms (pNN50) were significantly increased after exercise. In frequency domain variables, high frequency (HF) (ms2) and HF (nu) were significantly increased while low frequency (LF) (ms2) were significantly decreased after exercise. But LF (nu) and LF/HF ratio were unaffected after exercise. These data suggest that thrice-a-week moderate intensity aerobic exercise for six months improves cardiac rhythm regulation as measured by HRV in obese adults with type 2 diabetes.

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Lung expression of free fatty acid receptor 2 is upregulated in a murine model of asthma

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¹AstraZeneca, Mölndal, Sweden and ²MRC Toxicology Unit, Leicester, UK

Asthma is a serious inflammatory condition of the lungs which affects 5.4 million people in the UK. Asthma patients have an altered lung microbiota (Huang and Boushey, 2014). Free fatty acid receptor 2 (FFAR2) is a G protein-coupled-receptor...
responding to short chain fatty acids, metabolites of commensal bacteria. This study aims to characterize the effect of a murine model of asthma on FFA2 expression levels in the lungs.

An asthma-like condition was induced in C57/B16NCR1 mice by inoculation with house dust mite (HDM) protein. Briefly, mice were anaesthetized with isoflurane, and a 20 µL drop of HDM or saline applied to the nose. Mice were treated in this manner for 10 days, then terminated at 1, 2, 5 and 9 days post treatment. These studies were performed at AstraZeneca, Mölndal, with local ethical approval. After termination, the lungs were lavaged with PBS and cells in the bronchoalveolar lavage fluid (BALF) counted. The colon and lungs were removed for RNA extraction, and Ffar2 expression analysis with SYBR green qPCR. Data are presented as means ±SEM.

HDM inoculation triggered an inflammatory response in C57 mice, with an increase of eosinophils, neutrophils and total lymphocytes in BALF. Cell numbers were highest after 24 hours, and decreased over time (table 1). Levels of FFA2 message in the lungs followed the same pattern (table 2), while FFA2 in the colon did not change after HDM treatment. Statistical tests were 2-way ANOVA and Student’s unpaired t-test, as appropriate.

This study demonstrates that FFA2 is expressed in healthy lung tissue, and that expression is dramatically increased during HDM challenge. It is likely that the FFA2 receptor is present on the white blood cells, as opposed to smooth muscle or epithelium. As a control, another tissue well known to express FFA2, the colon, did not show any increase in FFA2 expression during asthmatic symptoms. To further this study, the microbiota of both gut and colon will be sequenced, and the FDM protocol repeated in Ffar2-/- mice to see if this inhibits its white blood cell infiltration.

Table 1. Mean cell counts (10^3 cells) of white blood cells in HDM-treated mice (n=8).

<table>
<thead>
<tr>
<th>Termination Day</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64.9 ± 28.9</td>
<td>344.7 ± 27.9</td>
<td>291.6 ± 19.6</td>
</tr>
<tr>
<td>2</td>
<td>60.5 ± 27.4</td>
<td>442.0 ± 25.6</td>
<td>226.0 ± 32.7</td>
</tr>
<tr>
<td>5</td>
<td>271.1 ± 62.2</td>
<td>193.3 ± 58.4</td>
<td>126.1 ± 12.2</td>
</tr>
<tr>
<td>9</td>
<td>34.4 ± 3.3</td>
<td>20.8 ± 3.3</td>
<td>91.5 ± 9.1</td>
</tr>
</tbody>
</table>

Table 2. Expression of Ffar2 relative to β-actin (2^-ΔCt) in HDM-treated mice (n=8).

<table>
<thead>
<tr>
<th>Termination Day</th>
<th>Lung</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.51 ± 0.268</td>
<td>0.1 ± 0.005</td>
</tr>
<tr>
<td>2</td>
<td>0.07* ± 0.0005</td>
<td>not measured</td>
</tr>
<tr>
<td>5</td>
<td>0.054 ± 0.002</td>
<td>**</td>
</tr>
<tr>
<td>9</td>
<td>0.005 ± 0.0002</td>
<td>**</td>
</tr>
</tbody>
</table>


Funding was from the BBSRC and AstraZeneca

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**PCA011**

**Why do athletes have heart block? Long-term endurance exercise causes electrophysiological remodelling of the atrioventricular node**

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Background: Veteran endurance athletes are prone to cardiac arrhythmias such as bradycardia and atrioventricular (AV) block, resulting in increased incidence of pacemaker implantation. Previously we reported that training-induced bradycardia is due to diffuse remodelling of pacemaking ion channels in the sinoatrial node(1). Here we examined the electrophysiology and ion channel profile of the AV node (AVN) in response to chronic endurance exercise.

Methods: 10 week old C57BL/6j mice were trained by swimming for 60 min/day, 5 days/week for 5 months and compared to control sedentary mice. At the end of the training period, animals were anaesthetised with 2% isoflurane and ventilated following which a thoracotomy was used for rapid atrial pacing with an octopolar electrode catheter. Mice were then killed by cervical dislocation under terminal anaesthesia and AVN preparations rapidly dissected and frozen. Total RNA was isolated from serial 20 µm sections of the AVN and right atrium (RA), identified by HCN4 immunolabelling and collected by laser capture microdissection. Preamplified CDNA was loaded onto TaqMan Low Density Array cards and the expression levels of 96 transcripts including key mediators of the membrane and Ca^{2+} clock mechanisms of pacemaking were measured by qPCR.

Results: Trained mice were bradycardic and had a prolonged PR interval (Trained, 44.0 ± 1.0 ms; Control, 40.0 ± 1.0 ms; P<0.05, n=12-14). In trained mice, in vivo programmed electrical stimulation protocols revealed a significantly increased Wenckebach cycle length (Trained, 99.6 ± 1.4 ms; Control, 85.5 ± 2.1 ms; P<0.001, n=11-14) and prolonged AV node effective refractory period (Trained, 80.1 ± 2.6 ms; Control, 62.6 ± 2.8 ms; P<0.05, n=11-13) suggesting altered conduction and electrophysiological remodelling of the AVN. 43.3% of the genes studied were downregulated in the AVN of trained mice (P<0.05). The trained AVN presented with lower expression of HCN4 (responsible for funny current, I_{fun}), RYR2 (SR Ca^{2+} release channel), NCX1 (Na–Ca^{2+} exchanger) and Cav3.1 and Cav3.2 (responsible for T-type Ca^{2+} current, I_{Ca,T}). Reduced expression levels of gap junction channels (Cx30.2, Cx40, Cx43) and inflammatory and fibrosis genes (interleukin 1β, NFkB, TGFβ1, Tnfr, collagen type 1 and 3, fibronectin 1, vimentin) were also observed. These changes might be explained by training-induced alterations in key transcription factors that can exert transcriptional control (Irx3, Klf4, Mef2c, Nlx2.5, Tbx3, Shox2). Only <8.9% of genes were significantly affected in the RA.

Conclusions: Chronic endurance exercise results in AVN dysfunction underscored by striking transcriptional remodelling of AVN genes responsible for action potential generation and propagation. Data provide a new paradigm for understanding the occurrence of heart block in veteran athletes.


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61P
Complexity of the human sinoatrial node: A computational investigation

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The sinoatrial node (SAN) is the heart’s pacemaker. The human SAN anatomy is complex. There is accumulating evidence that the mammalian cardiac anatomy consists of an electrical insulating border between the SAN and surrounding atrium [1]. The insulating border provides discrete SAN exit pathways that permit electrical coupling between the SAN and the atrial muscle. In addition, another anatomical feature that our laboratory have uncovered is a secondary pacemaker, named the paranodal area, in close proximity of the SAN [2]. The effects of these anatomical features on SAN electrical function have not been studied. Using our 3D anatomical model of the human SAN, we examined the significance of the anatomy in cardiac electrical wave dynamics.

A functional 3D model of the human SAN was constructed using the detailed anatomy from our previous study [2]. The model consists of a SAN primary pacemaker surrounded by an insulating border. The insulating border provides discrete exit pathways to permit electrical coupling between the SAN and atrial muscle. The paranodal area is a column extending along the length of, but not in contact with, the SAN. Each tissue type was assigned excitation properties using validated variants of the Fenton-Karma cell model [3]. Parameter gradients within the SAN ensure that the leading pacemaker location is located at the centre of the SAN during physiological heart beats. Simulation experiments were performed using this dynamic model. In each experiment, the cases with and without the exit pathways as well as the cases with and without the paranodal area were simulated to permit comparison between various anatomical constrictions. Several simulation experiments involved re-entrant wave dynamics. To initiate the re-entry, the phase distribution method that we developed previously was exploited [4]. A spectrum of codes were implemented to permit data analysis.

Our results show that cell-cell coupling gradients regulate the leading pacemaker location. The complex excitation initiation and propagation in the SAN region as observed experimentally [5] was possible by inclusion of the insulating border and could not be reproduced without the insulating border and exit pathways. When the SAN was made inactive, the paranodal area was capable of pacing the atrial muscle at a slower rate. The existence of an insulating border and discrete exit pathways promoted re-entrant wave breakup, and the arrhythmia breakup was aggravated by the presence of the spatially extended paranodal area. In addition, the paranodal and SAN oscillators may influence each others pacemaking propensity that may promote arrhythmia.

It was seen that the experimentally observed erratic activation of the SAN region could be reproduced in our simulations that had discrete exit pathways and paranodal area. Multiple re-entry based mechanisms could explain clinical atrial tachycardia.

Efimov et al. Circ Res. 2010; 106: 255-271

Cofirst authors: SN&AD

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but ECG changes are not, in medically managed CAD patients. Severity of CAD is the most important factor affecting the development of collaterals.


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PCA014

Low-level vagus nerve stimulation protects against ventricular arrhythmias in the isolated innervated rabbit heart

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Motivation/problem solving statement: Vagus nerve stimulation (VNS) is being trialed clinically to treat patient with heart failure. 1 In preclinical studies, our group has shown that VNS protects the heart against ventricular arrhythmias by increasing ventricular fibrillation threshold (VFT) and prolonging effective refractory period (ERP) in an isolated innervated rabbit heart preparation. 2 This protection however, is accompanied by a large bradycardia, which would not be advisable in patients. To reduce unwanted excessive vagal-bradycardia, VNS at low levels (LLVS) has been tested to suppress atrial fibrillation in patients. To reduce unwanted excessive vagal-bradycardia, which would not be advisable

Table 1. HR, ERP, and VFT changes during High Voltage – Low Frequency LLVSs

<table>
<thead>
<tr>
<th>Condition</th>
<th>Hz</th>
<th>ERP</th>
<th>VFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>110</td>
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</tr>
<tr>
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<td>20 Hz</td>
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</tr>
<tr>
<td>30 Hz</td>
<td>110</td>
<td>110</td>
<td>52</td>
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</table>

Conclusions: LLVSs displayed arrhythmogenic protection using both methods and shows potential to protect the heart against ventricular fibrillation and bradycardiac levels that should be safe in patients.

Table 2. HR, ERP, and VFT changes during Low Voltage – High Frequency LLVSs

<table>
<thead>
<tr>
<th>Condition</th>
<th>Hz</th>
<th>ERP</th>
<th>VFT</th>
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<tbody>
<tr>
<td>Control</td>
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<td>30 Hz</td>
<td>110</td>
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Circadian rhythm in QT interval is preserved in mice deficient of potassium channel-interacting protein 2

L.A. Gottlieb, A.F. Lubberding, A. Larsen and M.B. Thomsen

Department of Biomedical Sciences, University of Copenhagen, Copenhagen N, Denmark

Background: Sudden cardiac death in heart failure patients occurs predominately in the morning hours. Delayed ventricular repolarization, measured as prolonged QT interval on the ECG, heralds increased susceptibility to sudden cardiac death. Potassium Channel Interacting Protein 2 (KChIP2) is a subunit of the K+ channel Kv4.3 responsible for conducting the fast transient outward current, (Ito), important for ventricular repolarization. KChIP2 is suggested to be responsible for the circadian rhythm in repolarization duration, ventricular arrhythmias and sudden cardiac death.

Objective: We made the hypothesis that there is no circadian rhythm in QT interval in the absence of KChIP2.

Methods: Implanted telemetric devices recorded ECG continuously for 5 days in conscious male C57Bl6 wild-type mice (WT, n=9) and KChIP2-/- mice (n=9) in light:dark periods and in complete darkness. QT intervals were determined from complexes of the K+ channel Kv4.3 responsible for conducting the fast transient outward current, (Ito), important for ventricular repolarization. KChIP2 is suggested to be responsible for the circadian rhythm in repolarization duration, ventricular arrhythmias and sudden cardiac death.

Results: RR intervals are 125±5 ms in WT and 123±4 ms in KChIP2-/- (p=0.81). QT intervals are determined from complexes of the K+ channel Kv4.3 responsible for conducting the fast transient outward current, (Ito), important for ventricular repolarization. KChIP2 is suggested to be responsible for the circadian rhythm in repolarization duration, ventricular arrhythmias and sudden cardiac death.

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Methods: Implanted telemetric devices recorded ECG continuously for 5 days in conscious male C57Bl6 wild-type mice (WT, n=9) and KChIP2-/- mice (n=9) in light:dark periods and in complete darkness. QT intervals were determined from complexes of the K+ channel Kv4.3 responsible for conducting the fast transient outward current, (Ito), important for ventricular repolarization. KChIP2 is suggested to be responsible for the circadian rhythm in repolarization duration, ventricular arrhythmias and sudden cardiac death.

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(p=0.96; Figure panel A). Circadian rhythms in QT_{100} intervals are present in both groups, but at small amplitudes: 1.6±0.2 and 1.0±0.3 ms in WT and KChIP2\(^{-/-}\), respectively (p=0.15; Figure panel B). A diurnal rhythm in QT_{100} intervals was only found in WT mice. On the other hand, QT_{mean-RR} intervals in both groups display clear diurnal and circadian rhythm, where the amplitude of the latter is 4.0±0.3 and 3.1±0.5 ms in WT and KChIP2\(^{-/-}\), respectively (p=0.16; Figure panel C).

Conclusion: In the present study, we falsify our hypothesis and conclude in contrast to previous findings that KChIP2 expression cannot underlie the circadian rhythm in repolarization, because mice deficient of KChIP2 have a preserved circadian rhythm in QT interval. The molecular link governing circadian rhythm in sudden cardiac death remains therefore unknown.


Martino TA, Young ME. Influence of the cardiomyocyte circadian clock on cardiac physiology and pathophysiology. J Biol Rhythms Jun 2015:30:183-205


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA016

Tbx3 controls the pacemaker function of the adult sinoatrial node via Ca\(^{2+}\) clock


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In the adult, in various conditions (e.g. myocardial infarction, heart failure and pulmonary hypertension) there is sinoatrial node (SAN) dysfunction as well as changes in the expression of Tbx3. Tbx3 is known to play an important role in the embryonic development of the SAN, but the role in the adult is not known. Here, we explore the effects of Tbx3 upregulation in the adult SAN. Tbx3 was upregulated in the SAN by crossing CAG-CAT-Tbx3 transgenic mice with heterozygous HCN4-kit transgenic mice: this generates double-transgenic mice conditionally expressing Tbx3 in the SAN. Tbx3 upregulation was induced by intraperitoneal injection of tamoxifen (Sigma T5648; 40 mg/kg) for 3 days into adult males. The mice were kept for 3 weeks after the last injection. Animal procedures were undertaken in strict accordance with the United Kingdom Animals (Scientific Procedures) Act 1986. Data are presented as means±SEM & statistical differences assessed by Student’s t test, one – way ANOVA or two - way ANOVA as appropriate. Differences were considered significant if P<0.05. Quantitative PCR revealed an upregulation of Tbx3 mRNA in the SAN as expected. The ECG measured in the conscious and anaesthetised animal showed significant sinus tachycardia (increase in heart rate of 85±29 and 120±43 beats/min, n=10). In isolated SAN preparations, beating rate was significantly shorter in Tbx3 upregulated mice by 7.5%. SAN pacemaking is determined by the membrane and Ca\(^{2+}\) clocks. The membrane clock may not be responsible for the sinus tachycardia: transcripts of three key ion channels (all involved in the membrane clock) were investigated - HCN1 and Na\(^{+}\)v1.5 were unchanged, whereas HCN4 was significantly downregulated by 45%. However, the Ca\(^{2+}\) clock may be responsible: transcripts of three key Ca\(^{2+}\) clock components were investigated - RYR2 and NCX1 were unchanged, whereas SERCA2 was significantly upregulated by 152%. We investigated transcripts for a number of transcription factors known to play an important role in the SAN. Mef2c and Tbx18 were unchanged, whereas HCN4 was significantly downregulated by 45%. However, the Ca\(^{2+}\) clock may be responsible: transcripts of three key Ca\(^{2+}\) clock components were investigated - RYR2 and NCX1 were unchanged, whereas SERCA2 was significantly upregulated by 152%. We investigated transcripts for a number of transcription factors known to play an important role in the SAN. Mef2c and Tbx18 were unchanged, whereas Rest1 (represses HCN4 expression) was significantly upregulated by 26%, Pitx2 was significantly upregulated by 87% and Nfat4 was significantly upregulated by 730%. A potential Tbx3 binding site on the promoter region of Nfat4 was identified by in silico analysis. Nfat4 is known to upregulate SERCA2 (Prasad and Inesi, 2011). In conclusion, Tbx3 is able to control SAN pacemaking in the adult possibly by controlling SERCA2 expression via Nfat4.

Caveolin-3 KO recapitulates changes in I_{Ca} density and distribution observed in ventricular myocytes following transverse aortic constriction in mice

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In cardiac myocytes, the scaffolding protein caveolin-3 (Cav-3) plays an important role in determining membrane structure and in localizing the function of key proteins, including the L-type Ca channel, and thereby L-type calcium current (I_{Ca}) to the t-tubules. Acute inhibition of Cav-3 binding to endogenous proteins decreases I_{Ca} density preferentially at the t-tubules resulting in more uniform distribution of I_{Ca} between the t-tubule and surface membranes. Heart failure is associated with decreased Cav-3 levels (1) and redistribution of ICa away from t-tubules (2). We have, therefore, compared the effect of caveolin-3 knockout (Cav-3 KO) and transverse aortic constriction (TAC) on I_{Ca} distribution in mice.

Animal procedures were approved by local ethics committee and conducted in accordance with UK legislation. Ventricular myocytes were isolated from: (i) 20 week-old male C57BL/6 mice that had been subjected to TAC at 12 weeks, which were compared with sham-operated and age matched controls; mice undergoing surgery were given analgesia (buprenorphine 0.1 mg/kg s.c) and anaesthetised with ketamine (75 mg/kg i.p.) and medetomidine (0.5 mg/kg i.p.). (ii) 12 week-old Cav-3 KO mice (3) which were compared with wild-type controls. The whole-cell patch-clamp technique was used to record I_{Ca} from intact ventricular myocytes, and following acute detubulation (DT) using formamide-induced osmotic shock (4), at 22-25°C. I_{Ca} recorded from DT myocytes represent I_{Ca} at the surface membrane. Data are expressed as mean ± SEM. Extracellular acidosis (to pH 6.3) caused a significant positive shift in activation of I_{Ca}, an acceleration in deactivation, as well as a reduction in macroscopic current conductance from 0.34 ± 0.04 nS/pF at pH 7.4 to 0.22 ± 0.04 nS/pF at pH 6.3 (n=8 cells; P = 0.003, two-tailed paired t-test). A further reduction was seen in I_{Ca} conductance at pHe 5.5 (from 0.36 ± 0.04 nS/pF at pH 7.4 to 0.10 ± 0.01 nS/pF at pH 5.5; n=8 cells; P < 0.0001, two-tailed paired t-test). Determination of the sensitivity of different current properties to pH revealed that more than one distinct pKa value were apparent for different processes measured. Thus, sensitivity of the end-pulse current to pH gave a pKa of 6.0 ± 0.09 (n=8 cells) while the amplitude of the peak tail current was sensitive to pH with a pKa of 5.6 ± 0.21 (n=8 cells). Current deactivation was best described by two exponentials, each displaying a different sensitivity to pH: the fast component of deactivation had a pKa of 6.53 ± 0.07 (n=8 cells), while the slow component exhibited a pKa of 7.08 ± 0.35 (n=8 cells) These data illustrate that more than one site of protonation might be responsible for the sensitivity of the end-pulse current to pH. In conclusion, these data show that cardiac hypertrophy/failure and Cav-3 KO are both associated with cellular hypertrophy and a decrease in I_{Ca} density at the t-tubule membrane with no change at the cell surface. Thus the decrease of Cav-3 expression that occurs in heart failure may underlie these changes.

Feiner EC et al. (2011). J Card Fail. 17, 253–263
Bryant SM et al. (2015). J Mol Cell Cardiol. 86, 23-31

This work was supported by the British Heart Foundation

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Hagiwara Y et al. (2000). Hum Mol Genet. 9, 3047-54

Multiple effects of extracellular protons on hERG potassium channels

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The human ether-a-go-go-related gene (hERG) encodes the pore-forming subunit of the rapid delayed rectifier potassium current (I_{Kr}), contributing to the repolarisation of ventricles. The hERG-mediated current is modulated by extracellular acidosis that occurs during certain pathological heart conditions such as ischemia (Du et al. 2010; Van Slyke et al. 2012; Jiang et al. 1999; Anumonwo et al. 1999; Bett & RASMUSSEN 2003). We have studied the effects of extracellular acidosis on macroscopic wild-type (WT) hERG channels, with the aim of resolving the identity of the amino acid(s) that interact with extracellular protons. Whole-cell patch clamp measurements of I_{hERG} were made at room temperature using either HEK-293 or CHO cells expressing hERG, with the effects of extracellular acidosis (pH 6.3 and 5.5) determined under voltage clamp. In addition, experiments were conducted to derive a pKa for a number of I_{hERG} properties. Data are presented as mean ± SEM. Extracellular acidosis (to pH 6.3) caused a significant positive shift in activation of I_{hERG}, an acceleration in deactivation, as well as a reduction in macroscopic current conductance from 0.34 ± 0.04 nS/pF at pH 7.4 to 0.22 ± 0.04 nS/pF at pH 6.3 (n=8 cells; P = 0.003, two-tailed paired t-test). A further reduction was seen in I_{hERG} conductance at pHe 5.5 (from 0.36 ± 0.04 nS/pF at pH 7.4 to 0.10 ± 0.01 nS/pF at pH 5.5; n=8 cells; P < 0.0001, two-tailed paired t-test). Determination of the sensitivity of different current properties to pH revealed that more than one distinct pKa value were apparent for different processes measured. Thus, sensitivity of the end-pulse current to pH gave a pKa of 6.0 ± 0.09 (n=8 cells) while the amplitude of the peak tail current was sensitive to pH with a pKa of 5.6 ± 0.21 (n=8 cells). Current deactivation was best described by two exponentials, each displaying a different sensitivity to pH: the fast component of deactivation had a pKa of 6.53 ± 0.07 (n=8 cells), while the slow component exhibited a pKa of 7.08 ± 0.35 (n=8 cells) These data illustrate that more than one site of protonation might be responsible for the sensitivity of the hERG to extracellular acidosis (Bett & RASMUSSEN 2003). Ongoing mutagenesis work is targeting a cluster of residues located in the S5 and pore-loop region of the hERG channels to test for attenuation of sensitivity to protons.


Intracolonic hydrogen sulfide, a gut-bacteria metabolite, lowers blood pressure in rat

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BACKGROUND: Research suggests that hypertension is associated with gut microbiota dysbiosis. Although hydrogen sulfide (H2S) is an abundant metabolite of gut bacteria, the effects of gut-derived H2S on the circulatory system have not yet been investigated. We studied the effects of intracolonic administration of Na2S, a H2S donor, on systemic hemodynamics.

METHODOLOGY: Hemodynamics were recorded in anesthetized rats at baseline and after intracolonic injection of either saline (controls) or Na2S±9H2O saline solution at a dose range of 10-300 mg/kg of BW. RESULTS: The H2S donor produced a significant, dose-dependent decrease in mean arterial blood pressure (MAP), which lasted several times longer than previously reported after parenteral infusions (>90 min). The effect was more pronounced in hypertensive than in normotensive rats. The Na2S-induced decrease in MAP was reduced by pretreatment with glibenclamide, an inhibitor of ATP-sensitive potassium-channels. Na2S did not affect mesenteric vein blood flow. Rats treated with Na2S showed increased portal blood levels of thiosulfate and sulfane sulfur, products of H2S oxidation. In contrast, rats treated with neomycin, an antibiotic, showed significantly decreased levels of thiosulfate and sulfane sulfur, and a tendency for greater hypotensive response to Na2S. The H2S donor decreased heart rate but did not affect ECG morphology and QTc interval. CONCLUSIONS: The gut-derived H2S may contribute to the control of arterial blood pressure and may be one of the links between gut microbiota and hypertension. Furthermore, gut-derived H2S may be a therapeutic target in hypertension.

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also occurred. Furthermore, 46 patients were diagnosed with depression (>10 points on Beck Depression Inventory). None of the participants used antidepressants or sedative agents. In studied group, chronic diseases affecting autonomic nervous system were not diagnosed. Total of 80 healthy (40 women and 40 men) in mean age 42.7 years old and with mean BMI=24.6 were formed in group of controls. All of human subjects were volunteers and gave informed consent to participate on the study. All of patients had 24-hour ECG monitoring with Holter method in order to evaluate the autonomic activity with time and frequency domain analysis (heart rate variability - HRV).

Results: Obese group showed a significant reduction of parasympathetic activity and a significant increase in sympathetic activity. No significant differences in cardiac autonomic modulation were noted between the Hypertensive-Diabetic patients and those, only with morbid obesity. However, in studied group, obese patients with depression had lower time and frequency domain parameters (p<0.05) except SDNN, SDANN and LF/HF ratio in contrast to obese non-depressive individuals. Additional load of diabetes and hypertension in depressed patients did not affect the cardiac autonomic modulation differences.

Further prospective study can be undertaken within the same subjects to evaluate the effect of weight loss on the cardiac autonomic activity.

Conclusions:
1. Extreme obesity altered cardiac autonomic activity independently of hypertension and diabetes.
2. Depression associated with morbid obesity intensified HRV reduction.

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PCA022

The hemodynamic changes during antigravity straining maneuvers assessed by impedance cardiography

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Introduction: The anti-G straining maneuver (AGSM) is still an important part of pilot protection for G-induced loss of consciousness. They affect peripheral resistance, chest pressure, baroreceptors and autonomic nervous system activity. In consequence AGSM have the influence on parameters that determine of heart systolic function and left ventricle work. AGSM are combination of muscles tension (MT) and breathing techniques, but up till now it is not known which component affect more the physical aspects of blood circulation and cardiac function. The aim of the study was comparative assessment of changes in selected hemodynamic parameters during classical Valsalva maneuver (VM), MT and AGSM series. Method: 20 healthy, volunteer, pilots were examined. Hemodynamic parameters were recorded by impedance cardiography in rest and during VM, MT and AGSM. Each test last 15 s. Cardiac output (CO), left ventricle work index (LVWI), pre-ejection period (PEP), left ventricular ejection time (LVET) and Heath index (HI) were measured. Results: means values of parameters in rest, VM, MT and AGSM; PEP became shorter during MT and AGSM; LVWI was higher during AGSM and MT and HI was higher only during AGSM. Conclusions: 1. During AGSM series the dynamic changes in preload, afterload and sympathetic activity significantly affect parameters of systolic function and left ventricle work. 2. The hemodynamic trend during AGSM similar to MT suggest higher input of muscles tension.

Mean values of hemodynamic parameters in rest, VM, MN and AGSM

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rest</th>
<th>VM</th>
<th>MT</th>
<th>AGSM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>5.34±0.8</td>
<td>7.2±5.0</td>
<td>7.46±1.7</td>
<td>10.58±0.5</td>
</tr>
<tr>
<td>AGI</td>
<td>63.3±13.7</td>
<td>62.9±23.9</td>
<td>72.3±20.3</td>
<td>136.4±31.8</td>
</tr>
<tr>
<td>LVWI</td>
<td>4.11±6.7</td>
<td>4.7±0.6</td>
<td>6.1±1.5</td>
<td>6.2±1.1</td>
</tr>
<tr>
<td>LVET</td>
<td>287±10.6</td>
<td>287±10.6</td>
<td>280±28.7</td>
<td>284±11.0</td>
</tr>
<tr>
<td>PEP</td>
<td>124±6.9</td>
<td>120±6.4</td>
<td>114±8.9</td>
<td>96±8.5</td>
</tr>
<tr>
<td>HI</td>
<td>0.52±0.4</td>
<td>0.27±0.1</td>
<td>0.25±0.1</td>
<td>0.51±0.1</td>
</tr>
</tbody>
</table>

Cardiac output (CO), left ventricle work index (LVWI), pre-ejection period (PEP), left ventricular ejection time (LVET) and Heath index (HI)

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PCA023

Nω-Nitro-L-Arginine Methyl Ester (L-NNAME) induced hypertension and cardiorenal oxidative stress: Modulatory effect of the methanolic extract of Azadirachta indica

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Hypertension, a sustained elevation of blood pressure is known to cause both structural and functional abnormalities in both the cardiovascular and renal systems. In this study, we investigated the modulatory effects of the methanol extract of the leaves of Azadirachta indica (AI) on L-NNAME-induced hypertension and cardiorenal dysfunction via oxidative stress in rats.

Fifty rats divided into five (5) groups each containing 10 animals were used in this study. Group A (Control) received only clean tap water for twenty-one (21) days. Group B was treated orally with L-NNAME alone at the dosage of 40 mg/kg for 21 days. Groups C and D were treated orally with the methanol extract of AI at 100 mg/kg and 200 mg/kg, respectively together with L-Name for 21 days, while Group E received Enalapril at the dosage of 25 mg/kg alongside L-NNAME for 21 days. Systolic, diastolic and mean arterial pressures were recorded in conscious animals before the termination of the experiment by tail cuff plethysmography. The animals were thereafter fasted overnight, sacrificed and the hearts and kidneys were removed from the animals in each group. Biochemical assays to assess the antioxidant defense system and oxidative stress markers were carried out on cardiac and renal tissues. Treatment with L-NNAME led to a significant (p <0.05) increase in blood pressure and markers of oxidative stress in cardiac and renal tissues. AI caused an improvement in antioxidant defense systems, reduction in markers of oxidative stress and the restoration of blood pressure comparable with that of Enalapril. This study suggests that the methanol extract of AI possesses modulatory functions in L-NNAME induced hypertension and cardiorenal oxidative stress.
RAGE and SAGE: Therapeutic Modalities for smoke-induced COPD

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Chronic obstructive pulmonary disease (COPD) is the third leading cause of death in the United States and it is characterized by debilitating inflammation and deleterious tissue loss in the respiratory compartment. Voluntary and involuntary exposure to tobacco smoke is the major cause of COPD. Past studies identified the receptor for advanced glycation end-products (RAGE) as a smoke-induced pattern recognition receptor with potent pro-inflammatory characteristics. Further research demonstrated that RAGE is increased in the lung following first and secondhand smoke (SHS) exposure and that transgenic mice that conditionally up-regulate RAGE over-expressing, and control mice compared to identical animal groups exposed to room air only. Briefly, mice were subjected to daily SHS via a nose only inhalation system (Sireq Scientific, Montreal, Canada) as approved by institutional review boards. Groups of mice were also co-treated with SAGES via weekly ip injections where indicated. Molecules of mice were also co-treated with SAGES via weekly ip injections where indicated. Molecular characterization of primary and SHS revealed significant pulmonary inflammation mediated at least in part by RAGE. Inflammatory cell behaviors were assessed by determining the activation of ras, intracellular signaling kinases, and cytokine synthesis and secretion. Furthermore, bronchoalveolar lavage fluid (BALF) was procured from our mouse models for assessment of inflammatory cells and secreted molecules. As a general theme, inflammation induced by tobacco smoke exposure was influenced by the availability of RAGE and significant amelioration of tobacco smoke-induced inflammation was observed in mice also treated with SAGES. These data reveal captivating information suggesting a role for RAGE signaling in lungs exposed to tobacco smoke and implicates plausible therapeutic modalities.

This work was supported by a grant from the Flight Attendant’s Medical Research Institute (FAMRI, PRR) and a BYU Mentoring Environment Grant (PRR).

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PCA025

Transcutaneous vagal nerve stimulation: Neuronal tracing in vivo and functional studies in the working heart-brainstem preparation

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Transcutaneous vagal nerve stimulation (tVNS) is achieved via electrically stimulating the peripheral endings of the auricular branch of the vagus nerve (ABVN). In humans, tVNS can influence cardiovascular function by improving heart rate variability (HRV) and decreasing sympathetic nerve activity (Clancy et al., 2014). We sought to understand, in a rat model, whether similar effects were observed and the pathways underlying these effects. Rats (n=10) were anaesthetised with isoflurane, decerebrated and prepared for anaesthetic-free working heart-brainstem preparation as described (Paton, 1996). Applying 5 minutes of right tragus stimulation via an alligator clip (100 Hz, 1mA) immediately reduced sympathetic activity recorded from the thoracic sympathetic chain (baseline = 4.87 ± 0.51 AU vs stimulation = 4.01 ± 0.54 AU ; p < 0.05). The effects of sympathoinhibition persisted for 5 minutes after cessation of stimulation. There was also a significant reduction in arterial pressure, starting at 2 minutes following the beginning of stimulation (T2) and persisting for 4 minutes after the stimulation stopped (T9) (Figure 1). These changes occurred without significantly affecting heart or respiratory rate. To study the central terminations of sensory afferent nerves from the auricular tragus, 7 Wistar rats (200-250g) of either sex were deeply anaesthetised. Animals were injected subcutaneously with cholera toxin subunit B (CTb) (5 µl, 5.3 mg/ml) into the tragus. Four days post injection animals were deeply anaesthetised with 80 mg/kg of intraperitoneal sodium pentobarbitone and perfused with 4% paraformaldehyde (PFA). Following overnight post-fixation the upper cervical spinal cord and lower brainstem were sectioned at 50 µm using a vibrating microtome and processed with CTb immunohistochemistry. Heavy central terminations from tragus injections were found in the dorsal horn of the upper cervical spinal cord covering laminae II and III. In the medulla, there was moderate terminal labelling in the cuneate fasciculus and paragigeminal nucleus. There was little labelling in the nucleus tractus solitaries (NTS) which is a primary sensory afferent integration site that also received inputs from baroreceptors (Andersen & Mendelowitz, 1996). The identity of post-synaptic cells was then sought by immunostaining for ChAT, Calbindin, GAD67, NK1R or Parvalbumin: CTb labelled close appositions were infrequently detected onto each cell type. TVNS in rats therefore induces a sympathoinhibition similar to that in humans. The pathways through which tVNS functions remains to be concluded, but limited labelling in the NTS indicates that this region is not the initial termination point for auricular afferents to exert their influence on sympathetic nerve activity.
Low birth weight (LBW) was confirmed as a risk of high blood pressure (SBP), diastolic blood pressure, and heart rate (HR) showed no significant increases in men born with LBW, whereas men born with NBW had normal responses (p < 0.01). In women, the LBW individuals have blunted responses in HR and SBP compared to the NBW individuals. Similar to the results of earlier studies, healthy young men have lower HDL-C and higher TG levels compared to healthy young women in this study. Our results also show that healthy young men with a LBW have lower parasympathetic nervous activities compared to their counterparts with a NBW. In addition, among healthy young Japanese adults, both men and women with LBW may be less sensitive to postural changes in HR and BP. In conclusion, we suggest that, for both men and women, those born with LBW have higher risk of hypertension, in healthy young Japanese adults. Barker Dj et al, (1990). BMJ, 301 (6746): 259-262. Marvar Pj et al, (2011). Curr Opin Pharmacol. 1(2): 156-161. Hart EC et al. (2011). Curr Hypertens Rep. 13(3): 237-243.  

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reduced glutathione (GSH), glutathione peroxidise (GPxs), protein thiol, non-protein thiol and serum nitric oxide (NO) were significantly decreased (p < 0.05) following intestinal ischaemia-reperfusion injury. However, pre-treatment with Azadirachta indica and vitamin C restored the level of cardiac and renal GSH, GPx, protein thiol, non-protein thiol and serum NO and significantly decreased (p < 0.05) the level of cardiac and renal H2O2, serum MPO and XO. All these findings suggest that pre-treatment with A.I (100 and 200 mg/kg) ameliorated the cardiac and renal injury induced by intestinal ischaemia-reperfusion injury by reducing oxidative stress and increasing the antioxidant defence system.


The authors acknowledge the technical assistance of Dr. E.R. Asenuga

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PCA028

Does 30° lateral position affect circulation dynamics, autonomic nerve activity, and body pressure distribution in healthy adults?

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Postural change has frequently been used in clinical practice for pain relief, drainage, and prevention of pressure ulcers. In particular, 30° lateral position is known to be effective for body pressure distribution. However, few studies have been conducted to estimate the effects of 30° lateral position and body pressure dispersion mattresses on circulation dynamics and autonomic nerve activity. A slight postural change may be associated with circulatory changes such as hypotension and bradycardia in critically ill patients and the elderly. The current study aimed to evaluate the effect of 30° lateral position on hemodynamics and autonomic nerve activity with three types of mattresses used in clinical practice. Eighteen healthy adult university student volunteers (8 males and 10 females) aged 18-22 years participated in the experiment. We measured blood pressure and heart rate (HR), analyzed heart rate variability based on electrocardiographic data, and evaluated autonomic nerve activity. The simultaneous measurement of body pressure distribution was performed on a standard mattress (Paracare KE-801Q) and two types of body pressure dispersion mattresses (Maxi KE-801A and Air MADV83A) using a sensor. These were analyzed before and after postural change using the paired t-test. p < 0.05 was considered significant. There were no significant differences in systolic blood pressure before and after postural change on any of the mattresses. In contrast, compared to the supine position, significant elevation of diastolic blood pressure was observed immediately following postural change (p = 0.02) and in the 30° lateral position (p = 0.02) on the Air mattress. HR was significantly lower in the 30° lateral position than in the supine position (Paracare: p = 0.002, Maxi: p = 0.01, Air: p = 0.001). HF, an index of parasympathetic nerve activity, was significantly increased in the 30° lateral position compared to immediately after postural change on the Maxi mattress (p = 0.003). However, no significant difference was found in LF/HF, an indicator of sympathetic nerve activity, between the supine and 30° lateral position on any of the mattresses. The sacral region was recorded as the maximum pressure point in the supine position, but this changed to the greater trochanter with a shift to the 30° lateral position. Our study indicates that postural change from a supine to 30° lateral position had little effect on autonomic nerve activity in healthy young adults. Although HR decreased slightly in the 30° lateral position, this did not have a major impact on autonomic nerve activity and the pressure applied to a point on the body.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA029

The neurocardiological effects of autonomic nerve stimulation in a rabbit model of heart failure

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Sympathetic and vagus nerve stimulation exert positive and negative responses in heart rate (HR) respectively. They modulate ventricular fibrillation threshold (VFt) and action potential duration restitution (RT), both of which are markers of ventricular arrhythmia vulnerability. Prominent features of heart failure (HF) are sympathetic overdrive, parasympathetic attenuation and left ventricular (LV) impairment yet confirmation of autonomic imbalance in an in-vitro model is lacking. Our aim was to assess the effect of autonomic nerve stimulation on HR, atrio-ventricular delay (AVD), VFT and RT in an in-vitro innervated heart preparation following coronary ligation induced HF.

Coronary ligation (HF; n=13) and sham (SHM; n=12) surgeries were performed using NZW rabbits following anaesthesia (ketamine, 10mg/kg; medetomidine hydrochloride, 0.2mg/kg; butorphanol, 0.05mg/kg; s/c). After 6 weeks recovery, transthoracic echocardiography was performed to measure LV ejection fraction (EF) and fractional area change (FAC). Following 8 weeks recovery, terminal in-vitro experiments were performed using the dual-innervated Langendorff perfused heart preparation obtained after anaesthesia (ketaset; sedator and torbugecis; doses as above; s.c.), propofol anaesthesia and euthanasia using sodium pentobarbitone (160mg/kg). The effect of nerve stimulation was determined from within the spiral cord at the level of the stellate ganglia (SS) and the right cervical vagus nerve (VS). HR responses were examined during stimulation between 0-20Hz, whilst AVD was measured during atrial pacing (300ms CL) at 10Hz (SS) and 5Hz (VS). VFT, defined as the minimum current inducing sustained VF by burst pacing and RT slope, determined using an extra-stimulus protocol, were performed at nerve frequencies for equivalent HR response (SS: HF-9Hz; SHM-8Hz / VS: HF-11Hz; SHM-8Hz). Data are mean±SEM, *P<0.05 taken as significant.
EF (28.8±1.3 [HF] vs. 53.6±2.4% [SHM]) and FAC (27.0±2.8 [HF] vs. 51.2±2.5% [SHM]) were lower in HF. In HF, sympathetic tachycardia was significantly exaggerated whilst the vagal bradycardia was attenuated at high frequencies (Fig 1A). SS-AVD shortening (-9.4±1.9 [HF] vs. -19.5±3.0ms [SHM]) and VS-AVD prolongation (11.4±3.1 [HF] vs. 42.7±7.7ms [SHM]) were both significantly attenuated in HF. VFT was lower in HF during SS and VS (Fig 1B). RT was significantly steeper during SS (4.3±0.3 [HF] vs. 3.2±0.2 [SHM]) and VS (0.9±0.1 [HF] vs. 0.6±0.1 [SHM]) respectively. Coronary ligation-induced HF in rabbits leads to adverse electrophysiological effects (Winter et al., 2012), which are described here conform with the Physiological Society ethical requirements.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA030

Direct effects from left and right sympathetic nerve stimulation on ventricular electrophysiology and arrhythmia inducibility

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Left and right-sided sympathetic paravertebral chains innervate the heart with each chain reported to have different electrophysiological effects (Winter et al., 2012), which are important factors in arrhythmia susceptibility. However, direct studies on differential left vs. right effects on electrical restitution and ventricular fibrillation (VF) inducibility are sparse. The effects of left (LSS) and right-sided (RSS) sympathetic chain stimulation on effective refractory period (ERP), action potential duration restitution (RT) and VF threshold (VFT) were studied. The innervated isolated heart preparation (Ng et al., 2001) from adult male New Zealand White rabbits (n=11, 2.0-3.2Kg) was used. Rabbits were sedated with Medetomidine Hydrochloride (0.2 mg/kg), Ketamine (10 mg/kg), and Butorphanol (0.05mg/kg) (s.c.) with anesthesia maintained using i.v. Propofol. Animals were a heparinized (1000 IU, i.v.) and euthanized with an overdose of Euthetal (111 mg/kg, i.v.). Preparations were perfused in constant flow Langendorff mode (100 ml/min). Left and right sympathetic chains were stimulated between T2-T3 at x2 threshold voltage (1-4V), at a frequency (4-6Hz) that produced a maximum heart rate increase. ERP and RT were performed with an extrastimulus protocol and VFT with a rapid pacing protocol respectively at baseline (BL), RSS and LSS. Data are Mean±SEM; compared using ANOVA or paired t-test.

LSS had a greater effect on left ventricular contractility (p<0.05) and RSS had a greater influence on sinus rate (p<0.001) (Table). There was a significantly greater change in ERP with LSS vs. RSS (p<0.001). LSS RT slope values were significantly larger than RSS at apex and base (p<0.05). The change in VFT was significantly greater for LSS than RSS (p<0.05).

This study holds importance for arrhythmogenesis in cardiac disease and conditions of sympathetic imbalance and shows evidence to suggest that LSS is more likely to induce ventricular arrhythmia then RSS.

<table>
<thead>
<tr>
<th>Condition</th>
<th>HR (bpm)</th>
<th>LVP (mmHg)</th>
<th>ERP (ms)</th>
<th>RT Slope (ms/V)</th>
<th>VFT (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL</td>
<td>147±7.7</td>
<td>133±7.6</td>
<td>34±1.0</td>
<td>33±1.4</td>
<td>4.1±0.8</td>
</tr>
<tr>
<td>LSS</td>
<td>200±5.9</td>
<td>152±6.7</td>
<td>136±7.3</td>
<td>45±6.2</td>
<td>21±0.7</td>
</tr>
<tr>
<td>RSS</td>
<td>195±6.5</td>
<td>142±7.3</td>
<td>34±1.0</td>
<td>34±1.4</td>
<td>4.1±0.8</td>
</tr>
</tbody>
</table>


Winter et al., 2012. Differential cardiac responses to unilateral sympathetic nerve stimulation in the isolated innervated rabbit heart. AN:R&C 166, 4-14.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA031

Direct effects of pravastatin or melatonin on cardiovascular function in the chronically-hypoxic fetus: A comparison of two antioxidant strategies

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Maternal treatment with either pravastatin or melatonin to protect growth in the hypoxic fetus in complicated pregnancy is currently undergoing multicentre human clinical trials (Constantine et al. (2013). Obstet Gynecol. 121: 349; Alers et al. (2013). BMJ Open 3(12):e004141). However, whether these treatments have additional beneficial or detrimental effects on the hypoxic fetus is completely unknown. The chick embryo is the only established animal model to isolate the direct effects of any therapy on the fetus independent of effects on the maternal or placental physiology. Therefore, this study investigated the effects on cardiac and vascular function of treatment with either pravastatin or melatonin in the hypoxic chick embryo.

Fertilised eggs (n=7-10 per group) were incubated under normoxia (N) or hypoxia (H, 14%) from day 1 (term: 21 days). Pravastatin (1 mg.kg⁻¹), Melatonin (1 mg.kg⁻¹) or vehicle was injected daily into the air cell from day 13 of incubation, which equates to 25 weeks of human pregnancy. At day 19, the embryo was euthanized and cardiovascular function was determined using a Langendorff preparation and a wire myograph.

Chronic fetal hypoxia impaired systolic (reduced left ventricular developed pressure, LVDP) and diastolic (elevated
LV end diastolic pressure, LVEDP) function (Fig.1). While treatment with melatonin rescued systolic function, pravastatin rescued diastolic function in hypoxic embryos. Chronic hypoxia induced endothelial dysfunction in femoral vessels (N: 7.0±0.1 and H: 6.3±0.1, sensitivity [pD2] to log [acetylcholine], P<0.05). Treatment with melatonin or pravastatin rescued endothelial function in hypoxic embryos (HM: 7.0±0.1, HP: 7.0±0.2).

We show that human clinically-relevant doses of antioxidants being trialled clinically at present to protect the fetus in high risk pregnancy also have direct beneficial effects on the developing cardiovascular system of the hypoxic fetus.

**Supported by the British Heart Foundation**

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Electrophysiological characterisation of a minimally structured hERG potassium channel inhibitor

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Potassium channels that are encoded by the human Ether-à-go-go Related Gene (hERG) conduct the rapid delayed rectifier (I_{hERG}) current which is important in controlling the duration of both the ventricular action potential and the QT interval of the electrocardiogram (Sanguinetti & Tristani-Firouzi 2006; Hancox et al. 1998). Pharmacological inhibition of hERG by structurally and therapeutically diverse drugs is associated with acquired long QT syndrome and Torsades de Pointes (TdP) arrhythmia (Sanguinetti & Tristani-Firouzi 2006; Hancox et al. 2008). Understanding the structural basis of drug-hERG interactions is therefore imperative for safer drug design. Recently, substituted diphenylpropanamines have been designed as “minimally structured” high affinity hERG channel inhibitors (Cavalli et al. 2012). This study was undertaken to characterise the underlying nature of the interactions with hERG of one of these inhibitors: “Cavalli-2” (Cavalli et al. 2012). Experiments were performed on HEK-293 cells stably expressing wild-type hERG channels. Whole-cell patch clamp measurements of I_{hERG} were made at 37°C. Data are presented as mean ± SEM, with at least 5 replicates per observation. Potency of I_{hERG} block by Cavalli-2 was assessed by measuring tail currents at -40 mV following 2s depolarisations to +20 mV; this yielded a half-maximal inhibitory concentration (IC_{50}) of 35.6 ± 0.06 nM (Hill coefficient 0.69 ± 0.08). Through the application of voltage commands to a range of test potentials, I_{hERG} inhibition by Cavalli-2 was found to be voltage-dependent, with an increase in block coinciding with the steep portion of the I_{hERG} activation curve; this is consistent with gating dependent block. During an ‘envelope of tails’ protocol, comparatively little I_{hERG} block was observed for short depolarisations, with inhibition increasing with duration of the applied depolarising command. Half-maximal inactivation of I_{hERG} exhibited a modest leftward shift (of 6.4 ± 0.5 mV) in the presence of Cavalli-2. Depolarisation to +40 mV in order to promote inactivation, during a sustained command to 0 mV, produced a statistically significant (two-tailed paired t test, P < 0.05) decrease in I_{hERG} inhibition. Collectively, the results of this study show that Cavalli-2 exhibits potent I_{hERG} inhibition that is contingent upon channel gating, involving drug interactions with both activated and inactivated channel states. This minimally structured hERG channel blocker thus exhibits actions similar to canonical high potency hERG blockers, despite being incapable of forming hydrogen bonds with amino-acid residues lining the drug binding pocket.


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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Beta 1 adrenergic receptors mediate cardiovascular effects of caffeine in rabbits

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In vivo cardiovascular actions of caffeine have been reported in some animals (Ilback et al; 2007) but not in rabbits. This study investigated the effects of caffeine on cardiovascular activities of rabbits in vivo and also examined the mechanism(s) involved in caffeine action. The study was carried out on adult male anaesthetised New Zealand rabbits (weighing 1.8-2.2Kg) divided into 3 groups (n=5). Group I rabbits were given 0.2ml/Kg of normal saline and served as control while groups II and III rabbits were administered with 2mg/Kg and 6mg/kg caffeine respectively for 28 days. Following anaesthesia, arterial pressure (AP) was measured using non-invasive oscillometric blood pressure device (Contec Medicals, China) and lead II ECG and heart rate (HR) were recorded using veterinary ECG machine by Edan Instruments. Anaesthesia was induced by i.v. injection of sodium pentobarbitone (30 mg/kg). Blood samples (3.0ml/rabbit) were collected by retro orbital puncture to determine plasma catecholamines. The animals were sacrificed by cervical dislocation and cardiac tissue biopsies were collected on dry ice for biochemical and immunohistochemical analyses. Cardiac tissue cAMP concentrations, adenyl cyclase 9 and beta1 receptor expression were determined by immunohistochemistry and colorimetry techniques respectively, with assay kits obtained from Biovision Inc. Plasma catecholamines were determined by the use of ELISA kit (LDN Lab.). The results showed that caffeine at 2mg/kg and 6mg/kg significantly increased AP from 69.6 ± 2.46 mmHg to 72.9 ± 4.28 and 81.8 ± 2.0 mmHg respectively. HR decreased from 258.6 ± 3.9 beats/min to 234.0 ± 2.5 beats/min for 2 mg/kg caffeine and increased to 290.2 ± 5.79 beats/min for 6 mg/kg caffeine. Caffeine at 6 mg/kg only significantly reduced the QT interval from 164.0 ± 8.7 miliseconds to 133.0 ± 1.23 miliseconds. Caffeine also significantly increased the plasma levels of dopamine, adrenaline and noradrenaline. Caffeine at the two doses significantly increased cardiac immunoreactivity for adenyl cyclase 9. Also caffeine at 2mg/kg and 6mg/kg increased cardiac tissue cAMP concentrations from 5.097 ± 0.29 to 6.840 ± 0.26 and 6.36 ± 0.14 pmol/well. Using immunohistochemical analysis, percentage beta1 receptor expression was significantly high in response to the two doses of caffeine administered. The study therefore showed that caffeine increased cardiovascular activities of the rabbit most probably by increasing plasma catecholamines and activation of beta1 adrenergic receptors in the heart tissue.
The effect of a novel highly selective inhibitor of the sodium/calcium exchanger (NCX) on cardiac automaticity and arrhythmia in \textit{in vitro} and \textit{in vivo} experiments

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**BACKGROUND**

In the present work the effects of ORM-10962 (a follow-up compound of ORM-10103, an earlier NCX inhibitor, \cite{1}), a novel highly selective NCX inhibitor were studied on the cardiac NCX current and automaticity in experimental arrhythmias. The selectivity of the drug on various transmembrane ionic currents (including measurements of L-type Ca$^{2+}$ current, the main repolarizing K$^+$ currents, late sodium current, Na$^+$/K$^+$ pump and pacemaker current) was also investigated.

**METHODS**

Ion currents and action potential recordings were investigated by applying the whole-cell patch-clamp technique in canine single ventricular cells (CM) and standard microelectrode technique in rabbit cardiac preparations, respectively. Effects of ORM-10962 were studied in ouabain (10 $\mu$g/kg i.v.) induced arrhythmias in anesthetized guinea-pigs (with pentobarbitone (45 mg/kg intraperitoneally), and ischemia-reperfusion (IR) induced arrhythmias in anesthetized rats (pentobarbitone, 60 mg/kg intraperitoneally). ORM-10962 significantly reduced both the inward and outward NCX currents with the estimated EC50 values of 55 nM and 67 nM, respectively. The compound, even at the high concentration of 1 $\mu$M, did not change significantly the amplitude of $I_{Ca}$ in CM. ORM-10962 (1 $\mu$M) had no influence on the inward rectifier, transient outward, rapid and slow delayed rectifier potassium currents, late sodium current and Na$^+$/K$^+$ pump. ORM-10962 slowed automatically in Purkinje fibres and sinus node in dogs and rabbits, without altering the ivabradine sensitive pacemaker current. The amplitude of pharmacologically induced delayed afterdepolarizations (by digoxin) was significantly decreased by 1 $\mu$M ORM-10962 in canine Purkinje fibres. ORM-10962 (0.3 mg/kg) pre-treatment (i.v. 10 min before starting ouabain infusion) significantly delayed the development of ventricular extrasystoles (by about 50%) or ventricular tachycardia (by about 30%) in anesthetised guinea pig Fig 1A). On the contrary, ORM-10962 pre-treatment did not result in any change on the development, or the severity of IR induced arrhythmias in anesthetised rat (Fig1B).

**RESULTS**

**CONCLUSIONS**

The present study provides evidence for the strong and highly selective NCX-inhibitory activity of ORM-10962. In addition it is suggested that specific inhibition of the NCX current can influence normal pacemaker function (Ca$^{2+}$-clock hypothesis) and also contribute to the prevention of DAD based arrhythmias \textit{in vivo}. However, its effect on ischemia-reperfusion arrhythmias is still uncertain.

**Figure 1.** Panel A. Effect of ORM-10962 (0.3 mg/kg) in ouabain (10 $\mu$g/kg min i.v.) induced arrhythmias in anesthetized (pentobarbitone, 45 mg/kg i.p.) guinea-pigs. Time to the development of ventricular arrhythmias was measured on the ECG. Panel B. Effect of ORM-10962 (0.3 mg/kg) in ischemia-reperfusion of ventricular arrhythmias during reperfusion after 6 min coronary artery ligation in anesthetized (60 mg/kg i.p.) rats. Time to the development of ventricular arrhythmias was measured on the ECG.


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**Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.**

PCA036

Phosphodiesterase 5 inhibition improves contractility and sympathetic sensitivity in the failing sheep myocardium

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Heart failure is a major cause of premature mortality and increased morbidity. It is characterised by perturbed excitation contraction coupling and associated with autonomic dysfunction, in particular altered cyclic nucleotide (cAMP/ cGMP) signalling. Classical therapies for heart failure such as beta-blockers attenuate cAMP signalling, however the current study aimed to test whether activation of the cGMP pathway may similarly improve cardiac function and $\beta$-adrenergic sensitivity in the failing myocardium.

Heart failure was induced in sheep. Animals were anaesthetised for pacemaker implantation (isoflurane, 1-3% in oxygen) and perioperative analgesia provided (meloxicam, 0.5 mg/kg). After 7 days recovery, right ventricular pacing (210-220 bpm) was applied until clinical symptoms of HF were evident. Cardiac dimension and function were measured in vivo using echocardiography. At end stage heart failure animals were sacrificed by I.V. injection of pentobarbitone (200 mg/kg). Post-mortem the heart was taken and individual myocytes isolated enzymatically. In vitro, whole cell patch clamping

**PCA036**

**Poster Communications**
with corresponding fluorescent imaging was used to measure intracellular calcium, which rises rapidly during systole, and provides a function of myocyte contractility. In heart failure, cardiac dimension was increased (p<0.01, n=7) and contractility was reduced both in vivo (p<0.01, n=7) and in vitro (p<0.01, n=9). Furthermore, in vivo response to sympathetic nervous system stimulation (dobutamine, 20 mg/kg/min) was attenuated (p<0.01, n=5). After 4 weeks of tachypacing a subset of heart failure animals (n=20), were commenced on a 3-week treatment with a phosphodiesterase 5 inhibitor (tadalafil, 20 mg/day), which aims to increase cytosolic cGMP. In these animals cardiac dimension was no different to before the commencement of treatment, showing no further change in chamber dilation than untreated animals. Furthermore, treated animals had augmented contractility in vitro (p<0.01, n=20), and increased sensitivity to sympathetic stimulation in vivo (p<0.01, n=5). Finally, treated animals lived longer than untreated animals (15.9±8.4%, p=0.03, n=20). In conclusion this study shows that chronic phosphodiesterase 5 inhibition is beneficial in heart failure, facilitating longevity and improved cardiac function at the whole heart and cellular level. The data presented here suggests a promising future target, which may have important implications for the management of heart failure in clinical practice. Experiments were carried out in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986.

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### PCA037

**Using cardiac electrophysiology models to predict drug-induced pro-arrhythmic risk as part of the Comprehensive in-Vitro Pro-arrhythmia Assay**

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Assessing the potential for novel compounds to increase pro-arrhythmic risk is a high priority for pharmaceutical companies. A recent initiative led by the US Food & Drug Administration aims to streamline this process and provide more accurate predictions based on in-vitro technologies rather than clinical trials. The Comprehensive in-vitro Pro-arrhythmia Assay (CIPA) [1] aims to link high-throughput automated ion channel patch-clamp screening in expression systems for multiple ion channel targets; stem-cell derived myocyte assays; and mathematical models of electrophysiology to understand whether results are consistent and extrapolate to the adult human situation [2].

In previous work we showed how information on multiple ion channels could improve prediction of human clinical risk [3]. Recently we have been studying how uncertainty in ion channel screening [4], and choice of mathematical model, can influence simulation predictions.

Here we show our latest results examining the variability that is inherent in ion channel screening, and how this might be quantified. Once the variability is described by a probability distribution it can be propagated through simulations of electrophysiological activity to provide a probability distribution of outputs. We demonstrate how this propagation works, and how a simpler model (or emulator) can be used to speed up this process.

We demonstrate the effect of this uncertainty using a dataset of ion channel interactions for reference (marketed) compounds of known risk [5], and show how this affects the conclusions we can draw about how dangerous a novel compound is likely to be.


My work is funded by the Wellcome Trust & Royal Society (Grant number)

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**PCA038**

**Mechanism of Moringa oleifera Amelioration of Atrial Fibrillation Induced by exposure to petrol fume in Wister Rats**

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**Introduction**

There is a problem of regulation in petroleum products’ distribution in My Country- the mode of filling cars using funnel and hose, vandalism, black marketing, storing fuel privately. Deliberate inhalation of hydrocarbons as a form of recreational drug use, has become a significant health issue affecting children and adolescents (Azeez et al 2015). The number of motor vehicles exhaust typically found in an urban disaster site can be a source of many hydrocarbon chemicals, including gasoline, many of these substances are a mixture of hydrocarbons (Lisa A. Murphy et al, 2003). The morbidity and mortality associated with Atrial fibrillation (AF) is substantial. AF is responsible for more hospitalizations and longer hospital stays than any other arrhythmia, and may also lead to stroke, congestive heart failure, myocardial infarction, and death; Many episodes of AF are asymptomatic or minimally symptomatic.

**Methodology**

Procedures involving animals and their care were performed in accordance with the National Institutes of Health (NIH) guideline for the care and use of animals (NIH publication No 85-23, revised 1996). 25 Adult male Wister rats were grouped to 5 with 5 rats in a group. Group 1 control was given feed and water ad-libitum but not exposed to petrol fume. Groups 2, 3, 4, and 5 were exposed to petrol fume 10 minutes every day for eight weeks. Groups 3, 4 and 5 had candesartan (16mg/kg body weight), captopril (25 mg/kg body weight) and Moringa oleifera extract (40 mg/kg body weight) before exposure to petrol fume. The petrol fume was generated by using human compressor nebulizer adopted for rats with the rats kept in fume chamber. At the end of the eight weeks, rats were anesthetised with 1% chloralose and 25% urethane intraperitoneally; the electrocardiography was done using EDAN 10.

**Result**

The ECG recording (ECG lead II) of group 2 (rats exposed to petrol only) showed no clearly detectable P-wave very narrow QRS complexes and very high heart rate. The ECG
Oxygen regulation of breathing through an olfactory receptor activated by lactate

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Animals have evolved homeostatic responses to changes in oxygen availability that act on different time scales. On the acute time scale, the carotid body (CB) is the major sensor of arterial blood oxygen that stimulates ventilation within seconds of hypoxia. While cellular and neuroanatomical pathways for the hypoxic ventilatory response mediated by the CB have been elucidated, the molecular mechanism of oxygen sensing within the organ is not well understood. We took an unbiased, genomic approach to identify putative oxygen sensors in the CB by comparing gene expression of the CB and adrenal medulla (AM) from wild-type adult C57BL/6 mice using microarrays and RNA sequencing. Looking for genes encoding fast signaling molecules, we found a gene encoding an olfactory receptor (Olfr78) expressed at 92-fold higher level in the CB versus AM. Using mice expressing reporters from the endogenous Olfr78 locus, we showed that Olfr78 is highly and selectively expressed in oxygen-sensitive glomus cells of the CB. Olfr78 null mice failed to increase ventilation in hypoxia (10% O2) but responded normally to hypercapnia (5% CO2) and appear structurally intact in the CB versus AM. Using mice expressing reporters from the endogenous Olfr78 locus, we showed that Olfr78 is highly and selectively expressed in oxygen-sensitive glomus cells of the CB. Olfr78 null mice failed to increase ventilation in hypoxia (10% O2) but responded normally to hypercapnia (5% CO2) by unrestrained, unanesthetized whole body plethysmography. Body temperature and metabolic responses to hypoxia were not different between wild-type and Olfr78 mutant animals. Visualized by immunohistochemistry and electron microscopy, glomus cells were present in normal numbers and appear structurally intact in Olfr78 mutant carotid bodies. However, carotid body activity in hypoxia was reduced by half in electrophysiological recordings of the carotid sinus nerve in Olfr78 mutant preparations. Lactate, a metabolite that rapidly accumulates in hypoxia and induces hyperventilation (Hardarson et al., 1998; Kirsch and D’Alecy, 1983; Lee et al., 1996; Marina et al., 2015), activated Olfr78 in heterologous expression experiments in a luciferase assay. In the CB, lactate induced calcium transients in glomus cells, as reported by the genetically encoded calcium indicator GCaMP3, and stimulated carotid sinus nerve activity through Olfr78. Based on these results, we propose a model for CB oxygen sensing in which Olfr78 acts as a hypoxia sensor by detecting lactate produced when oxygen levels decline. All experimental details were previously described (Chang et al., 2015). Chang, A.J., Ortega, F.E., Riegler, J., Madison, D.V., and Krasnow, M.A. (2015). Oxygen regulation of breathing through an olfactory receptor activated by lactate. Nature 527, 240-244.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA040

Cardiovascular response to squat test among young healthy Nigerians

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Black Africans have a greater prevalence of cardiovascular disease (CVD) than White Europeans. Exaggerated responsiveness to postural stress has been implicated in development of CVD. Squatting, imposes one of the most potent orthostatic stresses. Chakrabarti et al., (2002) reported high incidence of stroke among Indians who squatted to defecate in the morning hours.

Methods

We examined responses evoked in 65 subjects (33 males and 32 females) aged 16-51 years during 2 minutes squat test. The protocol involved 3 minutes of standing followed by 2 minutes of squatting. Arterial blood pressure and heart rate were recorded at end of 3 minutes of standing (baseline) and at 30 seconds, 60 seconds and 2 minutes during squatting. The mean arterial blood pressure (MAP) and Pulse pressure (PP) were calculated. Changes from baseline were determined. The values were presented as mean and standard error of mean. Independent sample T test was used to test differences between standing and squatting, p level of 0.05 was taken as significant.

Result
At 30s of squatting, systolic blood pressure (SBP) increased by 8.569 ± 1.243 mmHg, and this increased further at 2 minutes of squatting by 10.569 ± 1.168 mmHg (p<0.000). The change in SBP ranged from -21 to 34mmHg. A little more than half of subjects (53.8%) had an increase in DBP, while 44.6% had a decrease and 1.5% had no change upon assumption of squatting position. The diastolic blood pressure (DBP) significantly increased at 2 minutes of squatting by 2.585 ± 1.168 mmHg (p=0.030). The change ranged from -37 to 13 bpm. Majority of the subjects (83.1%) had a decrease in pulse rate.

Conclusion: Squatting significantly increased SBP, DBP and Pulse Pressure with decrease in heart rate. At 30s the change in DBP was not significant, but at 2 minutes DBP increased significantly.

References:

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Angiotensin II contributes to augmented myocardial ischaemia-reperfusion injury after chronic nicotine administration
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Background: Angiotensin II (Ang II) is a peptide hormone that positively regulates mitochondrial superoxide (O2⁻) generation and its role in myocardial ischaemia-reperfusion (I/R) injury is well documented [1]. We have shown that chronic administration of nicotine augments I/R injury in rat hearts, however its mechanisms are unresolved. Aim: We sought to identify role of Ang II in the augmented myocardial I/R injury after chronic nicotine administration. Method: Male Sprague-Dawley rats (180-230g, n=14) were given nicotine (0.6 mg/kg in saline i.p.) for 28 days [2]. In addition, the rats were orally administered with Irbesartan (10 mg/kg in DMSO, n=7) or DMSO vehicle alone (n=7) for 28 days. Age-matched rats (n=6) were given vehicle alone to serve as control. After 28 days, the hearts were excised and mounted on Langendorff apparatus with constant flow perfusion (12 mL/min). After 20 minutes of steady-state perfusion, the hearts were subjected to 20 minutes of global ischaemia followed by 60 minutes of reperfusion. Results: Administration of nicotine for 28 days resulted in elevated plasma Ang II and ACE levels as compared to the controls (p<0.05 for both). The recovery of left ventricle developed pressure (LVDP) was markedly lower in nicotine group (Table). Besides, left ventricle end diastolic pressure (LVEDP) was increased in nicotine group at end point. Blockade of Ang II type I receptor with Irbesartan improved the recovery of LVDP and lowered LVEDP at end point. We also demonstrated that LDH release after I/R from rat hearts of nicotine group were higher than the controls, however this was attenuated by Irbesartan. Freshly isolated rat heart mitochondria from nicotine group exhibited lower SOD2 activity and enhanced Ca²⁺-induced swelling rate, which were also attenuated by Irbesartan (p<0.05 for both). Conclusion: Our results suggested that Ang II contributes to the augmented myocardial I/R injury evident after chronic nicotine administration. Therefore, Ang II type I receptor blockers may offer clinical advantage for management of cardiac I/R injury associated with nicotine addiction.

Table 1: Effects on Irbesartan on rat heart tolerance to I/R injury after chronic nicotine administration

<table>
<thead>
<tr>
<th>Group</th>
<th>Captopril (ng/kg in plasma)</th>
<th>LVDP (% recovery)</th>
<th>LVEDP (mmHg at end point)</th>
<th>LDH Release (% AUC to Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10±2.1</td>
<td>42 ± 1.4</td>
<td>15 ± 2.4</td>
<td>67 ± 7.8</td>
</tr>
<tr>
<td>Nicotine</td>
<td>160±21</td>
<td>14 ± 1.6</td>
<td>51 ± 3.5</td>
<td>125 ± 28</td>
</tr>
<tr>
<td>Nicotine + Irbesartan</td>
<td>142 ± 24</td>
<td>37 ± 3.4</td>
<td>12 ± 1.2</td>
<td>136 ± 15</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM for n=6-7. *p<0.05 vs Control, #p<0.05 vs Nicotine using one-way ANOVA.


This work was financially supported by Ministry of Education, Malaysia.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Does myocyte orientation in the atrial septum underlie a preferential pathway between the sinoatrial node and atrioventricular node?
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The action potential generated in the sinoatrial node (SAN) propagates through the atria to the atrioventricular node (AVN) and then onto the ventricles. The action potential is conducted from the SAN to the AVN via two ‘interodal pathways’: posteriorly down the crista terminalis and anteriorly down the interatrial septum (Spach et al., 1971; Li et al, 2014). Although the septal pathway is the dominant (i.e. faster) pathway during normal sinus rhythm (Spach et al., 1971; Li et al, 2014), there is no anatomical evidence for it. We have now studied the interatrial septum in New Zealand White rabbits. After iodine contrast enhancement, micro-CT was used to visu-
alise the high resolution (~20 μm spatial resolution) 3D anatomy of the right atrium between the SAN and AVN, including the interatrial septum. From this dataset, we extracted the orientation of myocytes. This showed the presence of a continuous pathway from the SAN to the AVN via the interatrial septum - the SAN in the right atrium is located next to the upper part of the crista terminalis and this is continuous with Bachmann’s bundle (which conducts the action potential to the left atrium). We now show that Bachmann’s bundle splits and one branch travels down the interatrial septum to the ‘fast pathway’ into the AV node. Along this internodal pathway, myocytes are arranged longitudinally, which means that conduction will be relatively fast (longitudinal conduction can be ~3 faster than transverse conduction). Masson’s trichrome staining confirmed the presence of the novel bundle down the interatrial septum. Immunohistochemistry showed that the myocytes making up the bundle do not express the nodal cell marker, neurofilament, indicating that the myocytes are not nodal in origin. Conduction velocity is also dependent on cell diameter and electrical coupling between cells (provided by connexins). However, the myocytes making up the bundle are similar in diameter to neighbouring less well-aligned myocytes (10.7 ± 0.4 versus 9.7 ± 0.9 μm, P<0.05) and expression of Cx40 and Cx43 (connexins responsible for fast conduction) is also similar. Is the longitudinal orientation of myocytes in the novel bundle sufficient to explain the importance of the septal internodal tract? Computer simulation of action potential conduction from the SAN to the AVN was carried out using the micro-CT generated structure of the right atrium. With incorporation of the novel septal bundle, simulations showed that the action potential was conducted to the region of the fast pathway of AVN via the interatrial septum as expected. In conclusion, the identified longitudinally arranged myocytes in the interatrial septum, although histochemically indiffent to the surrounding atrial myocytes, are arranged in a way that favours faster conduction of the action potential to the AVN.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA043

Action potential duration restitution affects contraction restitution in right ventricular myocytes from pulmonary hypertensive rats

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Action potential duration (APD) restitution describes the relationship between APD and the preceding diastolic interval. Steep negative APD restitution is pro-arrhythmic and steep negative contractile restitution is a manifestation of an inability to cope with increased demand and is a defining characteristic of heart failure. In this study we wished to investigate the interdependence of APD restitution and contraction restitution in myocytes from rats with right ventricular failure (RVF) induced by pulmonary arterial hypertension (PAH). Male Wistar rats were injected with saline (controls, CON) or 60mg/kg of monocrotaline (FAIL) to induce PAH and RVF. Single RV myocytes were isolated from hearts on the day of heart failure signs (FAIL) or a time matched day (CON). Myocytes were whole cell patch clamped using discontinuous voltage clamp at stimulation frequencies of 1Hz and 5Hz using voltage waveforms based on previously measured APD restitution (Benoist et al., 2012). Cell shortening and time to 50% relaxation were simultaneously measured by video edge detection. All experiments were performed at 36°C. Statistical analysis was performed by repeated measures analysis of variance. When voltage clamp protocols mimicked the respective APD restitution (in CON myocytes depolarisation duration was 50ms at both 1Hz and 5Hz, in FAIL cells depolarisation duration was 125ms at 1Hz but 50ms at 5Hz) an increase in stimulation frequency did not significantly affect the amplitude or time course of contraction in CON myocytes (6.57 ± 1.05 μm and 33.16 ± 3.08 ms at 1 Hz vs 5.91 ± 1.12 μm and 38.42 ± 6.87 ms at 5 Hz, P<0.05, n=11 myocytes). In contrast both contractile parameters were significantly reduced in FAIL myocytes (5.72 ± 1.36 μm and 35.03 ± 2.82 ms at 1 Hz vs 4.29 ± 1.16 μm and 28.50 ± 1.48 ms at 5 Hz, P<0.05, n=11 myocytes). However, when stimulation frequency was increased but depolarisation duration was held constant, at either 50ms or 125ms, contractile parameters were not changed in either group of cells (e.g. for FAIL cells with 125ms depolarisation at 5Hz (5.43 ± 2.14 μm and 31.99 ± 4.88 ms, P>0.05 vs above data for 1Hz at 125ms depolarisation, n=11 myocytes, P<0.05).

Voltage clamp protocols mimicking steep negative APD restitution provoked negative contractile restitution in FAIL cells, however the effect of increasing stimulation frequency on contraction was attenuated with fixed depolarisation duration. We therefore conclude that negative APD restitution is at least in part responsible for negative contractile restitution in FAIL myocytes.


Supported by the British Heart Foundation

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PCA044

Development of engineered xenogeneic patch for potential use in cardiac regenerative medicine

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Cardiovascular diseases are the leading global cause of death. Stem cell-based treatment approaches for injured heart represent an important frontier in cardiovascular medicine. Injection of cells to the damaged heart has been largely utilized, although cell retention remains the biggest challenge associated to this technique. Tissue engineered grafts have been shown to have a more favourable outcome for cell delivery. Decellularised xenogeneic tissues constitute promising naturally occurring scaffolds for regenerative medicine. The aim of this study was to develop decellularised engineered-scaffolds from different porcine tissues that could be used as patch for cardiovascular repair and regeneration of damaged tissue.
Porcine pericardium and myocardium were collected from 60 Kg pigs. Pericardium was decellularised with TritonX or Trypsin, while myocardium was decellularised with SDS. All treatments were followed by nuclease incubation. The differentiation of human thymus-derived mesenchymal stem cells (hTMSCs) was achieved using our established protocol based on a combination of growth factors. The expression of cardiac markers was assessed by immunostaining and qPCR. Differentiated and undifferentiated cells were seeded onto the decellularised scaffolds and cell viability was detected using a viability/cytotoxicity assay kit after two weeks of tissue culture. Histological characterization of the seeded and unseeded scaffolds was carried out with Hematoxylin and Eosin, Elastic Van Gieson and Alcian blue staining. Scanning electron microscopy allowed for visualization of engrafted cells' topography at high magnification.

Successful decellularisation was achieved in all used protocols as shown by cell removal from the matrix, preservation of the extracellular tissue content and collagen structural network. The cardiac-like MSCs (hCL-TMSCs) positively expressed the extracellular tissue content and collagen structural network. The cardiac-like MSCs positively expressed the extracellular tissue content and collagen structural network.

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PCA045

The effects of Hibiscus sabdariffa Linn. Aqueous Extract in obese rat hearts after myocardial infarction

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Obesity increases risks for hypertension and myocardial infarction (MI), which accounts for high rate of mortality globally. Therefore, agents targeting obesity and its cardiovascular complications are highly necessitated. Hibiscus sabdariffa Linn. (Roselle) is rich with antioxidants and we have recently shown that Roselle extract attenuate nicotine-induced cardiac injury1. Nevertheless, its effect on obese hearts after MI have not been shown. The aim of this study is to determine effect of Roselle extract on cardiac function in Langendorff-perfused obese rat heart after MI. Male Sprague-Dawley rats (300-350g) were fed with either standard diet (control) or obese diet with high-fat diet (HFD) (OB) for 12 weeks. Rats were given isoproterenol (ISO, 85 mg/kg s.c. 2 days, n=30) after 8 weeks of diet to induce MI. Roselle was given orally (100 mg/kg, n=12) after induction of MI for 4 weeks with continued diet.

Control rats received either vehicle (n=12) or ACE inhibitor Enalapril (10 mg/kg, n=6). After 12 weeks, rats were sacrificed and their hearts were mounted on Langendorff apparatus in constant flow mode. Readings were taken after 20 minutes of steady-state perfusion. Values are expressed as mean ± SEM, analysis by one way ANOVA. In this study, 12 weeks of HFD significantly increased systolic blood pressure (SBP) and was further aggravated by ISO-induced MI. Rat hearts from obese rats had significantly lower LVDP (Table 1) as compared to the controls, suggesting impaired contractile function. ISO-induced MI similarly worsened LVDP level. Interestingly, Roselle was able to improve LVDP and its derivatives in obese rat hearts, however its effects were abolished in rats with MI. Conversely, administration of Enalapril was able to attenuate LVDP and its derivatives in obese rat hearts after MI. In addition, ISO-induced MI in obese rats also lowered coronary flow, suggesting diminished vasodilatory response, which was ameliorated by Roselle and Enalapril (Table 1). The conclusion of this study was supplementation of Roselle (100 mg/kg, 4 weeks) improves cardiac function in a rat model of HFD-induced obesity, however, its effects are abolished in presence of prior MI.

Table 1: Systolic blood pressure from in vivo at the end-point of experiment and the heart characteristics on Langendorff apparatus.

<table>
<thead>
<tr>
<th>Group</th>
<th>SBP (mmHg)</th>
<th>LVDP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>End-diastolic volume (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>129±5 (n=6)</td>
<td>103±6 (n=6)</td>
<td>171 ± 12 (n=6)</td>
<td>157 ± 10 (n=6)</td>
</tr>
<tr>
<td>OB</td>
<td>163±5 (n=6)</td>
<td>95±2 (n=6)</td>
<td>190 ± 9 (n=6)</td>
<td>182 ± 12 (n=6)</td>
</tr>
<tr>
<td>OB + Roselle</td>
<td>147±5 (n=6)</td>
<td>72±3 (n=6)</td>
<td>186 ± 14 (n=6)</td>
<td>170 ± 15 (n=6)</td>
</tr>
<tr>
<td>OB + MI</td>
<td>159±5 (n=6)</td>
<td>76±3 (n=6)</td>
<td>170 ± 12 (n=6)</td>
<td>157 ± 10 (n=6)</td>
</tr>
<tr>
<td>OB + Roselle+MI</td>
<td>152±5 (n=6)</td>
<td>72±3 (n=6)</td>
<td>164 ± 10 (n=6)</td>
<td>151 ± 10 (n=6)</td>
</tr>
<tr>
<td>OB + MI + Enalapril</td>
<td>153±5 (n=6)</td>
<td>76±3 (n=6)</td>
<td>134 ± 10 (n=6)</td>
<td>151 ± 10 (n=6)</td>
</tr>
<tr>
<td>OB + MI + Roselle+Enalapril</td>
<td>150±5 (n=6)</td>
<td>72±3 (n=6)</td>
<td>146 ± 10 (n=6)</td>
<td>151 ± 10 (n=6)</td>
</tr>
</tbody>
</table>

This work was supported by Ministry of Agriculture, Malaysia

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PCA046

The direct effect of Roselle polyphenols to Langendorff-perfused rat hearts

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Introduction: Growing body of evidence has proven the use of natural polyphenolic compounds as nutraceuticals for cardiovascular diseases. Polyphenols have been shown to protect against cardiovascular-related diseases such hypertension, diabetes and atherosclerosis. Roselle (Hibiscus sabdariffa Linn.) is local natural source of bioactive polyphenols which was shown to exert protective effects on cardiovascular system e.g. vasodilator in aortic rings and antihypertensive via ion channels in the heart. We have recently shown in our laboratory that Roselle extract attenuate nicotine-induced cardiac injury in rats1. Nevertheless, effects of Roselle polyphenols on cardiac physiology are unresolved. The aim of this study was to investigate the effect of Roselle polyphenols (RP) on healthy Langendorff-perfused rat hearts. Method: Adult Sprague-Dawley rat (n=12, 14 weeks old) hearts were perfused in constant flow Langendorff mode at 10 ml/min
(baseline perfusion pressure 77mmHg, LVDP 106mmHg, heart rate 241bpm). The hearts were validated by positive inotrope isoprenaline (1 nM) and negative inotrope sodium nitrite (100 mM). RP (125-2000 mg/mL) was given via Krebs-Henseleit buffer perfusion to the heart and changes in cardiac function were compared against time-matched vehicle controls. Values are given as means ± S.E.M., compared by one-way ANOVA and t test. Results: HPLC profiling of RP extract revealed twelve flavonoids and seven phenolic acids confirming its polyphenolic content. Direct perfusion of RP suppressed rat heart systolic function as shown by lowered LVDP and maximal velocity of contraction (+dP/dt\text{max}). Besides, RP also reduced heart rate while simultaneously increasing maximal velocity of relaxation (–dp/dt\text{min}). RP significantly increase cardiac injury markers troponin T and lactate dehydrogenase (LDH) in coronary effluent at 1000 and 2000 mg/ml only. To further investigate involvement of calcium channels, inotropic responses were firstly enhanced by agonists of different receptors: L-type Ca\textsuperscript{2+} channel (±-Bay K 8644), ryanodine receptor (4-chloro-m-cresol), β-adrenergic receptor (isoproterenol), and also a SERCA blocker (thapsigargin) prior to administration of RP, which blunted all inotropic responses. Conclusion: Altogether, RP negatively regulates cardiac contractile function (inotropism), heart rate (chronotropism) but positively regulates venticle relaxation (lusitropism). Cardiac actions of RP may involve at least in part, modulation of calcium channels but further studies are warranted to strengthen this observation.

Changes of (A) LVDP (B) HR (C) maximal velocity of contraction (D) maximal velocity of relaxation in response to RP (mean±SEM). * vs. time-matched vehicle (p<0.05); ** vs. time-matched vehicle (p<0.001).


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PCA047

The effects of clopidogrel on contractility in rat aorta
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Clopidogrel is a tienopiridin derivative and is an inactive pro-drug that inhibits ADP stimulated thrombocyte aggregation. It is used in the treatment of atherothrombosis, stroke, myocardial infarctus and cardiovascular diseases. This study investigates the prospective effect of Clopidogrel on the contraction-relaxation mechanism of aorta, the artery that is exposed to the highest amount of pressure with a role in the regulation of blood pressure.

All experiments in this study were approved by the local committee on animal research (local committee decision number: 2016/01-02). Thoracic aorta tissues were removed from adult male Wistar albino rats after decapitation (n=7 each group). Thoracic aorta tissues were attached to an organ bath containing 5 mL Krebs-Ringer bicarbonate solution with a tension of 2 g. In vitro thoracic aorta studies were performed under the presence of endothelium. Clopidogrel was administered in 2 different doses; 10nM, 100nM and contraction-relaxation protocols were applied. Delta values of Clopidogrel were analysed before and after treatment. Results obtained after analysis were evaluated using student T test on SPSS program.

The inhibition contractions of thoracic aorta by Clopidogrel was statistically significant (p<0.05). This effect was strongest at the dose of 10nM. Clopidogrel also demonstrated statistically significant increases effect in endothelium dependent and independent relaxation (p<0.05).

The study examined the effect of a clinically used antiagregant, Clopidogrel, on aorta contractions in rats. It was recorded that, next to its antiagregant effect, Clopidogrel also leads to vasodilatation. Taking its vasodilation effect into consideration, new approaches can be developed in the use of Clopidogrel in the treatment of cardiovascular system pathologies.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA048

The investigation of the relationship between body mass index and coronary artery calcium index
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Obesity is a clinic entity, which is the excessive fattening of body. Its prevalence is increasing all over the world, and it is becoming a major health problem in many countries. There is a close link between obesity with vessel wall calcification in coronary artery and atherosclerotic coronary artery disease. This study aims to investigate the relationship between body mass index and coronary artery calcium score.
The patients file and records belonging to ones who underwent multi-detector computed tomography coronary angiography between 1 March 2012 and 31 December 2015 in our clinic were examined retrospectively. Those who were diabetes and malignity or with a chronic disease were not included in the study. The patients were divided into five groups according to their body mass index (BMI). The coronary artery calcium (CAC) score of each patient was calculated according to Agatston’s method. For the statistical analysis of the data, One-way Anova was used for the differences between the groups, and fit gaussian analysis was used for the relationship between BMI and CAC scores, and p<0.05 was accepted as significant. The average age of the 200 patients, 117 of them are male and the rest are female, and their ages range is 45 between 20 and 71. All of the patients were divided into five according to their BMI. The average calcium score was found as 0.62±0.15 for group 1, 21±3 for group 2, 126±25 for group 3, 340±17 for group 4, and 887±32 for group 5. There was a significant positive correlation between BMI and CACs value for the group 3, group 4 and 5 (Grup 3 r=0.34, Grup 4 r=0.62, Grup 5 r=0.53, p<0.05).

In the study, it was determined that there is a relationship between BMI and CAC scores indicating that as long as BMI increases, CAC scores increases prominently as well.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA049

The effect of stretch on noradrenaline induced activity of rat pulmonary veins: an electrophysiological study

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Atrial fibrillation (AF) is the most common sustained arrhythmia. Pacemaker-like cells within cardiac muscle of the left atria that extend into the pulmonary vein (PV) to form a sleeve of cardiac muscle and are thought to contribute to the generation of ectopic beats that initiate and sustain AF (Haissaguerre et al., 1998). Noradrenaline (NA) which is a combined α-β adrenoceptor agonist is known to induce ectopic activity in rat PV (Maupoil et al., 2007). Stretch is a main contributor to structural remodelling which opens stretch-activated ion channels (SACs) which enhances cellular Ca²⁺ influx and Ca²⁺ release from sarcoplasmic reticulum. It is suggested that SACs may promote AF by causing Ca²⁺ overload (Bode et al., 2000). This study aimed to investigate the effect of stretch on NA induced ectopic activity of the rat PV.

Male Sprague Dawley rats (180-300g) were euthanized, in accordance with the UK Home Office regulations, by cervical dislocation and their PVs carefully dissected and place in normal Tyrode solution, all experiments were carried out at 37°C. Action potentials (APs) were evoked at twice the threshold voltage, with a pulse duration of 2ms, at a frequency of 0.1Hz and stretched by applying 1g horizontal load, n = 5-6 for all experiments. Under resting conditions no ectopic APs were observed, with or without NA. Electrically evoked APs displayed the following characteristics at rest and in the presence of NA respectively: peak amplitude (PA) 99.18±2.66mV and 94.69±3.65mV; resting membrane potential (RMP) –80.56±3.11mV and –77.65±3.91mV; rise time (RT) 0.67±0.07ms and 0.71 ±0.1ms and action potential duration at 90% repolarization (APD90) 53.07±3.46ms and 51.60±4.34ms. Under stretch, the PV displayed some spontaneous activity, which had the following characteristics: PA 95.51±8.52mV, RMP –96.45±0.18mV, RT 0.6±0.06ms, APD90 59.58±2.11ms. Combining NA with a 1g stretch significantly (p<0.05) increased the frequency of ectopic activity by 10 fold compared with the stretching condition alone. The ectopic AP characteristics were: PA 97.18±1.37mV, RMP –98.09±7.96mV, RT 0.67±0.11ms, APD90 62.6±6.35ms. In PV with ectopic APs induced by NA plus stretch, gadolinium (Gd, 80µM) significantly (p<0.05) decreased the frequency of ectopic APs. In addition, Gd significantly increased APD90 by 41.18% compared to stretching plus NA. PA, RMP and RT were not significantly altered by Gd (p>0.05).

These findings suggest that stretch can induce ectopic activity. However when combined with NA, ectopic activity significantly increased in the rat PV, which can be reduced by a SAC blocker. In conclusion, stretch may induce abnormal electrical activity in the PV through the action of stretch-activated ion channels, which is enhanced by NA. This stretch-induced arrhythmogenic activity may be involved in the generation of AF.


Maupoil, V. et al. (2007). Br J Pharmacol. 150, 899–905


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA050

Bradycardia in hypothyroidism is caused by intrinsic remodelling of the sinus node

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Bradycardia (slow heart rate) is a characteristic cardiac phenotype seen in clinical hypothyroidism. The mechanism underlying the bradycardia is unknown. Potential causes include altered autonomic nerve activity and/or intrinsic remodelling of the sinus node. We have characterised the hypothyroid (HT) sinus node and identified the important mechanisms responsible for the bradycardia.

Sprague Dawley rats (male, 260-350g, n=18) were made HT with 6-n-propyl-2-thio-uracil (10 mg/kg/day for 15 days). Control cohort (n=20) received vehicle only. ECGs were recorded in anaesthetised animals (ketamine, 100 mg/kg) under control conditions and after complete autonomic block with propranolol (2 mg/kg) and atropine (1 mg/kg). Animals were sacrificed by cervical dislocation, hearts extracted and Langendorff perfused with Tyrode’s solution at 37°C and intrinsic heart rate measured. In sinus node, atrial and ventricular tissue biopsies mRNA abundance was measured using qPCR. Data are presented as mean±SEM and one-way ANOVA was used for statistical comparison. Animal work was approved by Marmara University animal experiments ethical committee.

HT animals showed a reduced heart to body weight ratio (3.9±0.2 vs. 4.7±0.1 mg/kg in control, P<0.05) and developed severe bradycardia (253±15 vs. 428±6 beats per minute, bpm, in control animals, P<0.05). After complete autonomic block
the intrinsic heart rate recorded in vivo was 223±9 bpm in HT and 404±19 bpm in control animals. In vitro, in the Langendorff perfused heart, the intrinsic rate measured in HT and control hearts was 186±9 and 238±7 bpm (P<0.01), respectively. Inherent automaticity in the sinus node is due to the voltage- and Ca2+-clock pacemaker mechanisms. In the HT sinus node, transcripts for key voltage-clock components were significantly downregulated: funny current (I\text{f}; HCN4 down by 79%, P<0.05), L-type Ca2+ current (I\text{Ca,L}; Cav1.2 down by 66%, P<0.05) and T-type Ca2+ current (I\text{Ca,T}; Cav3.1 down by 77%, P<0.05). Similar downregulation was observed in Ca2+-clock components: sarcoplasmic reticulum Ca2+ ATPase (SERCA2a, down by 90%, P<0.01) and ryanodine receptor (RyR2, down by 77%, P<0.05). The plasma membrane Ca2+ ATPase (PMCA1) responsible for Ca2+ extrusion out of the cell was downregulated by 85% (P<0.05) in the HT sinus node. Consistent with these findings, we identified potential thyroid hormone response elements in the 10 kb promoter region of HCN4 using in-silico analysis; it is already known that thyroid hormone binds to thyroid hormone response elements in the promoter region of SERCA2a and regulates its expression.(1) In the HT sinus node, downregulation of HCN4, Cav1.2 and Cav3.1 coupled with significant loss of SERCA2a and RyR2 is likely to compromise the voltage- and Ca2+-clock pacemaker mechanisms. Thus, ion channel remodelling intrinsic to the sinus node is the likely cause of bradycardia in hypothyroidism.


Our results indicate that TRIC-A can modulate RyR1 gating by affecting the ability of the channel to open in response to activating ligands in a manner that is not related to flux of monovalent current through SR K+ channels. The underlying mechanism appears to be due to altered sensitivity of RyR1 to Mg2+ inhibition. These results may, at least in part, explain why SR Ca2+-release is impaired in TRIC-A KO mice and demonstrate that TRIC-A may influence RyR1 function by multiple mechanisms.

Funded by the BHF and JSPS.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA052

Simvastatin differentially modulates single cardiac and skeletal ryanodine receptor channels

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Statins have been the standard treatment for hypercholesterolemia and the prevention of cardiovascular disease for more than 20 years. They function as competitive inhibitors of the HMG-CoA reductase, the rate-limiting enzyme in the synthesis of cholesterol. Although effective in lowering cholesterol levels, statin users experience side effects that are primarily
associated with muscle pain and weakness. Evidence suggests that sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} homeostasis is altered in statin-treated skeletal muscle fibres (1, 2), however, the reasons why statin toxicity is reported in skeletal but not cardiac muscle are not understood.

To examine whether simvastatin, a commonly prescribed statin, can directly modulate RyR channel function, we incorporated single sheep cardiac RyR2 or mouse skeletal RyR1 into planar phospholipid bilayers under voltage-clamp conditions as previously described (3). Solutions were 250 mM HEPES, 80 mM Tris, 10 mM free Ca\textsuperscript{2+}, pH 7.2, on the cis (cytoplasmic) side and 250 mM glutamic acid, 10 mM HEPES, pH to 7.2 with Ca(OH)\textsubscript{2} (free [Ca\textsuperscript{2+}] approximately 50 mM) on the trans (luminal) side of the bilayer. RyR1 open probability (Po) significantly increased after cytosolic addition of 1 µM simvastatin from 0.016±0.011 in control conditions to 0.064±0.021 (mean ± S.E.M., n=14, Student’s t-test, p<0.05). 10 µM cytosolic simvastatin produced further RyR1 activation (0.224±0.059, mean ± S.E.M., n=16, p<0.001). The increase in RyR1 Po was readily reversed to control values after washout of the cytosolic chamber (0.028±0.025, mean ± S.E.M., n=7). Simvastatin did not affect single-channel conductance under these conditions nor were significant changes after washout of the cytosolic chamber (0.028±0.025, mean ± S.E.M., n=7). Simvastatin did not affect single-channel conductance under these conditions nor were significant changes after washout of the cytosolic chamber (0.028±0.025, mean ± S.E.M., n=7). Simvastatin did not affect single-channel conductance under these conditions nor were significant changes after washout of the cytosolic chamber (0.028±0.025, mean ± S.E.M., n=7).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Poster Communications

PCA053

Cardiac action of the first G protein biased small molecule apelin agonist

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Pulmonary arterial hypertension (PAH) has a poor prognosis and is associated with pulmonary vasoconstriction, right ventricular hypertrophy and right heart failure. Current therapies aim to reduce vasoconstriction but do not benefit the heart and more efficacious treatments are required.

The peptide apelin signals through the apelin receptor to produce vasodilatation and cardiac inotropy, while its expression is decreased in PAH. Importantly, the apelin receptor is not down-regulated and infusion of apelin is beneficial in animal models\textsuperscript{1}. As a peptide, apelin is not an optimal drug-like molecule owing to its lack of bioavailability, limited half-life and rapid internalisation of the receptor through β-arrestin signalling. We hypothesise that a G protein biased small molecule apelin agonist could replace the missing endogenous peptide to produce vasodilatation and an improvement in cardiac remodelling of the right ventricle without receptor desensitisation. We characterised, \textit{in vitro} and \textit{in vivo}, the pharmacology of a novel small molecule agonist, CMF-019, demonstrating G protein bias at the apelin receptor.

In competition radioligand binding experiments in heart homogenates CMF-019 bound to human, rat and mouse apelin receptors with high affinity (pK\textsubscript{D}= 8.58±0.04, 8.49±0.04 and 8.71±0.06 respectively). In cell-based functional assays, whereas CMF-019 showed similar potency for the G\textsubscript{a}i pathway to the endogenous agonist [Pyr\textsuperscript{1}]apelin-13 (pD\textsubscript{2}=10.00±0.13 n=11/4 and pD\textsubscript{2}=9.34±0.15 n=8/4 respectively), in β-arrestin and internalisation assays it was much less potent (pD\textsubscript{2}=6.65±0.15 n=13/4 vs pD\textsubscript{2}=8.65±0.10 n=12/4 and pD\textsubscript{2}=6.16±0.21 n=6/2 vs pD\textsubscript{2}=9.28±0.10 n=6/2 respectively). Experiments were performed in triplicate where possible and results are expressed as mean±SEM. For cell-based assays, n-values are given as the number of replicates/number of experiments. Bias analysis was performed using the methodology of van der Westhuizen et al. (2014)\textsuperscript{2} and bias factors of ~400 for signalling through the G\textsubscript{a}i compared to the β-arrestin pathway and ~5800 compared to receptor internalisation were obtained.

Normotensive male Sprague-Dawley rats (273 ± 6g) were induced and maintained under anaesthesia with inhaled isoflurane (3% and 1.5% respectively) carried by oxygen (1.5l/min) and a pressure-volume catheter placed in the left ventricle to measure cardiac parameters. Intravenously injected CMF-019 (2500µg) caused a significant increase in cardiac contractility (dP/dt\textsubscript{max}, 833±152mmHg/s n=9) compared to saline (88.7±94.4mmHg/s n=3) (p<0.001, student’s t-test).

CMF-019 is the first biased small molecule identified at the apelin receptor and displays activity \textit{in vivo}, the pharmacology of a novel small molecule agonist, CMF-019, demonstrating G protein bias at the apelin receptor.

C. Read, C. Fitzpatrick, P. Yang, R. Kuc, J. Maguire, R. Glen, R. Foster and A. Davenport


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**PCA054**

Cardiac and skeletal sarcoplasmic reticulum K⁺ channels from TRIC-A knockout mice show distinct gating characteristics to those derived from wild type mice

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Evidence suggests that the proteins termed TRIC-A and TRIC-B behave as K⁺ channels in the sarcoplasmic reticular (SR) of cardiac and skeletal muscle and that there is approximately 10 fold more TRIC-A than TRIC-B present in both muscle types (1-3). We here compare the single channel properties of SR K⁺ channels derived from wild type (WT) and TRIC-A knockout (KO) cardiac and skeletal muscle SR as we might expect to observe only TRIC-B channels from KO tissue but a more heterogeneous population of channels from WT tissue.

Single SR K⁺ channels from cardiac or skeletal WT and TRIC-A KO SR were incorporated into planar lipid bilayers in symmetrical 210 mM KPIPES, pH 7.2, under voltage-clamp conditions as previously described (4). SR vesicles incorporated in a fixed age-clamped at potentials relative to the luminal side which was held at ground. Mean values ± SEM were compared by Student’s t test. The single channel conductance of SR K⁺ channels from WT and TRIC-A KO mice were similar for both cardiac (WT=210.1±1.4 pS, n=7; TRIC-A KO=211.8±1.1 pS, n=15) and skeletal muscle (WT=210.8±2.7 pS, n=25; TRIC-A KO=211.1±1.8 pS, n=36).

A characteristic of SR K⁺ channels is that they gate into multiple sub-conductance levels and this was observed for cardiac and skeletal channels from both WT and TRIC-A KO mice, however, the percentage of time that the channels dwelt in a sub-conductance level was higher in channels from TRIC-A KO mice. For example, at -30 mV, the sub-conductance openings contributed only 41% (n=16) towards the total open probability (Po) of skeletal WT channels in comparison to 86% (n=18, p<0.001) of the total Po in skeletal KO channels. The overall Po of single (lone) channels was also significantly lower in channels derived from the skeletal muscle of TRIC-A KO mice (for example, at +30 mV, WT Po=0.22±0.06, n=17 and TRIC-A KO Po=0.09±0.01, n=22; p<0.01). When multiple channels were gating, however, it was found that the channels derived from TRIC-A KO mice behaved in a positively cooperative manner exhibiting higher Po than when a single channel was gating in the bilayer; this was not the case for channels from WT mice. When both TRIC subtypes are present (SR from WT mice), since TRIC-A is much more abundant, it is likely that TRIC-A will predominantly incorporate into the bilayer. When TRIC-A is not present (TRIC-A KO), the remaining SR K⁺ channels, presumably TRIC-B, gate with lower Po, gate more in sub-conductance open states and exhibit positive cooperativity in their gating. The physiological relevance for the different gating behaviour of these two populations of SR K⁺ channel is not yet understood but may be linked to the different expression patterns for TRIC-A and TRIC-B in different cell types.

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**PCA055**

Contribution of nitro-oxidative stress on carotid body chemosensory potentiation and hypertension induced by chronic intermittent hypoxia

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Obstructive sleep apnoea (OSA) is recognized as an independent risk factor for hypertension. Among disturbances produced by OSA, chronic intermittent hypoxia (CIH) is considered the main factor for hypertension. Although the link between CIH and hypertension is well known, the mechanisms responsible for the hypertension are not entirely known. It has been proposed that CIH produces oxidative stress, inflammation, and sympathetic hyperactivity, which contributes to the hypertension. However, a growing body of evidence show that carotid body (CB) chemoreceptors play a crucial role in the augmented sympathetic drive and the hypertension. We aimed to determine whether peroxynitrite (ONOO⁻) formation contributes to enhance the CB chemosensory activity and the hypertension during CIH exposure. Accordingly, we study the effects of Ebselen, a specific ONOO⁻ scavenger, on 3-nitrotyrosine immunoreactivity (3-NT-ir) in the CB and the chemosensory discharges in rats exposed to CIH. In addition, we tested whether chronic treatment with Ebselen may reverse the CIH-induced hypertension. Experimental procedures were approved by the Bio-ethical Committee of the Biological Sciences Faculty, P. Universidad Católica de Chile, and were performed according to the National Institutes of Health Guide, USA. Male Sprague-Dawley rats (200g) were implanted with indwelling catheter for radiotelemetric BP measurements. After one week of recovery, rats were exposed to CIH (5% O₂, 12 times/h for 8 h) for 7 days. Then, rats received either Ebselen (10 mg/kg/day) or vehicle treatment (DMSO in saline) via subcutaneous implantation of osmotic pumps while the animals continue to be exposed to CIH for 7 days. At the end of the experiments, under sodium pentobarbital (40 mg/kg i.p.) anesthesia the CB chemosensory response to hypoxia was recorded, and then rats were perfused with paraformaldehyde 4% for 3-NT-ir assay. Exposure to CIH increased 3-NT-ir within
the CB, enhanced CB chemosensory responses to hypoxia, and produces diurnal hypertension. Ebselen treatment produced a significant reduction in CB 3-NT-ir levels (60.8±14.9 vs. 22.9±4.2 a.u., CH+veh vs. CH+Ebs, respectively; p<0.05), reduced the potentiated CB chemosensory response to 5% O2 (266.5±13.4 vs. 168.6±16.8 Hz, CH+veh vs. CH+Ebs, respectively; p < 0.05) and reversed the elevated mean BP (116.9±13.2 vs. 82.1±5.1 mmHg, CH+veh vs. CH+Ebs, respectively; p< 0.05). Thus, Ebselen prevented the CH-induced CB chemosensory potentiation and reversed the elevated BP, suggesting that CH-induced CB chemosensory potentiation and hypertension are critically dependent on ONOO- formation.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA056

Acute exposure to the uremic toxin indoxyl sulphate can increase reactive oxygen species and contraction in isolated rat ventricular myocytes

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Protein-bound uremic toxins such as indoxyl sulphate (IXS) accumulate in the blood of patients with chronic kidney disease (CKD) and are poorly cleared by dialysis. IXS is associated with cardiovascular mortality and incidence of heart failure in CKD. Its mechanisms of action are unclear but it has been reported to increase reactive oxygen species (ROS) in endothelial cells (Dou et al., 2007). In this study we tested whether IXS generates ROS and modulates contractility in ventricular myocytes.

Single ventricular myocytes were isolated from the hearts of male Wistar rats. Cells were loaded with the ROS indicator CM-H2DCFDA (DCF) for 20 minutes at a concentration of 2 µM. Cells were superfused with HEPES-buffered Tyrode’s solution then exposed to this solution in the presence or absence of IXS (200 µM) or H2O2 (50 µM) for 2 minutes at 37 °C. Cells were briefly exposed to excitation light of 475 nm (Optoscope, Cairn Research) and emitted fluorescence was measured at 510 nm. DCF fluorescence and sarcomere length were simultaneously measured in myocytes that were stimulated to contract by external Pt electrodes at a frequency of 1Hz. Sarcomere length was measured by fast Fourier transform of a video image of the cell (IonWizard, IonOptix).

After 2 minutes exposure, DCF fluorescence in response to Tyrode fell to 0.91 ± 0.04 of initial fluorescence, (n = 20 myocytes), in response to IXS, mean fluorescence was 1.00 ± 0.03 (n = 35 myocytes) and after exposure to H2O2 fluorescence was increased to 1.08 ± 0.05 (n = 29 myocytes), (P < 0.05 Tyrode vs H2O2, 1-way analysis of variance). However in response to IXS, there was a statistically significant, positive correlation between the change in fluorescence and change in sarcomere length shortening (Pearson’s correlation coefficient 0.53, P < 0.05). In 22 of 31 myocytes exposed to IXS, fluorescence was above the mean value for Tyrode exposure (0.91). In these myocytes sarcomere shortening increased by 16 ± 6 %. In contrast, in 9 myocytes exposed to IXS where fluorescence was less than 0.91, sarcomere shortening fell by 19 ± 6 % (P = 0.002, unpaired t-test).

We conclude that when exposure to IXS leads to generation of ROS in ventricular myocytes there is an associated increase in contraction. This may be linked to a cAMP-independent activation of protein kinase A (Burgoyne et al., 2012). Chronic stimulation of ROS production or PKA activation by IXS could be detrimental to the myocardium in CKD. Further study to understand the variable response of myocytes to IXS is in progress.


Supported by the Yorkshire Kidney Research Fund

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA057

Characterising hERG channel kinetics using sinusoidal voltage protocols and mathematical modelling

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The KCNH2 gene (known as hERG in humans) encodes the primary subunit of the rapid delayed rectifier potassium current IKr. IKr modulates cardiac action potential repolarisation and hERG expression affects action potential morphology. Consequently, the representation of hERG channel kinetics within mathematical action potential models has a great influence on simulations of cardiac electrical activity. This is true in control conditions but is also particularly evident when simulating the effects of drug block.

Mathematical electrophysiology modelling forms a core part of a new proposal for routine preclinical cardiac safety assessment of pharmaceutical compounds (Sager et al., 2014). It is therefore important that hERG channel kinetics are accurately represented within mathematical models of cardiac electrophysiology to be used for such simulations.

Many different mathematical models have been proposed to describe hERG channel kinetics. These models demonstrate a wide variety of behaviours when used to simulate both standard voltage-step and action potential protocols. We question whether all observed behaviours are expected experimentally; and if so, which model should we select to represent hERG within an action potential model in order to simulate cardiac electrical activity as accurately as possible?

We have designed novel sinusoidal voltage protocols to rapidly explore hERG channel kinetics. Using a Bayesian inference approach we assessed the ability to use the protocols to parameterise hERG channel models. We recorded currents in response to these protocols in patch clamp experiments using hERG-transfected HEK-293 cells.

The aim of this study was to determine the most appropriate model to describe hERG channel kinetics. We used experimental current recordings in response to the sinusoidal voltage protocols to parameterise a selection of candidate model structures. We then validated each model by assessing its ability to predict currents in response to traditional voltage-step protocols recorded from the same cell.
We identify model structures which are both able to describe the experimental data to which they were fitted and additionally make representative predictions of the validation data. This provides some indication that the selected models encapsulate the kinetics of the ionic current, and are not simply a description of the data to which they were fitted. This study demonstrates the necessity of careful consideration of experimental design, model parameterisation and model selection when constructing mathematical ion channel kinetic models. Such considerations are likely to be important in determining more predictive mathematical models of cardiac electrophysiology.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**PCA058**

**Distribution of 7 ion channels’ transcript level on 18 left ventricle segments**

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A tremendous amount has been learned over the last decade regarding the ionic and molecular basis of cardiac regional electrical specialization. Regional variations of ion channel subunits in specific cellular subtypes in complex regions of the left ventricle remained largely unknown. There is no debate over the importance of these ion channels in the normal functioning of the heart and in arrhythmogenesis. Therefore, the aim of the present study was to explore the ion channel distribution of KvLQT, minK, ERG, NCX, Cx43, SERCA2a, Nav1.5 in 9 - 9 segments of the left ventricle epi- and endocardial wall. Hearts were isolated from adult male NZW rabbits (n=8-5, 2.0–2.5 kg) following pre-medication with medetomidine hydrochloride (0.2 mg/kg), ketamine (10 mg/kg), and butorphanol (0.05mg/kg) (sc.). Animals were sacrificed by an overdose of pentobarbitone sodium (111 mg/kg, iv.)\(^1\). All procedures were undertaken in accordance with ethical guidelines set out by the UK ASPA. LV tissue samples were dissected 18x1mm² pieces and were cleaned from free running Purkinje fibers, and Papillary muscles. Q-PCR was performed using SYBR green technology\(^2, 3\). Experimental data points in triplicate were analysed using ΔCt method to compare the relative abundance of different transcripts with 28S, 18S or GAPDH. Statistical significant differences were accepted at p values of *p<0.05, **p<0.01 using ANOVA with Tukey’s Post hoc test.

Using ΔCt method in the distribution of KvLQT, ERG, Nav1.5 and Cx43 genes there were tendency differences at a non-significant level. In SERCA2a and NCX there were no differences transmurally and through the base-apex axis. Using ΔΔCt method comparing minK expression level in base to apex or epi- to endocardium, there were no significant differences through 8 transmural and 5 base/apex sites. MinK was 2.019±0.25 times more abundant in the epi-, than in the endocardium in the mid posterior wall, and 1.77±0.31 times higher in the apex than the base in the anterior apical region, which is in contrary with our previous finding, where protein level of minK was lower in the apex than the base\(^1\). Presumably this discrepancy can be attributable to differences in location in the LV. Most of the no differences are in line with ion channel distribution found in the neonate rabbit ventricle\(^2\). In previous findings it has been shown\(^2\) that KvLQT, ERG, Nav1.5, minK expression level decreased transmurally in basal free wall of the adult rabbit LV. Reason for the different finding can be amongst lots of other criteria the different tissue collection method.

However, the aim of the present study was to clarify the previous findings and give a deeper understanding of the transmural and base-apex differences in ion channel distribution, it still remained incompletely understood. Further studies are planned for reaching success.
Seasonal changes in cholinergic response in the atrial myocardium of Arctic navaga cod (Eleginus navaga, Gadidae)

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Several freshwater fishes of north-temperate latitudes exhibit marked seasonal changes in electrical activity as an outcome of temperature-dependent changes in the density of major ionic currents: delayed rectifiers (IKr, IKs) and inward rectifiers (IK1). In winter-acclimatized (WA) navaga could be well explained with respective seasonal changes in IK1 density. The density of IKACh induced by 10⁻⁵M CCh and measured at 0 mV was 2.8±0.5 pA/pF in SA navaga, but only 0.26±0.11 pA/pF in WA navaga. Thus, WA induces more than 10-fold decrease in IKACh density, which results in blunted cholinergic response of atrial myocardium in comparison with SA fish. To our knowledge IKACh is the only current in the fish heart, which decreases during the winter acclimatization.

This study was supported by the Russian Science Foundation (14-15-00268).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA060

Contrast-enhanced magnetic resonance imaging of the human foetal heart reveals its myocardial architecture

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The normal cardiac development of the human foetal heart is poorly described quantitatively. Animal and cell models have been used in the past to describe cardiac physiology; however their developmental processes and cellular properties have not been in complete agreement with human development requiring further optimisations. In this study, we have used human datasets to study foetal cardiac (specifically ventricular) changes occurring during gestation.

Human foetal hearts were obtained from elective termination of pregnancy, with informed written parental consent. Temporary storage followed by imaging of the hearts was in premises licensed by the 2004 Human Tissues Act (UK) and all protocols had ethical committee approval.

We apply magnetic resonance imaging (MRI) in combination with a contrast agent (gadolinium; Gd-DTPA) and specialised protocols to visualise the ex vivo human foetal heart at different gestational stages at high resolution. Hearts were fixed in 4% formaldehyde and immersed in Fomblin prior to MRI.
scanning. 3D Fast Low Angle Shot (FLASH) MRI was used to obtain detailed geometry of foetal hearts ranging from 90 to 143 days of gestational age (DGA) with isotropic cubic voxels 0.05mm³, 300 averages, echo time = 5.3ms, repetition time = 15ms and a flip angle of 30 degrees. Scanning time was an average of 120 hours per heart. All acquisitions took place in a Bruker BioSpin 9.4T vertical MRI/S system. All the 3D reconstructed hearts showed a clear laminar alignment of local tissue architecture within the foetal ventricles. Images analysed with Seg3D (https://www.sci.utah.edu/) and OsiriX (http://www.osirix-viewer.com/) demonstrated that the direction of maximum image contrast (as illustrated via the use of Gd-DTPA) corresponds to the direction of the tectal eigenvector of the measured diffusion tensor i.e. the normal to the sheet architecture direction [1]. FLASH MRI has confirmed the orientation of foetal ventricular myocytes and visualised their sheet directions and distributions. The concentric architecture observed in DT MRI [1] is also observed here with a 4-fold higher resolution.

FLASH MRI confirms the early isotropy and the later development of anisotropy in the human foetal heart. Average fractional anisotropy of a human foetal ventricular myocardium at 100DGA is 0.1 and at 143DGA is 0.6, with smooth transmural changes developing after 126DGA.

Pervolaraki E, Anderson RA, Benson AP, Hayes-Gill B, Holden AV, Moore BJR, Paley MN, Zhang H (2013). Antenatal architecture and increased cardiorespiratory morbidity and mortality. A classic diseases of “dusty” occupations may be on decline, but they are not yet extinct. Studies have found associations between changes in ambient particulate air pollution and increased cardiorespiratory morbidity and mortality. A cross-sectional comparative study design was employed on 127 male nonsmoker cobblestone paving workers and 194 matched employed office workers as a reference in order to assess changes in pulmonary function related to dust exposure among cobblestone road paving workers of Jimma zone, Ethiopia. Data were collected using structured questionnaires and spirometric measurements after ethical clearance was obtained. Unpaired t-tests was used to examine the differences between the groups. P-values equal or less than 0.05 were considered statistically significant; odds were calculated at a 95% confidence interval. Cobblestone road paving workers were found with higher odds of respiratory symptoms, dry cough (p < 0.05), cough (p < 0.01) and sore throat (p < 0.001) compared to the reference. The forced expiratory volume one (FEV1) cobblestone road paving workers ranged between 3.12 - 4.73 L with a mean of 3.96 ± 0.6 L, significantly lower than the reference groups who had a range of 3.3 - 4.78 L and a mean of 4.01 ± 0.6 L (p < 0.05). The mean value of the ratio of FEV1 and forced vital capacity ratio (FEV1/FVC) was significantly decreased in the cobblestone road paving workers compared to the controls, 87.2 ± 4.3, 89.5 ± 5.4, respectively (p = 0.01). In conclusion, the study revealed clear evidence of the need for health education and for the promotion of activities directed towards mitigating respiratory hazards in order to foster a safe and healthy work environment.

### Table 1

<table>
<thead>
<tr>
<th>Respiratory symptoms</th>
<th>Cobblestone workers</th>
<th>Reference group</th>
<th>OR</th>
<th>CI</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry cough</td>
<td>17 (13.4%)</td>
<td>11 (5.9%)</td>
<td>2.571</td>
<td>1.000 - 6.119</td>
<td>0.028*</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>9 (7.6%)</td>
<td>10 (5.2%)</td>
<td>1.403</td>
<td>0.506 - 3.872</td>
<td>0.634</td>
</tr>
<tr>
<td>Cough with sputum</td>
<td>12 (9.4%)</td>
<td>4 (2.1%)</td>
<td>3.497</td>
<td>1.438 - 8.689</td>
<td>0.007**</td>
</tr>
<tr>
<td>Sore throat</td>
<td>17 (13.4%)</td>
<td>5 (2.6%)</td>
<td>5.842</td>
<td>1.956 - 16.800</td>
<td>0.001*</td>
</tr>
<tr>
<td>Chest wheezing</td>
<td>8 (6.4%)</td>
<td>6 (3.1%)</td>
<td>1.490</td>
<td>0.578 - 4.309</td>
<td>0.372</td>
</tr>
<tr>
<td>Diagnosed LRTI*</td>
<td>9 (7.6%)</td>
<td>7 (3.0%)</td>
<td>1.907</td>
<td>0.629 - 5.807</td>
<td>0.316</td>
</tr>
</tbody>
</table>

* Significant, **Severe respiratory tract infection.

### Table 2

<table>
<thead>
<tr>
<th>Pulmonary functions</th>
<th>Cobblestone workers</th>
<th>Reference group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC</td>
<td>4.4 (0.5)</td>
<td>4.5 (0.6)</td>
<td>0.16</td>
</tr>
<tr>
<td>FEV1</td>
<td>3.96 (0.6)</td>
<td>4.01 (0.6)</td>
<td>0.03**</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>87.2 (4.3)</td>
<td>89.5 (5.4)</td>
<td>0.01*</td>
</tr>
</tbody>
</table>

**Significant.

### PCA061

#### Exposure to occupational dust and changes in pulmonary function among cobblestone paving workers of Jimma, Ethiopia

K.H. Abate and M.I. Sadik

Biomedical Sciences, Jimma University, Jimma, Ethiopia

The classic diseases of “dusty” occupations may be on decline, but they are not yet extinct. Studies have found associations between changes in ambient particulate air pollution and increased cardiorespiratory morbidity and mortality. A cross-sectional comparative study design was employed on 127 male nonsmoker cobblestone paving workers and 194 matched employed office workers as a reference in order to assess changes in pulmonary function related to dust exposure among cobblestone road paving workers of Jimma zone, Ethiopia. Data were collected using structured questionnaires and spirometric measurements after ethical clearance was obtained. Unpaired t-tests was used to examine the differences between the groups. P-values equal or less than 0.05 were considered statistically significant; odds were calculated at a 95% confidence interval. Cobblestone road paving workers were found with higher odds of respiratory symptoms, dry cough (p < 0.05), cough (p < 0.01) and sore throat (p < 0.001) compared to the reference. The forced expiratory volume one (FEV1) cobblestone road paving workers ranged between 3.12 - 4.73 L with a mean of 3.96 ± 0.6 L, significantly lower than the reference groups who had a range of 3.3 - 4.78 L and a mean of 4.01 ± 0.6 L (p < 0.05). The mean value of the ratio of FEV1 and forced vital capacity ratio (FEV1/FVC) was significantly decreased in the cobblestone road paving workers compared to the controls, 87.2 ± 4.3, 89.5 ± 5.4, respectively (p = 0.01). In conclusion, the study revealed clear evidence of the need for health education and for the promotion of activities directed towards mitigating respiratory hazards in order to foster a safe and healthy work environment.

### PCA062

#### Sex differences in cardio-renal functions of Sprague-Dawley rats fed a high salt diet

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¹Physiology, University of Lagos, Idi-Araba, Surulere, Nigeria and ²Biochemistry, University of Lagos, Idi - Araba, Surulere, Nigeria

High Dietary salt intake is an important risk factor for cardiovascular and renal diseases (1). However, responses to a high salt diet exhibit sexual dimorphism (2). To determine how sex affects cardio-renal functions response to a high salt diet (HSD), 20 weanling Sprague-Dawley rats (10 male and 10 females) were divided into 4 groups of 5 rats each that were fed a normal diet (0.3% NaCl) or HSD (8% NaCl)
for 12 weeks. Fluid balance (FB) was determined from 24hrs water intake and voided urine. Terminal BP (mmHg) was measured via arterial cannulation under anaesthesia (25% w/v urethane and 1% w/v α-chloralose; 3ml/Kg, i.p.). Serum levels of troponin I (ng/ml), creatinine (μmol/l), urea (μmol/l) and electrolytes and urinary concentration of albumin (gm/24hr), creatinine (μmol/24hr), urea (mmol/24hr), and electrolytes were measured using appropriate assay kits. Values are mean ± S.E.M, compared by one-way Anova. In the male rat, HSD increased BP (112.3 ± 3 vs. 139.4 ± 4; p < 0.001), serum: troponin I (0.42 ± 0.07 vs. 3.24 ± 0.06; p < 0.001), LDH (744.8 ± 20.6 vs. 970.4 ± 38.8; p < 0.001), urea (9.91 ± 0.56 vs. 15.89 ± 0.78; p < 0.001) sodium (131.4 ± 2.40 vs. 142.0 ± 2.33 (mmol/l); p < 0.01), urinary: albumin (0.48 ± 0.01 vs. 1.13 ± 0.05; p < 0.001), urea (0.38 ± 0.002 vs. 1.03 ± 0.02; p < 0.001), sodium (0.24 ± 0.01 vs. 1.12 ± 0.06 (mmol/24hr); p < 0.001), potassium (0.097 ± 0.001 vs. 0.24 ± 0.06 (mmol/24hr); p < 0.05) and FB (4.34 ± 0.4 vs. 10.27 ± 1.49; p < 0.01). In the female rat, HSD increased BP (108.0 ± 3 vs. 126.3 ± 3; p < 0.01) serum: troponin I (4.22 ± 0.07 vs. 2.49 ± 0.04; p < 0.001) LDH (579.28 ± 24.2 vs. 794.0 ± 19.87; p < 0.001), urea (8.80 ± 0.95 vs. 17.41 ± 0.53; p < 0.001) sodium (133.6 ± 1.44 vs. 143.6 ± 1.50; p < 0.01), creatinine clearance (0.013 ± 0.02 vs. 0.20 ± 0.07 (ml/min); p < 0.05), urinary: albumin (0.75 ± 0.03 vs. 1.01 ± 0.01; p < 0.001), urea (0.59 ± 0.001 vs. 1.55 ± 0.01; p < 0.001), sodium (0.39 ± 0.004 vs. 2.4 ± 0.18 (mmol/24hr); p < 0.001) and potassium (0.14 ± 0.001 vs. 0.32 ± 0.004 (mmol/24hr); p < 0.01). However, HSD increased BP (139.4 ± 4 vs. 126.3 ± 3; p < 0.05), serum: troponin I (3.24 ± 0.05 vs. 2.49 ± 0.04; p < 0.001) LDH (790.4 ± 38.8 vs. 799.0 ± 19.87; p < 0.001), urinary albumin (1.13 ± 0.05 vs. 1.01 ± 0.03; p < 0.05) and FB (10.27 ± 1.49 vs. 6 ± 1.55; p < 0.05) in male rats, while HSD increased urinary: urea (1.55 ± 0.011 vs. 1.03 ± 0.02; p < 0.001) and sodium (2.4 ± 0.18 vs. 1.12 ± 0.06; p < 0.001) more in female rats. Basal values in male vs. female of serum: LDH (744.8 ± 20.6 vs. 579.2 ± 24.2; p < 0.001), urinary: albumin (0.48 ± 0.01 vs. 0.75 ± 0.13; p < 0.001) and urea (0.38 ± 0.002 vs. 0.59 ± 0.001; p < 0.001) were significantly different. Thus sex plays an important role in the response of the heart and kidney to salt stress and male rats show a greater deleterious effect on HSD as compared to CONT rats. Increased salt intake lowered number of granulocytes and their phagocytic activity in ANG but not in CONT rats. No differences between groups were found in plasma levels of TNFa and IL-6 suggesting that systemic inflammation was not developed at this stage. In conclusion, our results demonstrate changed peripheral and tissue distribution of immune cells in adult male progeny of female rats with experimentally elevated ANG II levels during pregnancy. Increased immune cell infiltration in the kidney of ANG rats indicates development of renal inflammation, which in turn may link adverse prenatal environment with altered cardiovascular functions in adulthood.

Supported by the grants VEGA 1/0557/15, APVV-0291-12.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**PCA063**

**Elevated angiotensin II during pregnancy promotes renal inflammation in male rat offspring**

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Suboptimal conditions experienced in utero can change the developmental program of an individual and result in an increased risk of cardiovascular diseases in adulthood. However, the potential physiological mechanisms beyond this link are still not recognized. Our previous results indicate that disturbed homeostasis of renin-angiotensin-aldosterone system in rats during pregnancy can lead to increased blood pressure of male progeny in adulthood. To investigate underlying mechanisms between adverse prenatal environment and development of increased blood pressure we analysed effects of sustained infusion of angiotensin II (ANG) in pregnant rats on the adult immune status of male offspring. Female Wistar rats were either sham operated (n=4) or implanted with osmotic minipumps (n=5) continuously releasing ANG II at a dose of 2ug kg-1 h-1 for 14 days from day 6 of pregnancy. A mixture of ketamine (75mg kg-1) and xylazine (10mg kg-1, i.p.) was used as anaesthesia. Progeny of these females was assigned to control (CONT, n=12) and ANG (n=13) rats, respectively. From age 3 till 4 months a half of the animals from each group was fed with an increased-salt diet (0.8% sodium). During this period, immunohistochemistry of venous blood and functional analysis of granulocytes were performed by flow cytometry. Finally, rats were killed under CO2 anaesthesia and pro-inflammatory cytokines, tumor necrosis factor alpha (TNFa) and interleukin 6 (IL-6), were measured in the plasma and renal inflammation was evaluated by immunochemistry. Reduced numbers of CD8+ T-cells and monocytes were found in circulation of ANG than CONT rats. This corresponded with higher renal infiltration of T-cells and macrophages in ANG as compared to CONT rats. Increased salt intake lowered number of granulocytes and their phagocytic activity in ANG but not in CONT rats. No differences between groups were found in plasma levels of TNFa and IL-6 suggesting that systemic inflammation was not developed at this stage. In conclusion, our results demonstrate changed peripheral and tissue distribution of immune cells in adult male progeny of female rats with experimentally elevated ANG II levels during pregnancy. Increased immune cell infiltration in the kidney of ANG rats indicates development of renal inflammation, which in turn may link adverse prenatal environment with altered cardiovascular functions in adulthood.

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**PCA064**

**Chronic phase advance shifts desynchronize endocrine rhythms and enhance responsivity of the cardiovascular system to norepinephrine in rats**

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*Animal Physiology and Ethology, Comenius University Bratislava, Faculty of Natural Sciences, Bratislava, Slovakia*

Disturbances of circadian oscillations can have negative effects on cardiovascular functions but epidemiological data are inconclusive and new experimental data from animal experiments elucidating critical biological mechanisms are needed. We studied consequences of chronic phase advance shifts of light/dark (LD) cycle on the endocrine and cardiovascular system control. The study was approved by the local ethical committee and veterinary authority. In experiment 1, mature male rats were exposed either to the stable LD 12:12 (CONT, n=36) or rotating 8-h phase advance shifts of LD three times per week (SHIFT, n=36) for 10 weeks. Blood pressure (BP) was monitored weekly by plethysmography. Rats were killed under CO2 anaesthesia in 4-h intervals over 24 hours and daily rhythms of plasma melatonin, corticosterone and leptin were determined. In experiment 2, male rats (CONT,
n=9 and SHIFT, n=5) were exposed to the identical LD treatment as in experiment 1 for 12 weeks and daily rhythms of BP and heart rate (HR) were measured by telemetry. Telemetry transmitters were implanted under ketamine (75mg kg⁻¹) and xylazine (10mg kg⁻¹, i.p.) anaesthesia. During week 12, animals were treated with norepinephrine (NE; 200 µg kg⁻¹, s.c.) in the middle of the light and dark phase. Circadian rhythms were evaluated with Lomb-Scargle Periodogram by Chronos-Fit software. Effect of NE was evaluated as an area under the curve (AUC) using a trapezoidal rule. Data were analysed with repeated analysis of variance. We found preserved melatonin rhythms in SHIFT rats with damped amplitude in comparison with CONT suggesting that the central oscillator could adapt to chronic phase shifts, but its power was attenuated. Daily rhythms of corticosterone and leptin were present in CONT and disappeared in SHIFT rats suggesting that disturbances of the circadian system may occur downstream of the central oscillator. SHIFT rats did not exhibit the BP increase as compared to CONT but disturbed circadian rhythms in BP and HR were observed. Administration of NE to CONT during the daytime resulted in a BP increase (+39%; 140 min) while saline increased BP only temporarily (+19%; 30 min). NE administration to CONT rats induced a higher increase of BP during the passive phase (+36%) in comparison with the active phase (+23%). In SHIFT rats we found a more pronounced response of BP after NE in comparison to CONT without difference between the passive (+35%) and active (+34%) phase. Our results revealed an internal desynchronization in the endocrine system and weakened circadian control of BP and HR under chronic phase advance shifts with a pronounced response to NE especially during the passive phase. We conclude that the attenuated circadian control can enhance a response of the cardiovascular system to sympathetic activation with a potential negative impact on physiology and behaviour.

Supported by APVV 0291-12 and VEGA 1/0557/15

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

**PCA065**

**hERG potassium channel modulation by mutant T58P/L59P KCNE1**

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Heritable Long QT Syndrome (LQTS) is genetically heterogeneous. The KCNE1 protein forms the accessory subunit of slow delayed rectifier current (Iₖₛ) potassium channels through interaction with KCNQ1; KCNNE1 mutations are responsible for the LQT5 variant of LQTS (Moss and Kass, 2005). There is evidence that KCNE1 can additionally interact with hERG which carries the rapid delayed rectifier K⁺ current, Iₖᵣ; (McDonald et al, 1997); clinically relevant KCNE1 variants can influence hERG current (Iₖₑᵣ) magnitude (e.g. Ohno et al, 2007; Du et al, 2013). The T58P/L59P KCNE1 double-mutant occurs in Jervell and Lange-Nielsen syndrome (JNS); it has been reported to impair assembly with KCNQ1 and causes a severe attenuation of Iₖₑᵣ through disruption of an interaction site located in the transmembrane region of KCNE1 (Harmer et al, 2010). This study was undertaken to investigate whether or not the T58P/L59P double mutant can influence Iₖₑᵣ modulation by KCNE1. Whole-cell patch-clamp measurements of Iₖₑᵣ were made at 37°C from HEK293 cells stably expressing hERG that were transiently co-transfected with wild-type (WT) or mutant KCNE1 together with Green Fluorescent Protein (GFP) as a transfection marker. With a conventional voltage-step protocol, Iₖₑᵣ end-pulse density at +20 mV was measured as 30.18±4.88pA/pF (mean±SEM; n=11) with WT KCNE1 and this was increased to 87.94±17.32pA/pF (n=8) for T58P/L59P KCNE1 (p<0.005; unpaired t test). Iₖₑᵣ tail density, observed at −40 mV following step depolarisation to +20 mV, was 58.64±7.56pA/pF (n=11) for WT KCNE1 and 157.92±32.68pA/pF (n=8) for T58P/L59P KCNE1 (p<0.005; unpaired t test). Under ventricular action potential (AP) clamp, maximal Iₖₑᵣ density during AP repolarisation was also greater for T58P/L59P KCNE1, without a significant voltage shift of peak current during the AP command. Iₖₑᵣ was also compared with WT and T58P/L59P KCNE1 using 2s depolarizing voltage steps from −80 mV to potentials between −40 mV and +60 mV. Mean current–voltage (I–V) relations demonstrated marked elevation of the Iₖₑᵣ tail density for T58P/L59P KCNE1 (Two way ANOVA, p<0.01 compared to WT KCNE1). In separate experiments, WT or T58P/L59P KCNE1 was co-expressed with KCNQ1 and, as demonstrated previously (Harmer et al, 2010), T58P/L59P KCNE1 markedly suppressed the measured current. Collectively, the results of these experiments provide evidence that the KCNE1 transmembrane segment T58P/L59P double-mutant, which causes severe attenuation of Iₖₛ, can markedly augment Iₖₑᵣ. This finding both provides further evidence for differential modulation of Iₖₛ by WT and variant KCNE1 and suggests that the nature of the interaction(s) with KCNE1 may differ between hERG and KCNQ1.


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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

**PCA066**

**Respiratory changes in rats during postnatal development (P0-26) following prenatal fluoxetine exposure**

V. Biancardi, K. Cardoso Bicego and L.H. Gargaglioni

Physiology, FCAV/UNESP, Jaboticabal, Brazil

Serotonin (5-HT) is a neurotransmitter involved in nervous developmental processes, being an important modulator of


respiratory rhythm via activation of 5-HT_{1A} and 5-HT_{2A} receptors on respiratory neurons that contribute to hypercapnic and hypoxic ventilatory responses. Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine act as antidepressants and are generally prescribed in depression therapy, including to pregnant women. This study investigated the effects of prenatal (E15-21) exposure to fluoxetine on the ventilatory and metabolic responses to 7% CO\textsubscript{2} (hypercapnia) and 10% O\textsubscript{2} (hypoxia) of male and female rats during postnatal development (P0-26). To this end, osmotic pumps were implanted subcutaneously in pregnant female rats at embryonic day (E) 15 and delivered vehicle (VEH) or fluoxetine (FLX, 10 mg/Kg/day) during 7 days. Respiratory frequency (FR), ventilation (VE) and O\textsubscript{2} consumption (VO\textsubscript{2}) of pups from these litters were studied. In P0 male rats, the FLX group showed a higher respiratory frequency (FR) in room air conditions [VEH: 95.1\pm7.0 bpm (breaths per minute)]. There was no difference in the responses to hypercapnia across the two groups, but during hypoxia, VE/VO\textsubscript{2} ratio was attenuated in the FLX animals (VEH: 79.2\pm5.5; FLX: 61.6\pm6.7). At P6, male FLX animals presented a higher VE/VO\textsubscript{2} ratio during hypercapnia (VEH: 56.6\pm9.3 vs FLX: 79.4\pm6.5), and an attenuated VE/VO\textsubscript{2} ratio during hypoxia (VEH: 96.9\pm4.5 vs FLX: 78.2\pm4.1). No differences were observed between male rats in the VEH and FLX groups at P12 and P26. In P0 female rats, FLX animals showed a higher hypercapnic ventilatory response (VEH: 2072.3\pm196.3 vs FLX: 2521.2\pm167.0 mL/Kg/min) and higher VE/VO\textsubscript{2} ratio (VEH: 45.5\pm5 vs FLX: 63.0\pm2.0) but no changes were observed during hypoxia. Fluoxetine-exposed females at P6 showed a higher FR in room air conditions (VEH: 126.7\pm5.1 bpm vs FLX: 160.9\pm8.5 bpm), and no differences were observed during hypercapnia and hypoxia across the two groups. At P12, FLX females showed attenuated hypercapnic ventilatory response (VEH: 3549.6\pm160.3 vs FLX: 2692.8\pm340.1 mL/Kg/min) and attenuated VE/VO\textsubscript{2} ratio (VEH: 97\pm11.9 vs FLX: 64.8\pm8.1), but no differences were observed during hypoxia. It is also of note that P26 females displayed a higher VO\textsubscript{2} in room air conditions (VEH: 49.8\pm4.5 vs FLX: 69.8\pm5.8 mL/Kg/min), and a higher ventilatory response to hypoxia (VEH: 4695.6\pm342.7 vs FLX: 5640\pm290.7 mL/Kg/min). During hypoxia, P26 female FLX animals presented an accentuated VO\textsubscript{2} drop (14% for VEH; 22% for FLX) and lower FR (VEH: 175.7\pm14.5 vs FLX: 152.6\pm8.5 bpm). Taken together, these data indicate that SSRI exposure during the prenatal period results in long lasting and sex specific changes in the ventilatory and metabolic responses to respiratory challenges.

Financial support: FAPESP, CNPq.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**Beneficial effects of exercise training in the Goto-Kakizaki type 2 diabetic rat heart**

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Physical exercise continues to be a cost-effective strategy to help in slowing the progression of diabetes-induced cardiovascular complications. However, the molecular mechanisms by which regular exercise can improve cardiac function in type 2 diabetes (T2DM) remain poorly characterised. This study investigated the physiological and molecular changes that arise in hearts of 10-11 month old Goto-Kakizaki (GK) type 2 diabetic rats following 2-3 months of daily treadmill exercise compared to sedentary counterparts. Shortening and intracellular [Ca\textsuperscript{2+}], transients were measured in ventricular myocytes with video edge detection and fluorescence photometry, respectively. Structure and gene expression were assessed in ventricular tissue with electron microscopy, immunohistochemistry and real-time RT-PCR. Ethical clearance for the project was obtained from the College of Medicine and Health Sciences, UAE University and the University of Central Lancashire.

Exercise training in GK rats significantly (mean\pmSEM; p<0.05; ANOVA; n=15-18) reduced blood glucose level (133.07\pm9.79 mg/dl vs 161.29\pm12.77 mg/dl) and body weight (418.93\pm6.80 g vs 443.64\pm7.94 g vs) and increased heart weight (1.64\pm0.03 g vs 1.60\pm0.04 g) and heart weight to body weight ratio (0.392\pm0.013 vs 0.361\pm0.008) compared to sedentary GK animals. The amplitude of shortening decreased slightly in ventricular myocytes from exercised GK compared to sedentary GK rats. However, the amplitude of [Ca\textsuperscript{2+}]\textsuperscript{0} transients increased in ventricular myocytes from exercised GK compared to sedentary GK rats. LV from sedentary GK rats displayed disorganised architecture which was characterised by hypertrophied cardiomyocytes, disarray of myofibres and irregular myofibril pattern compared to exercised GK animals. Exercise training in GK rats significantly (p<0.05) reduced interstitial fibrosis, caspase-3-mediated apoptosis, ANP, BNP, TGF\textsubscript{beta}1, and gene expression of extracellular matrix components and regulators associated with cardiac fibrosis compared to sedentary GK animals. Moreover, exercise training significantly (p<0.05) increased the expression of genes encoding sodium–calcium exchanger, phospholamban and Calmodulin2 (CALM2), but not SERCA2a,ryanodine receptor, Ca\textsubscript{1.2}, Ca\textsubscript{1.3} and CaMKd compared to sedentary GK rats. It is concluded that exercise training can reduce blood glucose concentration, thereby improving the integrity of the myocardium against diabetes.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Pulmonary Epithelial Cells are a Source of Gremlin1 in the Lung

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University College Dublin, Dublin, Ireland

Hypoxic pulmonary hypertension (HPH) is a common complication of chronic lung diseases. Current treatments are only partially effective. Gremlin1 contributes to pulmonary vascular homeostasis by antagonizing Bone Morphogenetic Protein signaling. Pulmonary vascular remodeling in response to hypoxia in the lung is accompanied by lung-specific changes in gremlin1 expression, suggesting gremlin1 antagonism may be a novel therapeutic target. Ubiquitous gremlin1 halopedeiciency in mice attenuates the structural changes seen in the lung in response to hypoxia [1]. This work aimed to identify cellular sources of gremlin1 in the lung and quantify gremlin1 expression in Type II alveolar epithelial cells (ATII) using surfactant protein C (SPC) as a cell marker.

Male mice (C57BL6/J, 8 weeks old) were exposed to normoxic and hypoxic conditions (FiO2=0.10) for 2 days or 3 weeks. After exposure, mice were sedated using 2% isoflurane, deeply anaesthetised via IP injection of sodium pentobarbitone (60mg/Kg) and euthanised by exsanguination. Lungs were fixed in paraformaldehyde and embedded in paraffin wax. Immunohistochemical (IHC) staining and double immunofluorescence (IF) were employed to explore in vivo gremlin localisation. ‘Threshold’ and ‘watershed segmentation’ features in EBImage (Biocorpus) were used to identify ATII [2]. This automated method of identification was tested by comparing the number of ATII identified to the number identified by three independent observers. Fluorescence intensity was used as an index of gremlin1 expression, and quantified using the R programming language in EBImage.

IHC staining of lung sections localised gremlin1 expression to small airway epithelial cells and endothelial cells of large vessels. Staining in alveolar walls suggested gremlin1 expression in alveolar epithelial cells and macrophages. Double IF staining for gremlin1 and SPC confirmed gremlin1 expression in ATII under normoxic and hypoxic conditions. The ATII identified by EBImage were also identified by three independent observers. EBImage reliably detected ATII when SPC expression was uniform throughout the cell. Differences in the number of ATII detected between EBImage and independent observers were attributed to cells where SPC staining was punctate. Such staining represented ~20% of ATII detected by independent observers. Gremlin1 fluorescence intensity within ATII was significantly greater than that in all other gremlin1 expressing cells combined in alveolar tissue (n=9; P<0.001; T-test).

We report in vivo confirmation that ATII and small airway epithelium express gremlin1. EBImage software successfully identified ATII in which SPC distribution was uniform, but not when punctate. Gremlin1 quantification suggests ATII are a major cellular source of gremlin1 in alveolar tissue. Future work will investigate the functional role of ATII-derived gremlin1 in HPH.


Science Foundation Ireland.

The Roughton-Forster extrapolation of DLCO versus PO2 does not give its diffusive component

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In 1957, Roughton and Forster proposed a decomposition of carbon monoxide diffusing capacity (DLCO), also called the transfer factor (TLCO), as a sum of two resistances in series namely a membrane plus plasma resistance (1/DmCO) and a blood resistance (1/DiVc). Based on oxygen partial pressure (PO2) dependence of DLCO at large pressure, Forster et al. (1957) suggested that the diffusive component (DmCO) could be obtained by linear extrapolation of 1/DLCO to zero PO2, where the blood resistance becomes zero.

Here, we mathematically examine the same process, i.e., capture of CO by haemoglobin (Hb) after diffusion from the gas, with a minimal model of the physico-chemical phenomena responsible for capture, namely diffusion and reaction. In the frame of diffusion-reaction, the diffusive component DmCO is supposed to be the value of DLCO when the reaction time of CO and Hb is so fast that the red blood cell plays a role of a sink for CO. The diffusion-reaction equations have been solved for three different morphology of red blood cells: flat-parallel surfaces, spherical RBCs in a cylindrical capillary and biconcave RBCs in a cylindrical capillary.

The results, obtained from analytical solutions for the flat case and numerical (Finite Element Method) solutions for the spherical and biconcave cases, demonstrate properties that are common to the three different RBC morphologies. First, 1/DLCO versus the CO-Hb reaction time (τco) is only approximately linear. Consequently, the linear extrapolation method proposed by Roughton and Forster does not give 1/DLCO(τco=0). Therefore, the supposed correspondence between DmCO and the value of DLCO extrapolated from large PO2 is erroneous. The figure below illustrates the dependence of 1/DLCO(τco) as a function of τco in the case of biconcave RBC in a cylindrical capillary. One observes that for small τco corresponding to small PO2, the dependence is not linear. Note that for standard PO2 = 100 mmHg, the value of τco = 0.5 ms (Kang and Sapoval, 2016).

As similar results are found for the other morphologies, one has to conclude that the classical interpretation of DLCO is not soundly based.

Thus, an alternative interpretation of DLCO is needed. Such an alternative has been proposed recently by Kang and Sapoval (2016). The new interpretation is based on the idea that for a CO molecule to be captured, it must travel by diffusion from the gas to the Hb molecule during a duration called Δ, then it has to react which needs on average a time τco. So the time for capture is simply Δ + τco.
Institute of physiology, Medical faculty, Ljubljana, Slovenia, and Institute of Pharmaceutical Biology, Faculty of Pharmacy, Ljubljana, Slovenia.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

**PCA070**

**Early heart rate variability alterations in rodent model of cardiomyopathy**

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Autonomic tone imbalance plays an important role in the pathophysiology of the heart disease (1). A noninvasive tool that allows study of the autonomic cardiovascular modulation is the analysis of heart rate variability (HRV) (2). The alterations in autonomic modulation may be an initiating mechanism underlying the onset of cardiovascular diseases (3). Thus we aimed to test the hypothesis that HRV could be used for early detection of cardiomyopathy. A rodent model of heart failure induced by doxorubicin (DOX), otherwise an effective drug for the cancer treatment, was used in our study. Adult male Wistar rats were treated for 2 weeks with saline (control, n=7) or doxorubicin (1.5mg DOX/kg per week) (DOX group, n=7). In the early period after DOX treatment (7 days after the last application) (4) rats were anesthetized with ketamine (75mg/kg) and xylazine (9mg/kg) and standard 6 channel ECG (Cardiax, IMEDE, Budapest, Hungary) was measured for 3 minutes using subcutaneous needle electrodes. During the autopsy, hearts were gathered for histological examination. The experiment was approved by the National Animal Ethical Committee and conducted in accordance with the European Convention for the protection of vertebrate animals used for experimental purposes. HRV was analyzed in time and frequency domain (SA aHRV, Nevrokard, Medistar, Slovenia) and One way ANOVA was applied to find significant changes between groups. Power spectrum analysis was evaluated by the total power and by the power of two spectral bands: low frequency component (LF: 0.25–0.75 Hz) and high frequency component (HF: 0.75–3 Hz) according to previous studies (5). The LF/HF ratio was also calculated. Administration of DOX significantly decreased parasympathetically mediated HF component of HRV expressed in normalized units (64.02±2.87 compared to 78.18±5.01 in control rats, P<0.05) and increased normalized LF component of HRV (31.85±2.24 compared to 20.28±3.44 in control rats, P < 0.05), resulting in an overall increased LF/HF ratio (0.51±0.05 compared to 0.28±0.08 in control rats, P < 0.05). No changes were found in heart rate and time domain HRV indexes. In all DOX treated animals early myocardial lesions were found, histologically classified as myofibrillar loss, parenchymal degeneration and lymphoid infiltration. Our study confirmed that the modulation of autonomic nervous system activity accompanied the development of heart failure in rodents and could reflect subtle microscopic changes characteristic for the early phase of the disease. Our results recommend HRV as a novel tool for early detection of cardiomyopathy.


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**PCA071**

**Electrophysiological effects of β-adrenergic stimulation on pharmacologically induced LQTS 1&2**

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Long QT Syndrome (LQTS) 1&2 are life-threatening conditions that arise from dysfunction of slow (IKs) and rapid (IKr) components of the delayed rectifier K+ current respectively. The consequential impairment of ventricular repolarisation predisposes individuals to ventricular fibrillation (VF). It is known that sympathetic surges often precede VF in LQTS, but mechanisms of initiation are not understood. We aim to use pharmacological models of LQTS 1&2 in combination with the β-adrenoreceptor (β-AR) agonist isoproterenol (ISO), to mimic sympathetic stimulation to examine effects on the ventricular electrophysiology.

Adult male Dunkin Hartley guinea pig hearts (400-600g, n=12) were used in constant flow Langendorff mode following a licenced non-Schedule 1 culling procedure. Ventricular Monophasic Action Potentials (MAPs) were recorded at apical and basal sites and measured at 90% decay (MAPD90). Effective refractory period (ERP) / action potential duration restitution (RT) and ventricular fibrillation threshold (VFT) were measured using an extrastimulus and burst pacing protocol respectively. All tests were performed at control, ISO (10nM), LQTS drug and ISO + LQTS drug. HMR-1556 (HMR; 0.5µM, n=6) and E4031 (0.05µM, n=6) were used to replicate LQTS 1 & 2 respectively. Data is shown as mean±SEM and analysed using ANOVA, p<0.05 was considered significant.
Both HMR and E4031 have a bradycardic effect, but the application of ISO to LQTS models increased HR significantly compared to control. β-AR stimulation had different effects on the LQTS models: in LQT1, the presence of ISO caused a flattening of the RT curve, but in LQT2 the RT curve gradient was significantly steeper than in control conditions. Both ERP and VFT were increased during HMR and E4031 perfusion, but decreased when ISO was applied to both models. The preliminary data suggest that both LQTS 1&2 are associated with an increase in VF susceptibility when sympathetic activity is enhanced, in terms of ERP and VFT. However, there is a dichotomy in the effect on RT slope gradient between the two models, where β-AR stimulation flattens the RT slope in LQTS 1 model but steepens the RT slope in LQTS 2 model; this implies that there may be different mechanisms involved in arrhythmogenesis.

Construction and evaluation of clinical, blood-derived and combined frailty models in sheep

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Frailty is correlated with but not inevitable in advanced age. Higher frailty is associated with longer hospital stays and worse risks of infection and postoperative complications (Partridge et al., 2012). The ageing population in western nations has lead to a rise in the frail population, but there is no consensus on assessment methods for frailty, many of which are time-consuming and involved. Furthermore, while murine models are key to our understanding of frailty there is a need to develop a Frailty Index (FI) in a large animal model for future translation to clinical practice. We have designed and evaluated clinical (FIClinical) and blood-derived (FILab) defict-accumulation Frailty Indices (see Mitniski et al., 2001) in sheep, and observe the effects of ageing on the FI and cardiac function as measured by fractional shortening. Old (>8 years, n=4) and young (~18 months, n=7) female welsh mountain sheep were monitored for blood biochemistry parameters and echocardiographic fractional shortening. The FIClinical was determined using a 49-item questionnaire, refined over the course of the study by removing consistently non-scoring elements. All measures were repeated weekly. Control young sheep (n=19) were used to calculate the norm range (±1.5 standard deviations from the mean) for each parameter of a standard metabolic blood panel.

For the FILab, blood values within the normal range scored 0 and outside the normal range scored 1. The average of these scores is the FILab. Data is presented as mean ± SEM. Statistical analysis was performed using a Student’s t-test or two-way ANOVA.

FIClinical increased with age in sheep (0.06 ± 0.02 young vs. 0.15 ± 0.03 old, n=4-7, p<0.05). FILab also increased with age (0.07 ± 0.02 young vs. 0.45 ± 0.03 old, n=4-7, p<0.001). An increase in FIClinical was associated with a loss of fractional shortening (p<0.05; Figure 1). Mean FILab score was higher than the FIClinical score, possibly reflecting earlier visibility of the effects of ageing on the biochemical (FILab) level before the phenotype (FIClinical) level.

Table 1: a – Control vs ISO, b – Control vs Drug, c – Control vs ISO+Drug, d – ISO vs Drug, e – ISO vs ISO+Drug, f – Drug vs ISO+Drug

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Cardiac sympathetic afferent denervation improves renal dysfunction in a post-MI rat model

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Renal impairment is a common and independent risk factor of morbidity and mortality in patients with chronic heart failure (CHF). We hypothesized that impaired renal function is mediated, in part, by the cardiac sympathetic afferent reflex (CSAR), a cardiogenic sympatho-excitatory reflex that is activated during an acute myocardial infarction (MI) or in CHF. We previously demonstrated that chronic and selective CSAR denervation at the time of MI by epicardial application of resiniferatoxin (RTX) markedly reduced renal sympathetic nerve activity (RSNA) in rats ~10 weeks post MI (1). We evaluated renal function by simultaneously measuring blood K⁺, creatinine and blood urea nitrogen (BUN) in sham-operated
or MI rats treated with epicardial application of RTX (50ug/ml) or vehicle at ~6 weeks, ~12 weeks and ~18 weeks post MI. Compared to sham-operated rats, MI+vehicle and MI+RTX rats had normal blood K+, creatinine and BUN at ~6 weeks and ~12 weeks post MI, indicating that renal dysfunction may less likely occur at the early and middle stages of CHF. However, compared to the 12 week post MI time point, plasma K+, creatinine and BUN were significantly increased at the 18 weeks post MI (K+: 4.10±0.08 to 4.70±0.07 mmol/L; Creatinine: 0.47±0.02 to 0.70±0.05 mg/dL; BUN: 22.9±0.7 to 30.8±1.7 mg/dL; P<0.05, n=13), suggesting that renal dysfunction occurs in the late stage of CHF. Compared to MI+vehicle at ~18 weeks post MI, MI+RTX rats at the same time point exhibited almost normal blood K+ (4.19±0.09 mmol/L), creatinine (0.55±0.04 mg/dL) and BUN (25.8±1.1 mg/dL), suggesting improved renal function in RTX-treated MI rats. Infarct size between MI+RTX and MI+vehicle groups were similar, excluding the possibility that differences in infarct size caused the difference in renal function between these two groups. In order to evaluate the potential molecular changes in the kidneys of the post-MI rats treated with epicardial RTX, we harvested the kidney tissues from the above sham, MI+Vehicle and MI+RTX rats (n=4-5 per group) at ~18 weeks post MI to run the mRNA sequence analysis. Our data demonstrated that compared to the age-matched sham rats, several renal injury and inflammatory gene markers (Havcr1 also named KIM-1, ATF3, CXCL10, IGFBP1, SOCS3, HMOX1, LCN2 also named NGAL, BTG2, LAMC2, RND1) were significantly upregulated by 20-66 fold in MI+vehicle rats ~18 weeks post MI. Compared to MI+vehicle, CSAR ablation by RTX selectively reduced the gene expression of Havcr1 (a specific proximal tubular damage marker) by 4 fold, indicating a potential protective effect by RTX on proximal tubular dysfunction in MI rats at ~18 weeks post MI. These data suggest that pathological activation of the CSAR in the post-MI state plays a critical role in mediating the potential renal damage occurring in the late stages of CHF.


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PCA075

Calcium-activated Nedd4-2 mediates the downregulation of cardiac sodium channel Nav1.5 in heart failure

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Ventricular arrhythmias are the major cause of heart failure (HF)-associated deaths. Previous studies demonstrated that HF is associated with raised diastolic Ca²⁺ (1) and decreased expression and dysfunction of the cardiac voltage-gated sodium channel Nav1.5 (2). These changes may provide both triggers and a slowed-conduction substrate for cardiac arrhythmias (3). However, the mechanisms of Nav1.5 down-regulation in HF have not been fully elucidated. The ubiquitin ligase Nedd4-2, which is well expressed in the heart and activated by elevated calcium concentration, is a major factor for Nav1.5 heart rate control is an important adaptive mechanism during passive heating, a sudden increase in the heart rate should induce heatstroke. Wistar-Kyoto male rats 13-week old were implanted with telemetric transmitters to monitor ECG, aortic pressure and body temperature. During the surgery, general anesthesia with ketamine 120 mg/kg and xylazine 6mg/kg was used. The rats were randomly divided into 2 groups (n = 10) and examined in a climatic chamber with an air temperature of 44.4 °C. Time-frequency analysis (Wigner-Ville) was applied to estimate the high-frequency power of RR-interval variability (HFRRi), a measure of cardiac parasympathetic activity. The spontaneous baroreflex sensitivity (sBRS) was estimated with the cross-spectral method. Values represent the mean (standard deviation) compared with the t-test. During the passive heating, RR-interval (RRI) became progressively shorter with an average speed of 1.4 (0.2) ms/min. The permanent decline in the arterial pressure that began at the core body temperature (Tc) of 43 (0.4) °C heralded the beginning of the heatstroke; the RRI reached 110 (14) ms and systolic pressure 218 (5) mmHg at this moment. Oxyphenonium (10 µmol/kg) i.p. injection at Tc of 40 °C abruptly reduced RRI from 183 (12) to 120 (7) ms (p<0.001), sBRS from1.5 (0.05) to 0.1 (0.03) ms/mmHg (p<0.001) and HFRRi from 13.8 (0.7) to 0.6 (0.06) ms²(p<0.001). Heart rate-pressure double product rose from 5.6x10⁶ (4.0x10⁵) to 8.4x10⁶ (7.0x10⁵) bpm.mmHg (p<0.001), suggesting a strongly elevated myocardial oxygen demand. Although hemodynamic remained stable and the arterial pressure and RRI rose steadily further, the time needed to develop the heatstroke was significantly (p<0.002) shorter by 4.2 (0.3) min. This was associated with the increased speed of Tc elevation from 0.093 (0.008) to 0.112 (0.009) °C/min (p<0.001), probably a consequence of reduced salivation after oxyphenonium parasympatholysis, which is documented by strongly diminished HFRRi. Conclusion: Muscarinic blockade mildly accelerated heatstroke development, however probably not as a consequence of tachycardia, but because of the interference with evaporation cooling.

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PCA074

Parasympatholytic tachycardia does not induce hypotension during the passive hyperthermia

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Baroreflex was repeatedly postulated as an essential mechanism of hemodynamic adaptation to elevated ambient temperature. However, the exact role of the baroreflex in this adaptation is less clear. Because tachycardia leads to increased myocardial oxygen demand, reduced myocardial perfusion, and ventricular filling, we have hypothesized that baroreflex restraining effect on heart rate elevation is an important component of the adaptation to the hot environment. To confirm the role of unharnessed tachycardia in heatstroke development, we used a muscarinic blocker to remove vagal restraint on the heart rate by desensitizing the baroreflex. If baroreflex
NaV1.5 expression but decreased pA/pF, catalytically inactive mutant, Nedd4-2-C801S. Furthermore, expression and current were decreased by Nedd4-2 transfection and internalization which may be either degraded or recycled back to plasma membrane.


Degradation (4). Thus, we hypothesized that elevated diastolic calcium in HF increases Nedd4-2 expression, leading to reduced membrane density of Nav1.5. Male Sprague-Dawley rats (140-160g, n=12) were used to establish a volume-overload HF model by producing abdominal arterio-venous fistula (5). Western blot and immunofluorescence assay were used to detect the expression and co-localization of Nav1.5 and Nedd4-2 in heart tissues and isolated cardiomyocytes. Cell biology and patch clamp were further performed to investigate the mechanisms of Nav1.5 downregulation in HEK293 cells stably expressing Nav1.5 (Nav1.5-HEK). Values are presented as mean ± S.E.M., and one-way ANOVA was used for multiple comparisons. Nav1.5 expression is decreased whereas Nedd4-2 expression during the development of hypertrophy and heart failure.

Calcium-mediated degradation (4) was elevated by ionomycin (IM) treatment. In Nav1.5-HEK cells, 24-hour IM treatment decreased Nav1.5 expression in whole-cell lysates and membrane fractions, and reduced the peak sodium current (I_{Na} density: IM 24h -153.30±13.76 vs. Ctrl -318.06±36.49 pA/pF, P<0.01). However, 6-hour IM treatment did not significantly alter total Nav1.5 expression but decreased I_{Na} density: IM 6h -134.05±16.74 vs. Ctrl -318.06±36.49 pA/pF, P<0.01. The gating properties of Nav1.5 were not significantly altered by 24-hour or 6-hour IM treatment. Nav1.5 expression and current were decreased by Nedd4-2 transfection and further decreased under 6-hour IM treatment. These effects were not observed in cells transfected with the catalytically inactive mutant, Nedd4-2-C801S. Furthermore, elevated [Ca\(^{2+}\)]\(_{i}\) increased Nedd4-2 expression and enhanced the interaction between Nav1.5 and Nedd4-2 indicated by co-immunoprecipitation in Nav1.5-HEK cells. In conclusion, Nav1.5 is downregulated and co-localizes with Nedd4-2 and ubiquitin in a volume-overload HF model. Calcium-mediated expression and activation of Nedd4-2 reduces Nav1.5 expression and currents in Nav1.5-HEK cells. These data suggest a role of Nedd4-2 in Nav1.5 downregulation in HF.

The atrioventricular node (AVN) is critical to normal cardiac conduction and can also take over ventricular pacemaking should the sinoatrial node fail. AVN activity is generated by multiple sarcolemmal ionic currents (Hancox et al., 2010). The present study was undertaken to investigate in AVN cells: the distribution of IP3 receptors, the effect of cell permeant IP3 analogue on ICa and IKr and effects of caged IP3 on spontaneous activity. AVN cells were isolated from adult male New Zealand White rabbits in accordance with UK Home Office legislation. Double immunocytochemistry was carried out using IP3-R2 and RyR2 antibodies and imaged using confocal microscopy. Spontaneous APs and currents were recorded using the whole-cell patch clamp method at 37 °C. Data are presented as mean ± SEM. Statistical comparisons were made using a paired t-test, with significance denoted by P<0.05. IP3-R2 labelling was observed in transverse bands inside the cell and at the cell periphery where it was partially co-localised with RyR2 (n=6). Release of caged IP3 (100 μM in pipette solution) by UV flash photolysis rapidly increased spontaneous AP rate by 32.1±9.5% (n=7; P<0.05). The slope of pacemaker diastolic potential of APs increased from 73.4±17.6 to 110.5±14.0 mV/s (n=7; P<0.05), whilst AP overshoot, maximal diastolic potential and duration at half-maximal repolarization were unaffected (n=7; P>0.05). Application of the cell permeant IP3 analogue Bt1-Ins(145)P2/AM (10 μM) did not significantly affect L-type ICa amplitude (-13.2±2.6 pA/pF and -11.7±1.6 pA/pF at +20 mV in control and 10 μM IP3/AM solutions, respectively; n=6; P>0.05), or I_{Kr} tails at -40 mV (2.2±0.3 pA/pF and 2.1±0.2 pA/pF, respectively; recorded following step.

Figure 1. Proposed scheme illustrating that calcium reduces Nav1.5 expression by activating Nedd4-2 in heart failure. Increased [Ca\(^{2+}\)]\(_{i}\) “leakage” through malfunctioned RyR2 channel elevated cytosolic Ca\(^{2+}\) level, enhances Nedd4-2 expression and activity. The WW domain of Nedd4-2 binds to the PY motif of Nav1.5 to initiate Nedd4-2-mediated ubiquitination and internalization which may be either degraded or recycled back to plasma membrane.
depolarization to +20 mV, n=6; P>0.05). Collectively, these data provide further evidence of functional IP$_3$-R expression in AVN cells. Modulation of spontaneous AP rate by IP$_3$-R activation is unlikely to involve direct effects on L-type ICa or IKr.


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Potential therapeutic role for erythropoietin mimetic, ARA 290, in a mouse model of emphysema

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Chronic lung diseases, including emphysema, are a leading cause of death worldwide. The pathogenesis of emphysema is extensively debated; however recent research indicates that vascular loss may play a key initiating role. Inhibition of vascular endothelial growth factor receptors lead to emphysematous changes in the adult rat lung (1), whilst co-administration of erythropoietin (EPO) prevented similar disease development in a neonatal mouse model (2). EPO administration may deleteriously augment haematocrit, therefore, a non-haematopoietic-EPO mimetic, ARA 290 (ARAIM Pharmaceuticals) has been developed which specifically activates the EPO tissue-protective signalling pathway via activation of the innate repair receptor (EPOR/µCR). We hypothesised that ARA 290 would attenuate disease development in a neonatal mouse model of emphysema, potentially by preventing the loss of alveolar capillaries. Values are expressed as mean ± S.E.M, compared by ANOVA with Bonferroni Post-Hoc analysis. * indicates significant difference from all groups (p<0.05).

Data generated to date suggests a potential therapeutic role for ARA 290 in this setting of emphysema, however further work is warranted.

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Data generated to date suggests a potential therapeutic role for ARA 290 in this setting of emphysema, however further work is warranted.
Heparin enhances the effects of Mesenchymal stem cell transplantation in a rabbit model of acute myocardial infarction
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Mesenchymal stem cells (MSCs) derived from bone marrow are now considered as powerful therapeutic cells to treat diseases such as myocardial infarction (MI) (1). However, stem cell transplantation in combination with administration of bioactive compounds has shown more promising results for treating MI (2). In the current study, we investigated the effect of MSCs injection followed by heparin infusion into the ischemic heart of MI induced rabbits. Male New Zealand white rabbits (n=35) were randomly divided into five groups: (1) sham (2) MI (3) MI + MSCs (4) MI + heparin and (5) MI + MSCs + heparin. Animals underwent general anesthesia (2% sodium pentobarbital; 40 mg/kg i.p.). Next, they were intubated and ventilated by room air using a rodent ventilator (tidal volume 2–3 ml, respiratory rate 65–70 per minute, Harvard rodent ventilator model 683, Holliston, MA, USA). Left intercostal thoracotomy (between the two and three costal space) was performed under sterile condition. MI was induced to 30 min left anterior descending coronary artery (LAD) ligation with a 5-0 silk suture. Successful performance of coronary occlusion is confirmed by observation of the development of a pale color in the distal myocardium after ligation as well as dyskinesia of the anterior wall. The animals of MSCs and MSCs + heparin groups were injected with 150 \( \mu \)l of cell culture containing MSCs (3 × 10^6) into both sides of coronary arteries.

Functional parameters of the left ventricle (LV) by echocardiography, serum levels of VEGF by enzyme-linked immunosorbent assay (ELISA), size of fibrotic area by Masson’s trichrome staining, amount of pyknotic nuclei by Haematoxylene-Eosin (H&E) and angiogenesis by CD31 immunostaining were measured and compared in treated and non-treated animals. Histological studies were done 8 weeks after surgery. Our data showed that the injection of MSCs with heparin infusion and injection of MSCs alone could significantly improve the LV functional parameters (Ejection Fraction (EF) and Fraction shortening (FS)) but not Left ventricular end diastolic dimension (LVEDD), and increase VEGF levels in serum of MI induced animals. Our data showed significant reduction of fibrotic tissue, infarct size and increased number of live cells in MSCs and MSCs + heparin treated rabbits compared to control animals. Although injection of MSCs and MSCs + heparin significantly restored normal function of fibrotic area, we found that administration of heparin after MSCs injection to MI animals could have better effects on LV functional parameters in fibrosis area.

Fig1: Comparison the amount of VEGF in serum of different study groups. Significant increase at the level of VEGF could be found in MSCs + heparin treated animals in compare to control.

Fig2: Quantitative analysis of infarct size at 8 weeks after myocardial infarction. There was significant difference between MI+MSCs and MI+MSCs+heparin with MI group


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True molecular scale analysis of the calcium release machinery of the heart with enhanced super-resolution imaging
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The calcium (Ca^{2+}) signals underlying the contractile function of cardiomyocytes originate from ryanodine receptors (RyR) located within intracellular signalling sites known as ‘couplons’. In the healthy heart, the spatiotemporal properties of the unitary Ca^{2+} signals (sparks) require rapid co-activation and subsequent closing of RyRs as a result of (a) the close packing of RyRs into clusters and (b) the structural organisation of the couplon by its primary molecular tether, Junc-tophilin-2 (JPH2). Recent super-resolution imaging has been unable to fully resolve single RyRs and partner proteins within the clusters due to resolution still limited to ~ 30 nm. We have used greatly enhanced super-resolution imaging based on the new DNA-PAINT method (1) to visualise the nanoscale
molecular arrangement of RyR and JPH2 in peripheral couplings of ventricular myocytes at a resolution of 5-10 nm. Male Wistar rats weighing ~300 g (n = 8) were euthanized using a Schedule 1 non-recovery procedure approved by the University of Exeter animal ethics committee. Hearts were then dissected; ventricular myocytes were enzymatically isolated, fixed and immunolabelled against RyR2 and JPH2 as described previously (2). DNA-PAIT imaging (1) was performed using a modified total internal reflection fluorescence (TIRF) microscope. In regions identified as RyR clusters in dSTORM images, DNA-PAIT revealed arrays of punctate labelling, each reporting single RyRs within a coupling. Analysis of these receptor locations revealed that RyRs are spaced at distances of 43.2 ± 0.4 nm (n = 1802 clusters), similar to recent measurements on limited electron tomograms (3) but greater than the in vitro RyR packing distances observed in artificial bilayers (4). This loose arrangement was not explained by the remaining RyR localisation errors. It manifested as (a) observable gaps within RyR clusters accounting for 35.5 ± 0.5% (n = 2062) of the cluster area and (b) fewer RyRs present within peripheral clusters (7.3 ± 0.2 RyRs per cluster; n = 2062) than previously estimated with super-resolution dSTORM (2). These new measurements need to be incorporated into simulations of Ca²⁺ spark genesis (5) and termination to understand the measurements need to be incorporated into simulations of Ca²⁺ spark genesis (5) and termination to understand the

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Intracellular tortuosity underlies slow cAMP diffusion in adult ventricular myocytes

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Poster Communications

PCA080

Many effects of cAMP on cardiac myocytes can only be explained in terms of microdomain signaling. Enzymes that make and break cAMP are involved in the formation of microdomains, and their role in cAMP signaling has been studied extensively. The precise geometry of microdomains depends critically on cAMP diffusion, but this is poorly understood. Earlier estimates have postulated fast cAMP diffusion, which argues against the microdomain hypothesis. Accurate determination of cAMP diffusivity requires high-resolution cAMP sensors, robust methods to evoke cAMP gradients, and appropriate protocols and analyses to dissect true diffusivity from the complex diffusion/reaction dynamics of cAMP.

[cAMP] dynamics in the cytoplasm of adult rat ventricular myocytes were imaged using a fourth generation genetically-encoded FRET-based sensor. Myocytes were enzymatically isolated from adult male Sprague-Dawley rats. Cells were infected adenovirally with the construct for H187, the cAMP sensor. The [cAMP] response to addition and removal of isoproterenol (ISO; β-adrenoceptor agonist) quantified the rates of cAMP synthesis and degradation. To obtain a read-out of DcAMP, a stable [cAMP] gradient was generated using a microfluidic device which delivered agonist to one half of the myocyte only. After accounting for phosphodiesterase activity, a [cAMP] gradient evoked by regional exposure to 1µM ISO produced an estimate of DcAMP of 35±3.4 µm²/s (n=11/4), an order of magnitude slower than cAMP diffusivity in water (444 µm²/s). Diffusivity was not dependent on the amount of cAMP generated (DcAMP measured with 10mM ISO was 32±8.7 µm²/s; n=15/4; P=0.78 vs 1µM ISO). Saturating cAMP-binding sites with the cAMP analogue 6-Br-cAMP (5µM, delivered as AM-ester) did not accelerate DcAMP (29±7.1 µm²/s, n=11/3; P=0.45 vs 1µM ISO) arguing against a role for buffering in restricting cAMP mobility. Molecules that are chemically-unrelated to cAMP but of comparable molecular weight (fluorescein, MagFluo4) had similar diffusivity when measured using fluorescence recovery after photobleaching (FRAP; fluorescein, 37.0±4.5 µm²/s, n=8/3; P=0.73 vs DcAMP; MagFluo4, 37.9±4.4 µm²/s n=8/3; P=0.66 vs DcAMP) suggesting that restricted mobility relates to a common physical barrier to diffusion consistent with tortuosity. Tortuosity was greater in adult myocytes compared to rat neonatal myocytes as measured by calcine diffusivity. Dcalcine (normalised to Dcalcine in pure water) was higher in neonatal myocytes (0.29±0.04, n=6/4 vs 0.13±0.01, n=16/3; P<0.05), in agreement with the 2.5-fold greater non-mitochondrial space in neonatal cytoplasm compared to adult myocytes (17±1.3, n=9/3 vs 44±1.2, n=12/3; P<0.05 determined by TMRE staining). In adult cardiac myocytes, tortuosity due to physical barriers, notably mitochondria, restricts cAMP diffusion to 32 µm²/s; a level that is more compatible with microdomain signaling.

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ISO produced an estimate of DcAMP of 35±3.4 µm²/s (n=11/4), an order of magnitude slower than cAMP diffusivity in water (444 µm²/s). Diffusivity was not dependent on the amount of cAMP generated (DcAMP measured with 10mM ISO was 32±8.7 µm²/s; n=15/4; P=0.78 vs 1µM ISO). Saturating cAMP-binding sites with the cAMP analogue 6-Br-cAMP (5µM, delivered as AM-ester) did not accelerate DcAMP (29±7.1 µm²/s, n=11/3; P=0.45 vs 1µM ISO) arguing against a role for buffering in restricting cAMP mobility. Molecules that are chemically-unrelated to cAMP but of comparable molecular weight (fluorescein, MagFluo4) had similar diffusivity when measured using fluorescence recovery after photobleaching (FRAP; fluorescein, 37.0±4.5 µm²/s, n=8/3; P=0.73 vs DcAMP; MagFluo4, 37.9±4.4 µm²/s n=8/3; P=0.66 vs DcAMP) suggesting that restricted mobility relates to a common physical barrier to diffusion consistent with tortuosity. Tortuosity was greater in adult myocytes compared to rat neonatal myocytes as measured by calcine diffusivity. Dcalcine (normalised to Dcalcine in pure water) was higher in neonatal myocytes (0.29±0.04, n=6/4 vs 0.13±0.01, n=16/3; P<0.05), in agreement with the 2.5-fold greater non-mitochondrial space in neonatal cytoplasm compared to adult myocytes (17±1.3, n=9/3 vs 44±1.2, n=12/3; P<0.05 determined by TMRE staining). In adult cardiac myocytes, tortuosity due to physical barriers, notably mitochondria, restricts cAMP diffusion to 32 µm²/s; a level that is more compatible with microdomain signaling.

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Increased spontaneous contractile activity in cardiac ventricular myocytes from caveolin-3 knockout mice
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Heart failure is associated with increased risk of arrhythmia (1) and, at the cellular level, transverse-tubule (TT) network disruption (2), increased Na-Ca exchange (NCX) activity (1) and reduced caveolin-3 (Cav-3) expression (3). Cav-3 is a scaffolding protein which is implicated in TT formation (4) and in regulation of ion channels involved in excitation-contraction coupling, including the L-type Ca channel. However, little is known about whether and how altered Cav-3 expression affects TT structure, ryanodine receptor (RyR) and NCX activities.

Ventricular myocytes were isolated from 3 month old Cav-3 knockout (KO) mice and wildtype (WT) C57Bl/6 control mice. Animal procedures were approved by the local ethics committee and conducted in accordance with UK legislation. Spontaneous contractile activity (SCA) was assessed at different [Na]o in intact and detubulated (DT) cells, to distinguish SCA from gross changes in TT morphology, it may be involved in TT formation. Cav-3 KO mice and wildtype (WT) C57Bl/6 control mice. Ventricle myocytes were isolated from 3 month old Cav-3 knockout (KO) mice and wildtype (WT) C57Bl/6 control mice. Animal procedures were approved by the local ethics committee and conducted in accordance with UK legislation. Spontaneous contractile activity (SCA) was assessed at different [Na]o in intact and detubulated (DT) cells, to distinguish SCA from gross changes in TT morphology. Cav-3 KO mice and wildtype (WT) C57Bl/6 control mice. Ventricle myocytes were isolated from 3 month old Cav-3 knockout (KO) mice and wildtype (WT) C57Bl/6 control mice. Animal procedures were approved by the local ethics committee and conducted in accordance with UK legislation.

SCA was 23±3% greater in intact Cav-3 KO cells compared to WT controls (p=0.01, n=80, unpaired t-test), but did not change upon DT (ns for WT, n=80, and KO, n=80, 3-way Chi-Squared); thus SCA is unaltered by loss of TT NCX activity, but KO appears to have greater propensity for spontaneous activity. To determine whether a change in spontaneous RyR activity may be responsible, Ca sparks were measured. Total Ca spark frequency was reduced in KO (from 0.10±0.01 100 μm-2.s-1 in WT to 0.03±0.01 100 μm-2.s-1 in KO; p=0.0002, unpaired t-test), but activity was increased in the cell centre (p=0.02, Chi-Squared). To investigate whether changes in cell structure might also contribute to the changes seen in SCA, colocalisation analysis of WGA and RyR labeling was used, indicating that Cav-3 KO was associated with a ~30% decrease in RyR colocalisation with WGA (p=0.01, n=10, unpaired t-test) and vice versa (p=0.005, unpaired t-test). Fourier analysis of WGA staining revealed no changes to TT regularity in KO cells. These data suggest that while Cav-3 KO may not be associated with gross changes in TT morphology, it may be involved in regulating RyR and NCX, such that KO promotes SCA.

Wei S et al. (2010). Circ Res. 107, 520-531.

This work was funded by the British Heart Foundation and the Faculty of Biomedical Sciences, University of Bristol.
**PCA083**

**Improved prediction of post-pneumonectomy lung function**

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An important problem in lung cancer surgery is the prediction of post-operative respiration during the respiratory cycle (Kang et al. 2015). A new approach to this question is made possible by the recent bottom-up model of oxygen capture during the respiratory cycle (Brunelli et al., 2013). This model allows for a quantitative calculation of the respective role of ventilation (VE) and cardiac output (Q) on oxygen uptake (VO₂). The results are shown in the figure that represents the VO₂ isolines of a healthy lung as a function of VE and Q.

The predictive method is based on the idea that the resection of a fraction of the lungs volume does not modify strongly Q so that the local blood flow rate in the remaining volume is increased accordingly. Consider for example the case where half the lung volume has been resected. As a consequence the local blood flow is doubled but the local ventilation would remain the same if the diaphragm motion is not modified. This is shown in the figure by shifting from A for which VE(normal) = 7.5 L/min; Q(normal) = 5L/min and VO₂(normal) = 220mL/min to B (same ventilation, local Q(B) = 2Q(normal) = 10L/min and VO₂(post) = (1/2)VO₂(B) = (1/2)(0.35) L/min = 0.175L/min which is insufficient. To recover a normal VO₂, one has to increase the ventilation of the remaining lung by shifting from B to C where VO₂(post) = (1/2)VO₂(C) = 220mL/min = VO₂(normal). Not shown here are the abacuses giving PAO₂ and saturation abacuses computed from Kang et al. (2015) that confirm that provided the increase in ventilation, normal values will be recovered.

The conclusion is that, a constant cardiac motion, a ventilation increase either spontaneous or artificial are needed to recover normal VO₂. Not shown here are the abacuses giving PAO₂ and saturation abacuses computed from Kang et al. (2015) that confirm that provided the increase in ventilation, normal values will be recovered.

**PCA084**

**A sexy approach to pacemaking**

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The sinoatrial node (SAN) is the primary pacemaker of the mammalian heart and is where the heartbeat originates. Clinically, there has long been evidence that the SAN function differs according to gender; males have a slower resting and intrinsic heart rate compared to females₁. It has also been shown that males have a longer corrected SAN recovery time and atrial refractory period than females².

The aim of this study was to investigate gene expression in the SAN of male and female rats in order to determine if changes in ion channel expression could underlie the observed functional differences. Ion channel gene (and related gene) expression was measured through quantitative PCR (qPCR) n=12 (n=6 from each group); GAPDH was used as the housekeeping gene. All results are given as mean ± SEM. Differences were evaluated by t test with Sigma Stat software. Differences were considered significant at the level of P<0.05. Intracellular action potential recordings were obtained using the sharp microelectrode technique (n=10, n=5 from each group). The amplitude of action potential was significantly less in the female SAN when compared with the male. Action potential duration (APD) at 15% and 75% was significantly shorter in the female SAN when compared with the male. The upstroke velocity (dv/dt₅₀max) was significantly higher in the female SAN when compared to the male. Out of around 100 different transcripts investigated, only two ion channels were significantly different between the two sexes. The expression of the L-Type Ca²⁺ channel Ca₄.1.3 mRNA was significantly higher in the female SAN compared to the male. Muscarinic K⁺ channel K₄.3.1 mRNA was significantly higher in the female SAN when compared to male. Following histological staining with Masson’s trichrome of SAN preparations (n=4, n=2 from each group) to identify the location of the sinus node artery, we performed immunohistochemistry on adjacent sections to those used for histology. Our pilot immunohistochemistry data showed that Ca₄.1.3 and K₄.3.1 proteins were high in the female SAN (n=2) when compared with the male SAN (n=2).

Both male and female SANs expressed HCN4 at mRNA and protein level but there was no difference between the two groups studied. All antibodies investigated in this study were tested for their specificity using the Western blot technique (n=4 male SAN). The research was conducted in accordance with the Guide for the Care and Use for Laboratory Animals in the UK.

This study identified differences in key pacemaker ion channels between male and female SAN. Ca₄.1.3 was recently
shown to play an important role in the pacemaker function of the SAN, therefore the higher intrinsic heart rate of the female SAN could be caused by the higher expression of Ca_1.3. The differences identified in this study advance our understanding of gender differences in cardiac electrophysiology and may have implications for gender specific design of biological pacemakers.


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peak power (14.70 ± 8.13 versus 6.03 ± 3.15 W/cm²), peak shortening (0.35 ± 0.06 versus 0.30 ± 0.08 L/Lo), and peak shortening velocity (4.78 ± 1.57 versus 3.56 ± 1.21 Lo/s) were decreased (p<0.05*) in CIH-exposed animals compared with normoxic controls. Values are mean ± S.D.

Our results reveal that exposure to CIH causes profound dia-phragm muscle weakness without overt disruption to resting ventilation (including pattern of breathing) and metabolism. The striking muscle phenotype in the C57 mouse provides a robust platform for the study of mechanisms of hypoxia-related muscle dysfunction, which may have relevance to respiratory conditions characterised by CIH such as sleep apnoea.

Supported by the Department of Physiology, University College Cork.

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**PCA087**

**An enhanced vein physiology practical using Doppler ultrasound imaging**

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Ultrasound technology uses sound waves to visualise body tis-sues including the vasculature. In this undergraduate medical practical demonstration ultrasound is used as an additional learning tool to aid the understanding of vein physiology. A 30 minute demonstration involving ultrasound investiga-tions of the veins occurred in a 2 hour practical class. Ethical approval was sought and received from Queen’s University Belfast. The ultrasound testing was performed by a trained member of academic staff and the test subject was also a staff member. 61 medical students were present in the classroom and completed a Likert scale questionnaire investigating student perception of the value of ultrasound technology in learning about venous pressure. A score of 5 indicated strong agreement while a score of 1 indicated strong disagreement with a statement. Data are expressed as mean ± SEM, paired Student t-tests were performed and p < 0.05 was deemed significant. Images were secured using a Sonoscape portable ultrasound and displayed on overhead projectors to the entire class.

**Distension of veins in different body regions:**

The veins above (neck) and below heart level (back of the hand and the feet) were visualised and their level of distension compared, as an indicator of pressure, by observing vessel diameter.

**The skeletal muscle pump:**

The subject contracted the leg muscles squeezing blood from adjacent veins towards the heart. The emptying of the veins and their filling when skeletal muscle contraction ceased was observed.

**Pressure in the veins on the back of the hand:**

The subject relaxed their arm and allowed the veins to fill up with blood. The arm was then slowly and passively raised. The point at which the veins collapse is an estimate of central venous pressure (CVP).

**Pressure in the neck veins (jugular venous pressure):**

The subject performed the Valsalva manoeuvre in a semi-recumbent position. This assessed how jugular venous pressure (as an assessment of CVP) changes in response to increased abdominal pressure.

A statistically significant difference was found between stu-dents’ perceived understanding of the physiology of venous blood pressure before (2.77 ± 0.12) and after (3.95 ± 0.11) the ultrasound demonstration (p<0.001). 89% of students strongly agreed that ‘ultrasound enabled (them) to visualise the fac-tors affecting venous pressure’ and 84 % strongly agreed that ‘ultrasound enable (them) to see clinical applications of under-standing venous blood pressure’. 88% of students enjoyed the teaching session that incorporated ultrasound and 82% of stu-dents strongly agreed that the use of ultrasound in teaching is more effective than conventional methods. Ultrasound enables students to visualise the vasculature and is perceived by students to aid their understanding of venous blood pressure and its clinical significance.

Supported by the Physiological Society (David Jordan Teaching Award).

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**PCA088**

**Acute exposure to cylindrospermopsin: Pulmonary and cardiac outcomes after a 7-day treatment with anti-inflammatories**

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**INTRODUCTION:** Cylindrospermopsin (CYN) is a cyanobacte-rial toxin of increasing worldwide environmental importance, as it can lead to disease if absorbed by human beings. LASSBio 596 (596) is a drug candidate with anti-inflammatory proper-ties. We aimed at evaluating the lung and cardiac outcomes of a 7-day treatment with 596 or dexamethasone (DEX) of mice exposed to CYN. METHODS: The experiments were approved by the Ethics Committee on the Use of Animals in Scientific Experimentation, Health Sciences Center, Federal University of Rio de Janeiro (IBCCF Protocol 063/15). Male BALB/c mice (n=40) received by gavage distilled water (200 µl, n=18) or a sub-lethal dose of CYN (70 µg/kg in 200 µl of distilled water, n=22 (under isoflurane nasal cone anesthesia). 18 h later, the animals were gavaged under isoflurane anesthesia with either distilled water (200 µl) [groups CA (water+water, n=6) and CYNA (CYN+water, n=10)], or 596 (50 mg/kg by gavage) [groups CL (water+596, n=6) and CYNL (CYN+596, n=6)], or DEX (2 mg/kg by gavage) [groups CD (water+DEX, n=6) and CYND (CYN+DEX, n=6)]. Mice were treated every 12 h for 7 d. EKG was run before and after treatments. On the 8th day mice were sedated (diazepam, 5 mg, i.p.) and anesthetized (sodium pentobarbital, 20 mg/kg, i.p.), which maintains general anesthe-sia for at least 1 h, paralyzed (pancuronium bromide, 0.1 mg i.v.) and underwent mechanical ventilation. We measured pulmonary mechanics (6-10 cycles per animal) and EKG. The experiments lasted 15 min. The animals were quickly eutha-nized by sectioning of abdominal aorta and inferior vena cava after an extra shot of pentobarbital. Then, we fixed the left lungs for morphometric measurements, and homogenated the right lung for determination of inflammatory mediators IL-6, IL-1β, KC and TNF-α. Statistical analysis used one-way ANOVA, followed by Bonferroni’s test when necessary, p<0.05
Lung tissue then was collected and prepared for histological cava under deep anaesthesia. The experiments lasted 20 min. and lung stiffness were calculated. The animals were quickly transce, and inertance were determined. Total lung capacity model. Hence, tissue damping and elastance, airway resis- could be triggered. Then they were paralysed (pancuronium bromide 0.3 mg/kg) and lung mechanics was evaluated by exposure, animals were anaesthetised (isoflurane 2.5% and midazolam 5 mg/kg), no podal reflex to nociceptive stimuli were able to block inflation. Furthermore, possibly a higher oral dose would be required to trigger more serious outcomes.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA089

Does a 20-day exposure to aldehydes impair pulmonary function and structure?

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There are conflicting and scarce results on the lung outcomes of exposure to airborne aldehydes. They are not only by-pro-ducts of fuel combustion but widespread materials can also continuously release them. Hence, we aimed to investigate the effects on mice lung and nasal epithelium of exposure (8 5 years) of the study investigating sleep architecture and breathing disor-terrans: (1) at Zhongshan Station (69°22′S, 76°22′E) has the highest ice peak in Antarctica, with an elevation of 4093m above sea level. It has a barometric pressure of 560-590 hpa, equivalent to about 5000m. Plenty of research at high altitude reported that hypoxia exposure is deleterious to sleep. This is the first study investigating sleep architecture and breathing disor-ners in healthy expeditioners from sea-level to high altitude at Dome A in Antarctica.

Subjects and Methods: The study was carried out on ten healthy male volunteers (mean age 28.3±5.5 years) of the 31st Chinese Antarctic expedition to Dome A, who had provided written informed consent. Sleep was monitored using Emblita X100 portable polysomnography (PSG) at three conditions: (1) at Zhongshan Station (69°22′S, 76°22′E, sea level) before departure to Dome A; (2) at 4093m on the 13th-14th days after arriving at Dome A; (3) descent back at Zhongshan Station. The following channels were recorded: two electroencephalogram (EEG); two electro-oculogram left and right (EOG); chin and tibial electromyogram (EMG); thoracic and abdominal effort using inductance plethysmography sensors; oronasal flow was evaluated by an oxygen cannula and a pressure transducer; oxygen saturation was recorded during the night with a finger pulse oximeter.

Results: The percentage of slow wave sleep (SWS) significantly reduced at Dome A (P<0.01). The percentage of REM (rapid eye movement) sleep remained in the normal range (15-25%) at both sea level and Dome A. There was no statistically significant difference in total sleep time, sleep latency and sleep efficiency. The respiratory disturbance index (RDI, /h)
and apnea/hypopnea index (AHI, /h) substantially increased (P<0.001) under high altitude condition. The central apnea index (/h) was normal (<1/h) at sea level and rose progressively to 26.0±27.3 at Dome A (P<0.05). Apneas were almost exclusively of periodic breathing type appearing mostly during NREM (none rapid eye movement) sleep. The oxygen desaturation index greatly increased (P<0.001). The minimum SpO₂ and mean SpO₂ during sleep significantly fell at Dome A (P<0.001).

Conclusion: Our field study reported novel data on sleep architecture, breathing patterns, and nocturnal oxygen saturation at Dome A (4093m) in Antarctica. High-altitude at Dome A reduced slow wave sleep and induced periodic breathing.

Corresponding author: Chengli Xu (xuchengli@pumc.edu.cn) Weil JV.(2004).High Alt Med Biol.5,180-189
Tellez HF et al.(2014)Am J Respir Crit Care Med.190,114-116

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what they learned in the course. Promoting deep learning in MOOCs is recognised as challenging (5) but we aim to overcome these limitations in future iterations.

From the instructors’ perspective, designing and delivering the course help bridge the gap between research and the public. The course allowed the dissemination of the underlying principles of our research to a wide audience in an innovative, dynamic and interactive manner. The course was also an effective professional development tool, especially for the PhD students, as it helped them better understand how to interact with non-specialist audiences and facilitated the development of key communication skills.


www.coursera.org/course/nudgeit

Bali M. 2014. MOOC pedagogy; Gleaning good practices from existing MOOCs. Journal of Online Learning and Teaching. 10(1):44

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
students to achieve. It would be great to have someone who can work through whole period of your new research proposal. I particularly encourage students to design experiments, to deliver ideas, to write scientific reports for conference, and even to draft paper for submission. This would not only help students for their future career but also help myself to quickly build up my own research. It is hard process but worth.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**PCA095**

**Cross-institutional student-led collaborative learning of physiology**

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Encouraging students to engage fully with the learning experience in higher education (HE) is a challenge. With secondary schools increasingly “teaching to the test” and large class sizes in HE often necessitating the use of more didactic teaching methods, there is a significant risk of students becoming passive consumers of information, rather than developing into self-regulated independent learners. Recently there has been a drive towards development of openly-shared online resources (e.g. MOOCs, iTunesU and Khan Academy) to support learning, but these are traditionally designed by academic staff based on their perceptions of what students need. A more beneficial approach is for students to work as partners in curriculum design and support of teaching. The main aim of this project is to establish and evaluate the effectiveness of cross-institutional student-led collaborative learning environments (CLEs) to support the learning of Physiology in HE.

We have evaluated the use of three online platforms (Facebook, Blackboard CourseSites and Learnium) for running Cardiovascular Physiology CLEs within one partner HE institution. These pilot studies involved three student cohorts: first year medical students (n=297), first year bioscience students (n=414) and second year biomedical students (n=137). Surveys and semi-structured interviews were conducted to evaluate the dynamics of engagement and explore student attitudes towards collaborative learning. 25% of the medical cohort, 34% of the first year bioscience students and 31% of the second year biomedical students signed up to the CLEs. The main reason for non-participation was “not knowing it existed” (84% medic; 76% bioscience). The main motivations for joining the online CLEs were to “get help with hard topics” (76% medic; 61% bioscience), “curiosity” (61% medic; 54% bioscience) and “support from peers” (55% medic; 50% bioscience). Students who joined a CLE but did not engage in online activities felt they “had nothing of value to post” (68% medic; 86% bioscience), but did like looking at other student’s posts. Other key reasons for lack of engagement were “need to focus on other coursework” and “haven’t started revision yet”. Other dominant themes emerging are that students want CLEs to cover more physiology topics and desire more input from academic staff. Organising small group meetings facilitated by near-peer students increased engagement with the online CLEs. Learnium (an online social network tool for learning and teaching) was the most popular platform, but training was required for staff and students to use the communities, boards and collaborative documents. Facebook and CourseSites were less popular due to their overly social and overly academic nature, respectively. We have recently built on this pilot study to establish three cross-institutional CLEs and will also present the outputs of these projects at the meeting.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**PCA096**

**Physiology at the heart of modern biomedicine: Evidence through oral testimonies**

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The History of Modern Biomedicine Research Group, funded by the Wellcome Trust, studies the history of recent biomedicine principally by employing oral history methodology. We generate resources such as individual interviews, Witness Seminars, and other publications and outputs, by collecting, transcribing, editing and undertaking research on oral testimonies from groups and individuals who have made significant contributions to the legacy of modern biomedicine. Physiology lies at the heart of our work, as biomedical developments depend on a thorough understanding of human physiology and knowledge of the molecular mechanisms and functions that define health and homeostasis throughout our lives. Oral testimonies provide invaluable accounts of the historical background of modern biomedical discoveries: the personal, social and scientific contexts in which physiological and pathological concepts develop; the development of experimental techniques; and the institutional, financial and political forces that shaped innovation, as well as the coincidental factors that have led to breakthroughs. To date, the Group’s systematic work has generated more than 60 Witness Seminar volumes and a large number of individual interviews and publications, all of which are freely available to consult and download from the website: http://www.histmodbiomed.org/. Much of this output is related to the physiological understanding of genetics, immunity and brain function, with an emphasis on the historical background of understanding basic physiology and the creation of novel treatments for diseases such as rheumatoid arthritis, migraine, depression, or even seasonal affective disorder. Our outputs illustrate the speed of scientific progress after World War II, especially the advances, conceptual and technological, that have allowed for a better understanding of physiological and pathophysiological processes. This evidence is accompanied by testimonies that shed light on the ways the scientific community has reacted to challenges; the nature and the complexity with which scientific collegiality has been shaped throughout the years; the bureaucratic and legal pitfalls; as well as the role of research funding and industrial relations. The materials gathered through these oral history methodologies are unique resources that inform our understanding, contextualization, reconstruction and communication of important aspects of the recent history of physiology as a discipline, and their significance in the framework of modern biomedicine.

We thank the Wellcome Trust for their support
Does gender bias exist in first year biology undergraduate module assessment?

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For many years it has been recognised that educational assessment strategies can be biased against one gender or another (1). For example, the use of negative marking in exams has been shown to have a more significant impact on female students compared to male students (2). However, the vast majority of these studies have been undertaken at primary or secondary school level. The aim of this current study was therefore to investigate whether any gender bias exists in the assessment of large-scale, first year undergraduate biology modules at University College Dublin. The study compared the academic performance of female and male students in two large first year modules during both the 2014-15 and 2015-16 academic years (i.e. four modules in total). These modules were assessed through a combination of negatively-marked MCQ exams, Mastering Biology on-line tests and laboratory practical class worksheets.

As expected from previous published studies, female students did not perform as well as male students in negatively marked MCQ exams (P<0.05, N=4, Mann-Whitney). Further analysis showed that this was due to a reduced number of correct answers and an increased number of passes. In contrast, females performed significantly better than males in Mastering Biology on-line tests and in laboratory practical class assessments (P<0.05, N=4, Mann-Whitney). However, combining all these different assessments together, there was no significant overall gender bias (NS, N=4, Mann-Whitney) in the four individual modules investigated.

In conclusion, although no overall gender bias has been detected, these detailed analyses have confirmed significant gender differences in various methods of undergraduate assessment. We therefore strongly recommend that consideration of potential gender bias should be taken into account during decisions on designing module assessment strategies.


This work was supported by The University College Dublin.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Enhancing the learning experience in physiology lectures, practicals and tutorials using ultrasound technology

C. Johnson, S.M. Roe and E. Tansey
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There are many areas of physiology that students have difficulty learning, including the cardiovascular system. We have been examining ways in which teaching of cardiovascular physiology can be improved. Ultrasound imaging and Doppler velocity measurement technology has been available for many years and is used clinically to identify abnormal physiology. Basic principles of ultrasound are beginning to be taught at undergraduate level, but with the specific intention of furthering its use in clinical medicine (1). However, due to the nature of ultrasound imaging, and the immediacy of actually seeing the beating heart within the body, we have hypothesised that ultrasound technology may be used to demonstrate basic scientific principles more effectively. Thus, we have evaluated its use in teaching several cardiovascular principles in practical classes.

We asked first year Medical students to fill out brief questionnaires regarding their general opinions on the use of ultrasound in teaching physiological concepts following cardiovascular practical classes. All questionnaires and experimental protocols were permitted by the ethics committee at School of Medicine, Dentistry and Biomedical Sciences, Queen’s University Belfast. These examined i) factors affecting cardiac output and the Frank-Starling law, in which 2-dimensional imaging was used to visualise heart chambers and make measurements of their dimensions to calculate ventricular volumes and ejection fraction, before and after exercise; ii) venous pressure was studied by 2-dimensional imaging of venous circulation, along with factors involved in arterial exercise hyperaemia using 2-dimensional imaging of artery dimensions and Doppler velocity measurements, before and after exercise. Questionnaires incorporated three questions requiring responses on the Likert scale (Strongly Agree = 5 – Strongly Disagree = 0; mean values ± SEM). Teaching sessions incorporating ultrasound were universally popular (Likert scores: (i) 4.28 ± 0.08, N=61; (ii) 4.39 ± 0.09, N=58), and the vast majority of students thought teaching incorporating ultrasound was more effective than conventional methods (Likert scores: (i) 3.98 ± 0.11, N=61; (ii) 4.08 ± 0.11, N=58). Students also deemed ultrasound technology as useful in calculating physiological parameters (Likert scores: (i) 4.23 ± 0.08, N=61; (ii) 4.24 ± 0.07, N=58). These highly positive scores were accompanied by very positive comments in open-ended question responses.

We conclude that the use of ultrasound technology enhances the learning experience of medical students in these aspects of basic cardiovascular physiology, and we are now investigating the effectiveness of this technology in improving learning, compared with conventional methods.

We thank the Physiological Society for the David Jordan Teaching Award that has supported this work

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA101

The design of animations and multimedia for teaching

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There have been very few studies on the effectiveness of multimedia as a learning tool (Rolfe & Gray 2011). Our hypothesis was that students would prefer animated presentations and that learning would be enhanced. It has previously been reported that static images worked just as well as animation (Paik & Schraw, 2013). These authors examined the ‘Illusion of Understanding’ in which students invest less cognitive effort when viewing an animation that appears to be easier to understand. Therefore we have investigated the use of animations versus static images in an instructional multimedia presentation.

We created two versions of a 3D animation describing vascular function. V1 had a full 3D moving animation whilst V2 had 17 static images from the animation (Fig 1). 54 Students (two groups of 27 level 3 physiology and pharmacology students) viewed V1 or V2 and then answered a short 8 min. question. A marking criteria assigned ‘core’ (essential material) and ‘bonus’ marks (correct use of terminology). Results showed a trend in favour of animation, but this was not statistically significant (table 1). The only significant difference was the lower bonus marks scored by the pharmacology ‘stills’ group vs the pharmacology ‘animation’ group.

Student feedback was 88% positive showing a clear desire for more animation content. Our results illustrate the ‘Illusion of Understanding’ as appetite for animation did not translate into more animation content. Our results confirm that 3D instructional animations per se will only be of value if appropriate multimedia and cognitive load theories are taken into account (Reed 2006).

Test Scores and bonus marks awarded for each group of students.

<table>
<thead>
<tr>
<th>Test Scores</th>
<th>Animation (All)</th>
<th>Stills Only (All)</th>
<th>Animation (Pharm)</th>
<th>Stills Only (Pharm)</th>
<th>Animation (Phys)</th>
<th>Stills only (Phys)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>3.7</td>
<td>3.2</td>
<td>3.5</td>
<td>3.3</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>SD</td>
<td>0.2</td>
<td>0.4</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>SEM</td>
<td>0.4</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Table 1. Test scores for Level 3 pharmacology (pharm) and physiology (phys) student groups viewing either a fully animated multimedia presentation (Animation) or a still-image-based (Stills Only) presentation. Test scores were marked 0-11 and unlimited 0.5 mark bonuses were awarded as described in the text. A significant difference (*, p=0.04) was detected in the bonus marks awarded to the pharmacology stills group vs the pharmacology animation group.

PCA102

Quality improvement measures applied to a Medical Physiology course

R.G. Carroll

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Medical physiology course directors will benefit from an understanding of how the quality improvement cycle is structures and applies, particularly when it is a component of the accreditation process. The Medical Physiology course at Brody School of Medicine recently underwent a 3 year review as a component of the overall curriculum quality assurance plan. Components of the review includes a 1) description (hours and instructional activities, assignment of grades and grades) of the course and changes over the past 3 years, 2) Student opinion surveys (at the end of course and at graduation), 3) Student performance on the physiology component of the licensing examination, 4) Student performance on a NBME subject exam administered as a course final examination, and 5) Narrative comments from student evaluations. Importantly, the student performance on the final examination, on the physiology component of the licensing examination and the student opinion at graduation can be compared to national norms, and the remainder of the student opinion compared to school-wide norms. The data was reviewed by a committee consisting of the physiology course director, 3 other course directors, and 2 past students. Overall, the multiple diverse methods used in this comprehensive review process provides a balanced description of the strengths and weaknesses of the course, and serves as a vehicle for guiding future development of the course.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
What makes your heart beat? A simple school curriculum-based outreach activity
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In recent years there has been a focus from Government, the General Medical and Medical School’s Councils on widening participation in medicine, such that the medical profession reflects the diversity of the population, increases social mobility and so that the medical profession has access to the best students from all backgrounds. At the University of Birmingham we developed Routes to the Professions: Medicine (R2P) which we believe is unique in the country in providing a complete package of support for potential applicants to medicine who have the academic potential to succeed but are from groups currently under-represented in medicine.

The R2P programme starts for students in Year 10 (14-15 years) with an Insight Day: Medicine, which brings them to the Medical School to participate in a day of interactive activities with staff and students. The day includes various talks related to medicine, however, to increase the value of the day to schools, it has been important to develop curriculum-based interactive activities.

Analysis of GCSE and A-level examination board syllabuses in England identified the cardiovascular system as a common topic and therefore the physiology of the heart provided an excellent basis for the development of a short activity that also included group work, numerical skills and data interpretation. The activity, What makes your heart beat?, introduced students to the electrical activity of the heart, relating it to their existing knowledge of the gross anatomy of the heart.

In small groups they measured their own electrocardiograms (ECG) and with the help of a staff facilitator were able to make a basic interpretation of the ECG using their own knowledge of the cardiac cycle. A short exercise required the students to measure R-R intervals and calculate their heart rate. They were encouraged to compare their results with others and consider the differences. Finally, students recorded their ECGs again during deep breathing to induce respiratory sinus arrhythmia, they calculated heart rate during inspiration and expiration and were able to determine when the heart sped up and slowed down.

Evaluation of the day shows that we have successfully targeted students from under-represented groups and that the sessions provided were very useful. What makes your heart beat? is a cheap and easy activity to run, it introduces Year 10 students to physiology as a discipline, and encourages them to apply their school-curriculum based knowledge to an activity that requires numerical and interpretative skills. Feedback from staff suggests that the inclusion of curriculum-based activities boosted students’ interest and engagement and therefore increased the value of the event. Making outreach and public engagement activities relevant to the school curriculum may increase uptake and engagement more widely, particularly in under-represented groups.

Selecting For Excellence (2013). Medical Schools Council, end of year report.
Routes to the Professions: Medicine, www.birmingham.ac.uk/routes

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

An enhanced cardiac physiology practical using ultrasound imaging technology
S.M. Roe, E. Tansey and C. Johnson
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For the medical and biomedical student, the importance of physiology education is enshrined within licensing bodies’ literature, which stresses the importance of an understanding of normal function with special emphasis on linking normal physiology to disordered activity. Students are encouraged to develop learning skills and so become “lifelong learners” (General Medical Council, 2015). To achieve this, it is essential that learning is an activity in which the student participates. In this way, knowledge is more likely to be retained by the student who could then apply it to different contexts (Michael, 2006). Our group has previously proposed practical activities as a way of making learning active by encouraging students to engage more with the material (Roe et. al., 2009). More recently, the use of ultrasound in the practical classroom has been mooted to help students visualise thoracic function (Paganini & Rubin, 2015).

We have added the use of a simple ultrasound scanner to our medical electrocardiography practical classes. A volunteer’s cardiac diameter, cross sectional area and length can be measured and used to calculate various cardiac volumes, ejection fraction and cardiac output (with heart rate). Students are given these data at rest and for a period of cycling on an ergometer. They then engage in directed self-learning where we ask them to link their calculations at rest and during exercise to the principles taught in the lectures involving the effect of increased venous return on cardiac output (Frank-Starling law).

To evaluate these classes effectiveness, students were asked to rate their understanding of the Frank-Starling law before and after the ultrasound class. Questions were also posed on the usefulness and clinical applications of the class. Questionnaires were completed by 61 students. A 5 point Likert scale was used to evaluate the response to each of the questions with 5 indicating strong agreement with a statement and 1 strong disagreement. Ratings are given as mean marks out of 5 ± S.E.M, n = 61.

There was a significant increase in understanding of the Frank-Starling law, with scores increasing from 2.8±0.1 before, to 3.8±0.1 after the class (student’s t-test, P<0.001, n=61). Students scored a statement on how ultrasound enabled better visualisation of the Frank-Starling law with a mean of 3.9±0.1. In response to a question on the usefulness of performing calculations with the data and another on the clinical relevance of the principles learned, students scored 3.9±0.1, and 4.3±0.1 respectively.

Our findings suggest positive engagement with the technology. Self-reported measures of learning are significantly improved after its use. Further work on more objective measures of learning would be appropriate.


Michael, J. (2006). Wheres the evidence that active learning works? Advances in Physiology Education 30 159-167

Creativity in bioscience teaching enriches the student experience

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Purpose
Nursing students often find engaging with bioscience modules challenging because they lack confidence in their ability to study science; consequently many students and qualified nurses have difficulty applying anatomical and physiological information, essential to providing safe and effective patient care (Rogers, 2014). Recent evidence highlights variation in the extent of bioscience teaching and assessment across nursing curricula (Taylor et al., 2015). However, nurse educators also need to develop innovative and creative approaches to enhance the teaching and learning of bioscience subjects. Given the links between art, science and nursing (Jasmine, 2009), this project aimed to explore the benefits and impact of engaging undergraduate nursing students in biosciences through the artistic medium of felt.

Methods
Year one undergraduate nursing students participated in a series of workshops designed to explore the cells, tissues and organs of the human body through felt. The project was facilitated by lecturers in nurse education in partnership with an artist from Arts Care, a unique arts and health charity in Northern Ireland. Felting engages all the senses and involves manually teasing out individual wool fibres, which are reconstructed to form intricate designs before being finally bonded together using warm soapy water. Evaluation was based on individual reflective journals completed by each student throughout the project.

Results
The creative process translated and transformed the students’ learning of cells, tissues and organs, creating striking, memorable art works, which have been exhibited across Northern Ireland. Analysis of student reflections revealed the project was associated with positive emotion, engagement, meaning, positive relationships, and accomplishment – elements which have been identified as contributing to overall well-being (Seligman, 2011) and improved student experience.

Conclusions
This paper reports on the positive impact the creative project had on the experience of year one nursing students and how it enhanced their approach to the professional nursing care of patients. This paper proposes that how we teach biosciences can enable students on any bioscience program to flourish as individuals, enhancing both knowledge and overall well-being. Rogers, K. M. A. 2014. A Preliminary Evaluation of a New Life Science Module for Year One Nursing and Midwifery Students. Health and Social Care Education, 3, 46-47.
the foundation for greater understanding later in practice. We have since adapted the session to create a data-handling based, plenary session for veterinary students. In future we also plan to embed the session in biomedical science teaching for undergraduate students of physiology and related disciplines.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA107

Physiology – histology integrated laboratory practice using case based learning

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Background Understanding key concept in physiology and histology as a part of biomedical science is important in early of learning process in undergraduate medical students. Students had difficulties in linking the content of biomedical learning content into the daily-process that occurs in the human body both in physiological processes and also in the cellular level. Therefore, physiology-histology integrated laboratory practice (PHILP) was planned to integrated students understanding on physiology and histology so that student understanding of the physiological processes as well as the cells involved in the process will increased.

Purpose The purpose of this study was to introduce an integrated laboratory practice using case based learning and determine its acceptability among students and faculties.

Interventions The PHILP was undertaken for first year medical student in Sensory Block. CBL case that prepared by experienced faculty in physiology was a basic biomedical case that included questions as learning objective guideline. The PHILP session conducted in 100 minutes. Limitations of histology slide require students devided in to two group. The first group conducted physiology laboratory practical session prior observed the histology slide. CBL case given to the student prior the physiology laboratory practical session. The content of physiology practical session was sensory receptors. Second group start the session with observed the histology slides continued with physiology practical session. In both group, the learning process followed by small group discussion, which refers to the CBL case.

Results Both students and faculties have positive perspectives towards these learning strategy. The CBL case improve students understanding of human body process both structure and physiological process. The PHILP process promoted integrated understanding between the abstract material in histology and the more pronounced content in physiology. Limitations of time was a constrain on the practical of PHILP.

Implication The integration should be developed more comprehensively in PHILP. It is important to develop consistent research on this learning strategy, especially on the effective-ness in supporting students’ understanding.

Setia, S., Bobby, Z., Ananthanarayanan, P. H., Radhika, M. R., Kavitha, M., & Prashanth, T. (2011). Case Based Learning Versus Problem Based Learning: A Direct Comparison from First Year Medical Students Perspective

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA108

Teaching acid-base physiology to first year veterinary science students using animal clinical case data

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1Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, UK and 2Langford Clinical Veterinary Service, University of Bristol, Bristol, UK

Acid base balance is a challenging concept for students to grasp but is an important concept for veterinary science students. At Bristol we have previously taught acid base by way of an interactive case based session which was adapted from a session using a Human Patient Simulator (CAE Inc, Canada) developed for the medical students. However, veterinary science students relate much better to veterinary related teaching. We have therefore developed a case based interactive session using data from real veterinary clinical cases. In the session student participation is encouraged via the use of audience response devices (Turning point), which have been demonstrated as an effective way of increasing student engagement (1). The session was delivered immediately following a lecture on the basis of pH and acid-base disturbances. In the interactive session 5 cases were presented to illustrate common simple acid-base disturbances. The values from blood analysis (pH, pCO2, HCO3⁻), that are typically available in veterinary clinics, were presented along with relevant clinical signs. The students used the data presented to work out the possible disturbance and underlying cause indicating their answers using turning point. Compensatory mechanisms for each case were also discussed. In one case (renal failure) the clinical data was modelled using paediatric settings of human patient simulator software (CAE Inc, Canada) to provide respiratory data (tidal volume and respiratory rate) and demonstrate the compensatory mechanisms. These data were then used to illustrate the respiratory compensatory mechanisms following a metabolic acidosis. In another case further data...
on renal function was used to encourage the students to think about how to determine the causes of acid-base disturbances. Students were provided with a handheld to record their findings for each case which included an acid-base nomogram to help aid their diagnoses.

The session was evaluated via a test and a questionnaire completed immediately before and after the interactive session. There was a statistically significant increase (P < 0.001) in the post session test scores (from a mean of 62.5% to 86.0%) indicating the students' knowledge improved in the session. Questionnaire data demonstrated that students improved their confidence in the topic and that they found the session useful and informative. The session will be incorporated into the first year timetable in future years in place of the human use of the public in a wide range of contexts including primary and secondary schools, colleges and public events, such as Skirting Science (http://skirtingscience.wordpress.com/), Nailsworth Festival, University Open Days and Bristol Neuroscience Festival. It has been possible to provide free or reduced cost visits to a selected proportion of these events for qualifying pupils using Widening Participation funding. This has allowed the widest range of students possible to access our resources and information.

In order to disseminate information about the scope of activities on offer, the webpages for outreach work in the Faculty (http://www.bristol.ac.uk/biomedical-sciences/outreach/) have been updated and linked to the Faculty homepage for ease of access. We are currently commissioning the production of short films for the website illustrating the activities on offer and are devising a series of outreach packages that schools can book. With the use of questionnaires and audience response devices, we are implementing a more rigorous evaluation and monitoring strategy to continually improve our work and provide a record of its impact linked to Widening Participation.


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**PCA109**

**Physiology Outreach in the Faculty of Biomedical Sciences, University of Bristol**

L.K. Goodhead, D. Davies, N. Cave and F. MacMillan

In the Faculty of Biomedical Sciences we continue to deliver a large number of outreach activities for a wide range of public groups. All our activities are coordinated by an outreach assistant, a role which has been in place since 2013, resulting in a substantial increase in the volume of outreach delivered and the diversity of activities offered. Our outreach includes visits to schools and science events and hosting visits in our teaching laboratories. New events in 2015 included a biomedical sciences ‘taster day’ for 60 local school children from widening participation backgrounds. Such was the success, this will be repeated annually with double the number of attendees. Aiding the expansion of our activities has been the provision of funds from the central University Widening Participation office.

Existing contacts with schools and established activities have also been maintained, including use of the Mobile Teaching Unit (MTU), a specially-equipped lorry that expands into laboratories. The MTU enables scientific equipment to be taken to schools, for example a Vitalograph to measure lung function, ECG monitors and pulse oximeters to demonstrate the diving reflex, and reaction timers to compare visual and auditory reaction times. Recently-developed sessions include an exploration of hearing and deafness and a practical session on size exclusion chromatography in the context of blood. The scope of visits to the University has been widened to include topics such as electrophoresis. A number of visits have also covered more general issues such as ethics and careers and also sought to engage all sectors of the community through ‘knit a neurone’ activities.

Our outreach work has involved school students and members of the public in a wide range of contexts including primary and secondary schools, colleges and public events, such as Skirting School (http://skirtingscience.wordpress.com/), Nailsworth Festival, University Open Days and Bristol Neuroscience Festival. It has been possible to provide free or reduced cost visits to a selected proportion of these events for qualifying pupils using Widening Participation funding. This has allowed the widest range of students possible to access our resources and information.

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**PCA110**

**Exploring hearing and deafness – an outreach workshop for all**

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Over the past few years we have endeavored to expand our outreach offering to reach as many sections of the community as possible from school pupils at primary and secondary level to members of the public at science festivals. Thus, it is important that we offer activities that are stimulating and accessible for all ages. With this in mind we developed a workshop to investigate aspects of sound, hearing and deafness that could be adapted to suit a wide range of ages.

Within the school context, this workshop is aimed mainly at Primary level KS1 and KS2. In the classroom, the workshop takes the form of short sections introducing concepts interspersed with activities. Starting at ‘what is sound?’ we investigate why sounds sound different introducing the major properties of sound (frequency and amplitude), the structure of the ear and deafness. The activities include identifying different sounds, making an ‘ear drum’ with kitchen utensils and ‘can we all hear the same things?’ - recording sounds made by the pupils (normally singing) and removing the high frequencies, using a commercially available android app, to mimic age-related hearing loss. This workshop links with the primary curriculum incorporating sound and hearing (Yr 2 - parts of the body and senses; Yr 4 - sound waves and the ear). In addition to introducing children to these concepts, it also encourages the use of scientific language, labelled diagrams and graphing data. But, possibly more importantly, it enthuses and generates questions. Evaluation is accomplished via the use of Turning point audience response software and handsets. Pupils are asked if they enjoyed the session and specific questions are directed to what they have learnt in the session. We also ask...
what will they go home and tell their family about the session, attempting to draw out the most memorable concept. In contrast, in the science festival setting we do not have a captive audience and participants are likely to spend only a very short period of time with any particular stand so the focus is much more on activities, for example testing hearing at high frequencies and adding data to a graph showing highest frequency perceived against age. This is interesting to all age groups as they can see their own data adding to a population survey during the festival. Additional information is provided, in this situation, with posters and by volunteers. In all settings, posing the question ‘can you hear through your teeth?’ and answering that question using a portable bone conduction apparatus, designed and constructed by technicians at the University of Bristol, proves to be the most popular activity. The overriding impetus when designing new workshops is to make our outreach contribution interesting and relevant. Achieving this through flexible content is both efficient and rewarding in that it highlights that interest in these concepts crosses many societal boundaries.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA111

Engaging and involving the public in shaping the future of research into cerebral palsy

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Research into the causes and consequences of cerebral palsy, is often ‘to’, ‘about’ or ‘for’ people with cerebral palsy i.e. what researchers or clinicians think they want rather than research that is ‘with’ them, their families or carers - research that is relevant, focused and directly addresses their needs. Patient/Public Involvement (PPI) groups, where patients and the public decide clinical care priorities or identify and prioritise research questions, are increasing being used by both Government and clinical or translational research funders to direct research and funding priorities. However, use within the biomedical sciences is extremely limited. Therefore, our aim was to use PPI methodologies to identify and prioritise research questions for CPRes (Cerebral palsy research), a newly created, multi-disciplinary research grouping the University of Leeds interested in increasing understanding of the causes and consequences of cerebral palsy, and in developing new, or enhancing existing, supportive therapies and treatments for people with the condition. People with cerebral palsy, their families and carers are at heart of our research strategy: equal partners with researchers, clinicians and other healthcare professionals in our research community.

To identify the initial research questions for the group, a workshop, attended by people with cerebral palsy, their family and carers, clinicians, healthcare professionals and researchers, was held. Delegates split into small groups, each led by a person with cerebral palsy, to discuss, amongst themselves, the area(s) where they thought research funds and effort should be focused. The outcomes of their discussions were collated, shared with the community, and uploaded onto the groups website (Lewis et al., 2016). An active, international twitter community (@CPResLeeds) has been developed to facilitate community engagement and involvement with the research going forward.

This project has shown that PPI methodologies are an effective and novel means of engaging and involving the public with research in the biomedical sciences, and in shaping translation research questions and priorities

Lewis Di et al. (2016) www.CPRes.leeds.ac.uk

This work was supported through the award of an Engaging Excellence Fellowship by the University of Leeds to DIL. Their support is gratefully acknowledged

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA112

Self-learning activities improve academic outputs in a second-year course in animal physiology

T. Carbonell, J. Blasco, A. Ibarz, N. Alva, I. Garcia Meilan, G. Viscor, T. Pages and J. Fernandez-Borràs

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When the degrees of the University of Barcelona were adapted to the European Higher Education Area (EHEA), we took the challenge to develop new methodologies promoting a student-centered learning process in the course of Physiology, partly replacing the traditional classroom lectures. We consider that students are active agents for scaffolding their learning process, by searching for relevant content and solutions, making good debates and solving complex problems or case-studies, mainly by self-regulating their own learning. Nevertheless, in the Biology degree, the majority of courses are taught as classroom lectures supported by PowerPoint presentations.

In the present study, we aim to explore if interventions targeting improvements in self-learning tools have a positive impact on the academic performance in a second-year course in Animal Physiology.

We used the Virtual Campus-UB based on Moodle with three kinds of tasks (Lessons, Forums, and Quizzes) which provide feedback information regarding students’ scores. Although all these tasks are formative assessments, to encourage the students in active learning, the activities scored up to a maximum of 5% of the final grade.

The analysis of the data showed that academic success was positively associated with the accomplishment of activities (R²=0.3201). Twenty-five % of students, however, conducted no one of these optional activities. Among these students, the number of those who failed the final test increased.

In addition, using Moodle as LMS, we have had complete access to the registries about how our students used these tools (only for statistical analysis). The results helped us 1) to determine the most meaningful activities, improving students’ skills, as well as the ineffective actions; 2) to identify unsolved problems, the poorly learned concepts, and misconceptions. This information will be used to improve our teaching in the near future.

Our findings show that recording students’ activities may be useful as a predictor of their academic performances. Learning analytics, treating an enormous amount of data, can
substantially benefit teachers, specially tutors, assisting their pupils to improve academic completion and social success.

Note the positive correlation between the score of the activities and the final grade.

This study has been funded with Redice-14-16/ 1342

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA113

The Mobile Physiology Laboratory: a tool to promote the understanding of Physiology by secondary school students

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Introduction: As a Department of Physiology in which teaching is a core activity and which also promotes the discipline as an undergraduate degree outlet, a concern is a lack of knowledge of this field among the public and in particular, among prospective students. This is often encountered during ‘open days’ at University College Cork, when secondary school pupils from throughout the Province of Munster (population of 1.25 million people) visit exhibits hosted by various academic units, aimed at promoting entry into their degree programmes. We considered the utility of pre-emptive, outreach approaches to address this perceived lack of knowledge of the discipline of Physiology among secondary school students. Specifically, we aimed to develop a means of demonstrating physiological experiments, in the form of a ‘Mobile Physiology Laboratory’ (MPL).

Methods: the MPL (http://www.ucc.ie/en/physiology/outreachprogrammes/mobilephysiologylaboratory/) consists of portable apparatus to allow demonstration of the measurement of a wide range of physiological parameters and the effects of diet and exercise on these, in situ within secondary schools. Available assays include electrocardiography, phonocardiography, respirometry, estimation of metabolic rate (using a bicycle ergometer and a ‘metabolic system’), nerve conduction velocity, pneumotachography and blood glucose monitoring. These demonstrations can be tailored to suit particular school years and lesson durations. The MPL was trialled to 5th Class pupils (typically 16 to 17 years old; equivalent to Key Stage 4 in the UK, or Grade 12 in the USA) at two secondary schools in Cork City: Bishopstown Community College (to about 50 students) and Mount Mercy College (30 students) during Autumn 2015. Pupil responses to these demonstrations were assessed using a classroom assessment test (a ‘minute test’, Angelo & Cross, 1993), in the form of written responses before and after the lesson, to the question ‘What is physiology?’

Results and Discussion: the MPL was viewed positively by many of the students within these cohorts, as indicated by recorded testimonials, for example: (https://www.youtube.com/watch?v=uBnMKfMils) and by informal comments. Moreover, document analyses of minute tests indicated an increase in informed responses to the question ‘What is physiology?’ On the basis of these preliminary findings, we plan to expand the use of the MPL as an outreach tool to secondary schools in the Province of Munster and to carefully assess its impact on secondary pupil understanding of Physiology and on the profile of intake to our degree programme.


We are very grateful to the Physiological Society for supporting our outreach activities

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA114

Use of smart phones for academic purpose among medical students of private medical colleges of Karachi, Pakistan

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Introduction/ Objective/s: There has been a paradigm shift of thoughts regarding the usage of mobile phones in the last few years. The utilization of smart phones is getting more common in healthcare day by day. Healthcare providers and students are widely using the smart phone for getting up to date Medical information within no time. The aim of the study is to determine the awareness and the extent to which medical students use smart phone apps in their academics and to determine the relationship between academic use of smart phones and final grades.

Methodology: It was a cross sectional study in which data has been collected from 400 medical students of two private medical colleges of Karachi. Through a self administered questionnaire questions such as demographic, usage of smart phones, type of smart phone being used, and awareness of any medical apps, awareness of any medical books, time spent on medical apps, purpose of using medical apps, no of apps being used, duration since when the apps are in use, etc has been asked.

Results: In this study 360 medical students participated in which 40 were male and 320 were female students out of which 96.1% (n=346/360) of medical students owned a smart phone. Out of these 360 students, 91.7% of medical students used smart phone for academic purpose. Majority of the students have 1 –3 medical related applications installed on their phones with a maximum of 13 apps in any phone. Majority of
the students were using free apps and only 28% of the apps were purchased. It has been observed that majority of the students spend less than 1hr on mobile devices for academic purpose. Most of them used it once or twice a day for multiple academic purposes that is for taking notes, regular study & revising exams. But there was no significant difference in the GPA among students who used the smart phones for more than 5 hours for academic purpose as compare to those who used it for less than 1 hour (p= 0.76).

Conclusion: It has been concluded that medical students are using smart phones for academic purpose as well in addition to telecommunication and the proportion is much greater that it was observed in a similar study a year back in a nearby city.


Department of Health Management, IoBM and MBQMDC for their support.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**PCA115**

**Use of LabTutor improves student engagement and achievement in ECG and EEG practical classes**

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When students are being taught physiological measurement techniques, they may find it difficult to stay enthused and engaged when trying to perform such novel/complex tasks. Problems with equipment setup, calibration, and perceiving relevance to real-life situations can mean that students become disheartened, overwhelmed or fail to understand the point of the exercise. This may be common where students are drawn from a variety of disciplines. The LabTutor computer-based system (AD Instruments, NZ) provides step-by-step instructions for the students to help learn such techniques. Patient cases are integrated into the practical tasks. Practical results and student answers may be uploaded electronically for instructor marking later. This study aimed to discover whether use of LabTutor could improve student engagement and achievement in practical classes.

Two different classes were studied – one teaching basic measurement/interpretation of ECG’s (n= 32 in 2013-14 and 39 in 2014-15), and the other EEG’s (n = 39 in 2013-14 and 46 in 2014-15). The ECG class was composed of students studying anatomy or neuroscience. The ECG class was composed of students studying physiology or sports science. Students could leave the class when they felt they had completed the assigned work satisfactorily. In 2013-14, equipment setup/technique was demonstrated at the start of the class, with paper-based instructions and submitted practical answers. In 2014-15, students followed the computer-based scenario/instructions provided by LabTutor, submitting their answers electronically. The mark achieved by students and time spent completing the exercise was recorded.

Use of LabTutor produced extremely significant increases in both the mark achieved by students and the time spent voluntarily in completing the practical tasks in both classes (P< 0.001, Mann-Whitney test). ECG class duration increased from 82.9±2.8 min to 109.7±2.0 min, and grade increased from 67.4±1.8 % to 90.6±1.3 %. ECG class duration increased from 148.7±3.48 min to 253.6±8.7 min, and grade increased from 68.2±1.1 % to 75.0±1.2 %. Error values represent standard error of the mean. Anonymised feedback from student course feedback questionnaires was overwhelmingly positive regarding use of LabTutor, compared to previous years’ comments where some students felt overwhelmed when trying to learn such measurement techniques. LabTutor may improve student engagement and achievement when learning physiological measurement techniques. Integration of clinical scenarios enhances student appreciation of the activities. Staff reported that students of all backgrounds required less help and found it much easier to work through the tasks, with the focus being more on understanding concepts rather than getting equipment to work. Use of LabTutor may enable increased provision of practical skills training to a wider range of students.

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**PCA116**

**Can high fidelity human patient simulators help biomedical science students better understand complex concepts such as anticholinergic burden?**

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High-Fidelity Human simulators (HFHS) are life-like mannequins used extensively for teaching within the clinical setting. They offer an exciting method of learning compared to conventional passive approaches to learning (e.g. lectures) where students may have little opportunity to engage with the material. This pilot study investigated whether HFHS could be an effective educational tool within medical sciences teaching. It explored whether simulators could enable students to better understand complex concepts such as anticholinergic burden that involve a knowledge of both physiology and pharmacology. The SimMan 3G (Laerdal, Norway) HFHS was used to imitate responses observed in a patient experiencing anticholinergic burden due to inappropriate prescribing. Volunteers (n= 28) were biomedical science undergraduates and teaching staff (n = 4). Before commencing, participants were given a scenario and revision sheet covering areas (Autonomic physiology, opioids and anticholinergic burden) relevant to the simulation/existing course material to assist them in their understanding and to help them ‘recap’. Multiple Choice Questions (MCQs) were given prior to and following the simulation. Participants also completed an anonymous questionnaire which asked them to grade aspects of the activity on a Likert scale.

Participants scored significantly higher in MCQs (P = 0.0057, Wilcoxon matched pairs test) following the simulation compared to pre-simulation (Increase in mean score achieved from 9.3±1.4 to 13.2±1.1, n = 16 since not all students completed
the questionnaire, error values represent standard error of mean). Participants found this novel experience an effective, highly engaging and realistic teaching tool. Themes identified from free text feedback indicated that duration of simulation, requirement for an introductory and debrief period, and the delivery style of the simulation leader were important to participants. Participants enjoyed the interactive nature of the simulation and being able to apply scientific concepts they had learnt in lectures to a ‘real-life’ case scenario. Staff highlighted that simulation ‘brings to life’ the material from classes and suggested ways to further integrate simulation into the current curriculum.

Simulation is a novel teaching method that enables clinically-related students to critically think beyond the textbook/lecture theatre, by relating to and being able to visualise/conceptualise real physiology. To further test how participants respond to lectures vs simulation, separate groups – one group given lecture-based material and the other the simulation alone, independent of each other - would be required. It is hoped this will provide further evidence that simulation can be successfully applied outwith traditional clinical teaching activities.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Poster Communications

PCA117

Thyroid hormones transporters and deiodinases mRNA expression are altered in placenta from gestational diabetes mellitus

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Gestational diabetes mellitus (GDM) characterizes by an abnormal maternal D-glucose metabolism is associated with reduced maternal circulating free tyroxine (fT4) [1]. The human placenta regulates thyroid hormone concentration in fetal circulation by modulating thyroid hormone transporters (THTs) and thyroid hormones metabolism mediated by deiodinases (Dio) [2]. Interestingly, THTs and Dio expression in the human placenta from GDM have not been studied, yet [3]. Methods: Human placentas were collected after uncomplicated pregnancy with full-term delivery from 20 normal and 20 GDM pregnancies. mRNA from placental cotyledon was extracted with Trizol reagent and used for real time PCR for measuring THTs and Dio using the 2ΔΔCt method. Results: Compared to normal pregnancy, GDM exhibited reduced maternal fT4 in the first trimester of pregnancy in GDM might be associated with placental alteration in the transport and metabolism of thyroid hormones.


Dirección de Investigación Universidad San Sebastián.

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PCA118

xCT, N-acetylcysteine uptake and volume regulated glutamate release in cell lines and from the MVM of human placenta

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The human placenta maintains high intracellular glutamate concentrations with no net glutamate transfer to the fetus. This study explores specific glutamate efflux mechanisms on the syncytiotrophoblast microvillus membrane (MVM) and demonstrates the expression of xCT and the Maxi chloride channel in the human placenta, the activity of which may be regulated by oxidative stress. Human term placental cotyledons were perfused with 58 nM 14C-glutamate in the maternal circulation. To stimulate glutamate efflux, 16 µmol boluses of glutamate, the antioxidant N-acetylcysteine (NAC), glutamine, taurine and serine were perfused into the maternal circulation. Volume regulated glutamate efflux was investigated by perfusing 50 mM urea into the maternal circulation and adding 50 mM urea to BeWo cells. Western blotting determined xCT expression in human placenta. Xenopus laevis oocytes were used to investigate NAC transport by xCT, by expressing human xCT-GFP/4F2hc. HEK293 cells were transfected with xCT-GFP/4F2hc and incubated with 14C-glutamate, with/without cystine (30-300 µM), NAC (30-5000 µM), glutamate (1 mM) and quisqualate (10 µM) to inhibit xCT mediated uptake. Glutamate efflux data are presented as median and interquartile range and were analysed using a Mann-Whitney U test. Xenopus oocyte/cell culture 14C-glutamate efflux data are presented as mean and SEM and assessed using a one-way ANOVA with a Dunnett’s posthoc test whereby the efflux of 14C-glutamate was compared to control (buffer alone). xCT protein was localised to the MVM of the syncytiotrophoblast (n=4 blots, 45 µg protein). In placental perfusions, glutamate efflux occurred in response to potential xCT substrate NAC (n=6, p=0.031). In Xenopus laevis oocytes expressing human xCT-4F2hc, glutamate (10 mM) and NAC (10 mM) but

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not glycine trans-stimulated $^{14}$C-glutamate efflux compared to buffer alone ($n=6$, $p<0.001$). In BeWo cells ($n=3$ experiments, $p=0.001$) and the isolated perfused placenta ($n=12$ experiments, $p=0.047$), addition of urea bolus to the maternal circulation stimulated release of $^{14}$C-glutamate. However pre-treatment with the antioxidant NAC decreased urea mediated glutamate efflux from both BeWo cells ($p<0.05$) and the placental perfusions ($p=0.018$).

This study demonstrates glutamate efflux from the MVM of human placental syncytiotrophoblast by the transporter xCT and a volume regulated mechanism, likely to be the Maxi chloride channel. The finding that the antioxidant NAC inhibits volume regulated glutamate release suggests that Maxi chloride channel function may be regulated by oxidative stress status. As xCT appears to transport NAC, it is possible that Maxi chloride channel function is thus indirectly regulated by xCT activity.

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PCA119

The ErbB3 receptor tyrosine kinase restricts intestinal Paneth cell numbers

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Paneth cells (PCs), a secretory population located at the base of the intestinal crypt, support the intestinal stem cells (ISC) with growth factors and participate in innate immunity by releasing antimicrobial peptides (AMPs), including lysozyme. Loss of or functional defects in PCs are seen in disorders such as inflammatory bowel disease and necrotizing enterocolitis. We recently showed that activation of the neuruglin receptor ErbB4 protects PCs against injury. However, the role of ErbB3, the other neuruglin receptor, in PCs is unknown. In this study we tested the effects of ErbB3 signaling on PC numbers, AMP production, and the ISC niche. METHODS: Ileal tissues from ErbB3$^{+/+}$ (functionally wild type) and ErbB3$^{flox/flox}$ mice were characterized by immunofluorescence, qPCR, and disaggregation/cytometry time-of-flight (CyTOF) analyses. In vitro, ErbB3 was activated with NRG1$^β$ (10ng/ml, 24 h exposure) or downstream signaling was inhibited (PI 3-kinase/Akt with NRG1$^β$ was activated with NRG1$^β$ aggregation/cytometry time-of-flight (CyTOF) analyses. In vitro, ErbB3flox/flox (functionally wild type) and ErbB3 flox/flox; Vil-2$^{-/-}$ mice had a significant increase in expression of the secretory cell markers (Chga, Muc2, Lyz1) (9.9 vs. 5.6% of epithelial cells, $p=0.008$), as well as increased Lyz1 (by 67.53%, $p<0.0001$) and ISC marker Lgr5 (by 46.65%, $p=0.012$) RNA expression. This appeared to be developmental, as lysozyme$^+$ cells were readily detectable in crypts of 7 day-old E3KOIE but not ErbB3$^{flox/flox}$ animals. Conversely, activation of ErbB3 with NRG1$^β$ resulted in reduced expression of Lyz1 in WT enteroids (by 29.47%, $p=0.03$) or HT-29 cells (by 31.0%, $p=0.02$). We did not observe differences in expression of other ErbB family members (Egfr, ErbB2, and ErbB4) or other secretory cell markers (Muc2 and Chga). Interestingly, E3KOIE mice had a significant increase in expression of the secretory regulator Atoh1 (by 59.20%, $p=0.0009$), which is required for PC development. With regard to the underlying mechanisms, CyTOF analysis of disaggregated crypts showed loss of basal Akt (1.8-fold decrease, $p=0.01$) and ERK MAPK (3.7-fold reduction, $p=0.02$) phosphorylation specifically in CK20$^+$/Lyz$^+$ transit amplifying/progenitor cells. Furthermore, in HT-29 cells, exposure to PI 3-kinase or MEK inhibitors resulted in a dose-dependent increase in Lyz1 expression. Interestingly, NRG1$^β$ exposure could overcome MAPK but not PI 3-kinase inhibition, suggesting differential requirement for these cascades. CONCLUSIONS: ErbB3 restricts PC numbers by a mechanism involving PI 3-kinase/Akt, MAPK, and Atoh1. Understanding the role of ErbB3 receptor could identify new therapeutic targets for regulating PCs and the ISC niche.

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PCA120

Mechanosensitive calcium-permeable ion channels in transformed fibroblasts: An evidence for Piezo1

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Living cells express a number of mechano-dependent molecules that transduce mechanical forces and trigger a variety of intracellular signaling events. One of the major players is mechanosensitive ion channels, whose activation and inactivation is regulated by the mechanical status of the cell. Calcium influx via non-selective Ca$^{2+}$-permeable channels activated by membrane stretch (stretch-activated channels, SACs) is thought to have an valuable impact on intracellular signaling and calcium-dependent coupling of cellular reactions. Previously we have identified typical SACs in 3T3B-SV40 mouse transformed fibroblasts with unitary conductance of 24±1.4 pS. Importantly, we showed that calcium influx via SACs triggered the activation of calcium-sensitive potassium channels (Chubinskiy-Nadezhdin et al., 2014). The goal of the present study was to identify the main molecular candidate for the role of SAC in 3T3B-SV40 cells using a combination of pharmacological approach and molecular biology techniques. The proposed candidates were proteins from TRP superfamily (TRPC1, C6, V2, V4 and M7), ASIC/ENaCs and Piezo1/2. The pharmacological blockers of putative mechanosensitive channels were added to the patch pipette in cell-attached single-channel patch-clamp experiments. The following inhibitors were tested: blocker of TRPC1/C6 lanthanum (100 µM), blocker of TRPV subfamily Ruthenium Red (10 µM), blocker of TRPC1/6 and activator of TRPV2/4 2-APB (100 µM), amiloride (200 µM, blocker of ASIC/ENaC and TRPC6). We showed that all tested compounds had no effect on stretch-induced activation of SACs and single channel conductance in the plasma membrane. It is known, that the unique fingerprint of TRPM7 is the dramatic increase of the unitary conductance in the
absence of Mg²⁺ ions (Macianskiene et al., 2012). Variation of Mg²⁺ concentration had no effect on the unitary conductance of SACs, thus TRPM7 was excluded from the possible correlates. In sum, the experiments with the use of pharmacological blockers allow us to suggest Piezo1/2 as the main candidates on the role of SACs in transformed fibroblasts. RT-PCR analysis detected the presence of PIEZO1 mRNA but not PIEZO2 mRNA in cells. Immunofluorescent staining with the specific anti-Piezo1 antibodies confirmed the expression of Piezo1 in the cell membrane. Importantly, a predominant localization of Piezo1 with in lamellipodia regions was detected by double staining of Piezo1 and F-actin. It is worth noting that actin disassembly by cytochalasin D did not prevent coupling of mechano-gated and calcium-activated channels in course of mechanotransduction. The role of Piezo1 in the signaling processes (e.g. cell migration, motility and calcium signaling) in 3T3B-SV40 transformed fibroblasts is planned to be studied using selective siRNA gene knockdown.

Chubinskiy-Nadezhdin VI et al. (2014). Biochem Biophys Res Commun. 451(3):421-4


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**PCA121**

**Bile acids potentiate proton-activated currents in Xenopus laevis oocytes expressing human acid-sensing ion channel (ASIC1a)**

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Acid-sensing ion channels (ASICs) are non-voltage-gated sodium channels transiently activated by extracellular protons and belong to the epithelial sodium channel (ENaC)/Degenerin family of ion channels. Bile acids have been shown to activate two members of this family, the bile acid sensitive ion channel (ASIC1) (1, 2) and ENaC (3). The aim of the present study was to investigate whether bile acids also can modulate ASIC function. For this purpose human ASIC1a was heterologously expressed in Xenopus laevis oocytes. Whole-cell currents and single-channel currents were measured using the two-electrode voltage clamp technique and outside-out patch-clamp recordings, respectively. Values are presented as mean ± S.E.M. Paired Student’s t-test was used for statistical analysis. Exposing oocytes to tauro-conjugated cholic (t-CA), deoxycholic (t-DCA) and chenodeoxycholic (t-CDCA) acid (500 µM) at pH 7.4 did not activate ASIC1-mediated whole-cell currents. This is in agreement with a report that rat BASIC but not rat ASIC1a could be activated by taurodeoxycholic acid (4). We hypothesized that bile acids may not activate ASIC per se but may modify channel activation by protons. Indeed, we observed that ASIC1a whole-cell currents elicited by pH 5.5 were significantly increased in the presence of bile acids. In the presence of t-CA the peak inward current elicited by pH 5.5 was increased by a factor of 1.41 ± 0.06, whereas t-DCA and t-CDCA increased the proton-activated currents by a factor of 2.07 ± 0.15 and 2.09 ± 0.09, respectively (p<0.001, n=18). Application of t-DCA was accompanied by a significant ~15% reduction of the single-channel current amplitude of ASIC1a (p<0.001, n=13). This suggests an interaction of t-DCA with a region close to the channel pore. Analysis of the chicken ASIC1 crystal structure (5) revealed the presence of co-crystallized n-dodecyl-β-D-maltoside detergent molecules in the pore region of the channel. Bile acids are amphiphilic substances and can behave as detergents. Therefore, we speculated that bile acids and maltoside detergent bind to ASIC1a at similar sites and may affect channel activity in a similar way. Indeed, application of malto-side detergent (10 µM) mimicked the effect of bile acids and led to a significant increase of proton-activated ASIC1a currents by a factor of about 1.4 (p<0.001, n=19). Finally, molecular docking analysis predicted binding of bile acids to the pore region in the open conformation of the channel. We conclude that bile acids potentiate proton-activated ASIC1a currents probably by stabilizing the open state of the channel.


Lefèvre CM et al. (2014). Pflügers Arch 466(2), 253-263.


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**PCA122**

**Co-expression of connexin 30 inhibits epithelial sodium channel (ENaC) in Xenopus laevis oocytes by a mechanism involving a critical region in the C-terminus of the channel’s γ-subunit**

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Genetically modified mice lacking connexin 30 (Cx30) have been reported to develop salt-sensitive hypertension (1) because of an increased activity of the epithelial sodium channel (ENaC) in the distal nephron (2). This suggests that ENaC is inhibited by Cx30. In the present study co-expression experiments were used to investigate a functional interaction of ENaC and Cx30. Human ENaC and human Cx30 were heterologously expressed in Xenopus laevis oocytes. In two-electrode-voltage clamp experiments ENaC-mediated whole-cell currents were determined by measuring amiloride (2 µM) sensitive currents. ENaC expression at the cell surface was estimated using a FLAG-tagged βENaC subunit and a chemiluminescence assay. Channel open probability (P_o) was assessed in outside-out patch-clamp recordings. Site-directed mutagenesis was used to identify channel regions relevant for the functional interaction of ENaC and Cx30. Values are presented as mean ± SEM. Unpaired Student’s t-test was used for statistical analysis. Co-expression of Cx30 significantly reduced ENaC currents by 56.3±3.0% (n=79, p<0.001) compared to those in control oocytes expressing ENaC alone. The inhibitory effect of Cx30 on ENaC was associated with a significant ~60% reduction of channel surface expression (p<0.001, n=72) without an apparent decrease in P_o. Each of the three
ENaC subunits (αβγ) contains a PY-motif within its cytosolic C-terminus. The PY-motifs are thought to be critically involved in Nedd4-2-dependent channel retrieval from the cell surface. Simultaneous truncation of the C-terminus of all three ENaC subunits essentially abolished the Cx30-mediated channel inhibition. Interestingly, truncation of the γ ENaC C-terminus alone was sufficient to prevent ENaC inhibition by Cx30, whereas mutation of the prolines in the PY-motif of γ ENaC (623Pαγ627) did not significantly reduce the inhibitory effect of Cx30. This finding indicates that the inhibitory effect of Cx30 is not mediated by increased Nedd4-2-dependent channel retrieval. A putative clathrin adaptor protein 2 (AP-2) recognition motif (YxxΦ) is also present in the C-terminus of the γ-subunit and shares the tyrosine with the PY-motif. Importantly, mutating the leucine residue in this 627YxxLγ30 motif of γ ENaC significantly reduced the inhibitory effect of Cx30 on ENaC which averaged 23.5±4.0% (p<0.001, n=50). We conclude that Cx30 inhibits ENaC by reducing channel expression at the cell surface. Our finding that the inhibitory effect of Cx30 involves a putative AP-2 recognition motif in the C-terminus of the channel’s γ-subunit suggests that the effect may be mediated by stimulating channel retrieval via clathrin-dependent endocytosis. Reduced channel retrieval in the absence of Cx30 may explain an increased ENaC activity in mice lacking Cx30.


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Compensatory role of the NBCn1 Na+/HCO3− co-transporter on Ca2+-induced mitochondrial swelling in spontaneously hypertensive rat heart mitochondria

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NBC Na+/HCO3− co-transporter and NHE1 Na+/H+ exchanger have been associated with cardiac disorders and recently located in mitochondria of coronary endothelial cells (CEC) and cardiomyocytes, respectively. Mitochondrial NHE1 blockade delays mitochondrial permeability transition pore (MPTP) opening and reduces mitochondrial-derived superoxide production, two critical events exacerbated in cells of diseased hearts. Conversely, activation of the NBC isoform, NBCn1, prevented apoptosis in CEC subjected to ischemic stress. We characterized the role of these transporters in mitochondria of hearts from adult spontaneously hypertensive (SHR) and control (Wistar) rats. Expression of NHE1 was analyzed in left ventricular mitochondrial lysates, by immunoblot. NHE1 expression increased by ~40% in hypertrophic SHR compared to control (P<0.05, n=4). To determine if increased expression of NHE1 in cardiac hypertrophy correlates with increase transport activity, mitochondria were loaded with BCECF-AM dye and the maximal rate of pHm change measured after addition of 50 mM NaCl. SHR mitochondria had greater changes in pHm compared to Wistar rats, 0.10±0.01 vs. 0.06±0.01, respectively (P<0.05, n=5). Additionally, mitochondrial suspensions from SHR and control myocardium were exposed to 200 mM CaCl2 to induce MPTP opening (light scattering decrease, LSD) with the consequent mitochondrial swelling (MS). Surprisingly, SHR rats showed smaller LSD and a reduction in MS, 67±10% (n=26), compared to control, 100±8% (n=23). Selective NBC inhibition with 1 mM of the high-affinity 50859 compound, significantly increased MS in both, control 139±10% (n=7), and SHR 126±10% (n=7) mitochondria. Finally, NBCn1 Na+/HCO3− co-transporter increased by twofold its expression in SHR heart muscle mitochondria, compared to normal (P<0.05, n=5). Together our data suggest that increased NBCn1 activity plays a compensatory role in hypertrophic hearts, protecting mitochondria from Ca2+-induced MPTP opening and MS.

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Impairment of transient receptor potential Vanilloid 4-Mediated dilation in Mesenteric arteries of spontaneously hypertensive rats

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Background: Hypertension is increasingly becoming a matter of medical and public health importance. The maintenance of normal blood pressure requires a balance between cardiac output and total peripheral resistance (TPR). The endothelium, through the release of vasodilating factors, plays an important role in the control of TPR and hence blood pressure homeostasis. Transient Receptor Potential Vanilloid type 4 (TRPV4) is a mechanosensitive non-selective cation channel that is expressed on the endothelium and contributes to endothelium-mediated vasodilation. So far, no data are available about the morphological and functional status of this channel in hypertensive cases.

Objectives: To compare the morphological and functional expression of TRPV4 in the mesenteric artery of normotensive and hypertensive rats.

Methods: Young and adult Wistar-Kyoto rats (WKY-Y & WKY-A), as well as young and adult spontaneously hypertensive rats (SHR-Y & SHR-A) were involved in this study. Second order mesenteric arteries were isolated from male rats anesthetized with an intraperitoneal injection of ketamine (140mg/Kg) and xylazine (40mg/Kg) mixture. Isolated arterial segments, 2 mm in length, were mounted in a four-chamber wire myograph and pre-contracted with 4 µM phenylephrine. Then the effect of 5 µM 4αPDD (TRPV4 agonist) was investigated in the presence and absence of 1 µM HCO67047 (TRPV4 antagonist), 100 µM L-NAME (nitric oxide synthase inhibitor), and endothelium. The morphological distribution of TRPV4 in the mesenteric arteries was investigated by immunostaining and the level TRPV4 mRNA expression was studied using Real-time PCR. Values are means ± S.E.M., compared by student t-test.
Results: 4cPDD induced a relaxation response in the mesenteric arterial preparations (WKY-Y: 85.98%±4.18; n=5) that was markedly inhibited by HC067047 (18.3%±2.86; n=5; p<0.05), endothelium removal (19.93%±1.5; n=4; p<0.05) and L-NNAME (28.18%±3.09; n=5; p<0.05). The 4cPDD-induced relaxation was significantly lower in SHR-Y compared to WKY-Y (SHR-Y: 70.96%±3.65; n=6, WKY-Y: 85.98%±4.18; n=5, p<0.05). Moreover, the 4cPDD-induced response was significantly lower in WKY-A than WKY-Y (WKY-A: 75.58%±1.3; n=5, WKY-Y: 85.98%±4.18; n=5, p<0.05).

Immunostaining study showed immunofluorescent signal confined to the endothelial layer of mesenteric arteries. The expression of TRPV4 mRNA in SHR-Y was significantly lower than in WKY-Y (SHR-Y: 0.67RU±0.34; n=4, WKY-Y: 2.34RU±0.15; n=4, p<0.05). Furthermore, TRPV4 mRNA expression in WKY-A was lower than its expression in WKY-Y (WKY-A: 0.62RU±0.37; n=4, WKY-Y: 2.34RU±0.15; n=4, p<0.05).

Conclusion: Stimulation of TRPV4, expressed on the endothelium of rat mesenteric artery, triggers an endothelium-mediated relaxation response that markedly decreases with hypertension and growing up due to down-regulation of TRPV4 expression.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA125

Calcium handling as a determinant of the podocyte injury during diabetic nephropathy

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An increase in intracellular calcium $[Ca^{2+}]_i$ in podocytes is one of the major causes of their loss. Podocytopenia results in impaired glomerular filtration barrier (GFB) and proteinuria, characteristic for chronic kidney disease (CKD). Diabetic nephropathy (DN) is the leading cause of CKD. Key mediators of $Ca^{2+}$ flux in the podocytes are ion channels of the transient receptor potential canonical (TRPC) family (1) (other sources were also reported). A number of stimuli can trigger an elevation of $[Ca^{2+}]_i$ in the podocytes, including ATP and Angiotensin II (Ang II), and growing evidence suggests that both Ang II and ATP play a role in DN development. The goal of this study was to characterize mechanisms resulting in elevated $[Ca^{2+}]_i$ in podocytes during DN.

Dahl SS rats injected with streptozotocin (STZ-SS) and type 2 diabetic nephropathy (T2DN) rats were used as type 1 and 2 diabetes models. Rats were anesthetized with 2.5% isoflurane for kidney flush, and sacrificed using thoracotomy. Glomeruli were isolated from excised kidneys by differential sieving (2). Patch-clamp and imaging analyses were performed on podocytes of the glomeruli to assess calcium handling. Basal $[Ca^{2+}]_i$ was enhanced in both STZ-SS and T2DN rats compared to respective controls (268.3 ± 30.9 nM in STZ-SS rats and 231.9 ± 20.2 nM in T2DN rats, vs Wistar (150.1 ± 10.8 nM) and untreated SS rats (131.1 ± 8.99 nM); p<0.01). Ang II application resulted in approximately 1.5-fold increase in calcium influx, and an elevation in the TRPC channels open probability in podocytes of STZ-SS compared to control rats (0.6 ± 0.1 vs 0.29 ± 0.12, p<0.01) (3). WB and IHC, along with the data obtained using TRPC modulators SKF 96365 and La$^{3+}$, and knockout mice (4), was consistent with TRPC6 being the channel mediating this effect. Data are reported ± SEM, significance tested with ANOVA.

Next, we assessed the effects of ATP on podocytes during DN. The response to ATP in Wistar rat podocytes was similar to what we reported for Sprague Dawley rats (EC50 = 10.7 ± 1.5 µM (5)). However, it was substantially enhanced in GK rats (diabetic but lacking pronounces DN), with dose-response curve shifted towards a potent activation with lower ATP concentrations. Importantly, calcium transient was further increased in T2DN, and dose-independent within the tested concentration range was shifted towards more potent activation by lower ATP concentrations. Western blotting revealed a significantly higher expression of ionotropic P2X7 and P2X4 receptors in the cortex of T2DN and GK strains compared to Wistar rats, and a decrease in the expression of metabotropic P2Y1 receptors.

Taken together, these studies have a strong potential for advancing our understanding of calcium-mediated effects on podocytopenia in DN, and identification of new pharmacological targets to maintain podocyte integrity and function. Ilatovskaya DV and Staruschenko A. (2015). AJP Renal Phys 309, 393-7.


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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA126

Actin-based regulation of ENaC-like sodium channels in leukemia cell lines.

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Actin cytoskeleton is one of the important cellular systems that are structurally and functionally coupled with ion channels in plasma membrane. We have previously shown that function of non-voltage-gated sodium channels in human leukemia K562 cells is critically dependent on actin dynamics. In recent years, leukemia-lymphoma cell lines are extensively used as appropriate cellular models to study the role of ion-transporting systems in hematologic malignancies. In the present work, we used different modes of patch clamp technique to examine ion channels involved in Na-transporting pathway in U937 human lymphoma cells. The activity of native sodium-selective channels with unitary conductance of 10–11 pS was revealed in cell-attached, inside-out and whole-cell configurations. Patch-clamp data indicated principal similarity of functional properties of the channels in K562 and U937 cells. Sodium channel activity in leukemia-lymphoma cell lines was directly controlled by submembranous actin cytoskeleton. Specifically, an activation of sodium channels in U937 cells in response to microfilament disruption by cytochalasin D was demonstrated on single-channel and integral current level. Inside-out experiments with the use of globular actin showed that filament assembly on cytoplasmic membrane surface caused fast inactivation of the channels. Bio-physical characteristics of non-voltage-gated sodium channels
in leukemia cell lines were similar to that of epithelial sodium channels (ENaCs). Consistently, an expression of alpha-, beta-, gamma-hENaC subunits in K562 and U937 cells was detected using RT-PCR and immunofluorescent staining. However, we showed that amiloride (up to 0.1 mM), known inhibitor of DEG/ENaC, did not block single sodium channels; whole-cell current measurements revealed no amiloride-sensitive component of membrane current. Interestingly, we found that sodium channel activity was drastically increased in response to extracellular application of trypsin (5 mg/ml). Taken together, our observations suggest that amiloride-insensitive sodium channels in K562 and U937 cells belong to the ENaC family. We conclude that cortical actin structures represent the main factor that controls the activity of ENaC-like channels in human leukemic cells.

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PCA127

Glucocorticoids activate the thiazide-sensitive Na-Cl transporter through glucocorticoid receptor stimulation

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The distal convoluted tubule (DCT) reabsorbs ∼7% of the filtered sodium load and contributes importantly to blood pressure regulation. The Na-Cl co-transporter (NCC) is the major route for apical Na entry into DCT cells. Thiazide diuretics inhibit this NCC and are a mainstay hypertension treatment. Previous studies have shown that both mineralocorticoids and glucocorticoids can increase NCC expression. Although glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) are expressed throughout the distal nephron, this part of the renal tubule is conventionally thought to be aldosterone-sensitive due to 11b-hydroxysteroid dehydrogenase (11bHSD2) coexpression. However, we find that 11bHSD2, which inactivates glucocorticoids, seldom colocalizes with NCC, suggesting that glucocorticoids may directly activate NCC. We therefore investigated whether glucocorticoids activate NCC in vivo and whether GR or MR is involved in this process.

Male C57BL6 mice were anaesthetized under isoflurane and implanted (s.c.) with silastic pellets containing spironolactone (50 mg, chronic GR blockade) or RU486 (60 mg, chronic GR blockade). Control mice received no implant. After 5 days, corticosterone (cort, 6 mg/kg, s.c.) or vehicle (veh, 2% DMSO, s.c.) was injected at ~8am, coincident with the nadir of the endogenous corticosterone circadian rhythm. 4 hours later mice were culled and kidneys snap frozen. NCC threonine phosphorylation (on T53, pT53-NCC) was measured by western blot analysis as an index of transporter activation. NCC abundance (1.0 ± 0.15 AU (veh) vs 1.9 ± 0.28 AU (cort), p=0.004, Student’s t-test, n=6 in each group). Chronic MR blockade reduced total NCC protein (0.39±0.21 AU (spironolactone; n=5) vs 0.80±0.18 AU (control; n=6), p=0.004, by post hoc Tukey tests) but acute cort still substantially increased pT53-NCC abundance (1.0±0.46 AU (veh; n=5) vs 3.7±1.4 AU (cort; n=7), p=0.0018, Student’s t-test). In contrast, chronic GR blockade did not affect total NCC abundance (0.59±0.15 AU (RU486) vs 0.80±0.18 AU (control) p=0.15, Student’s t-test, n=6 in each group) but did prevent phosphorylation induced by acute cort injection (1.0±0.19 AU (veh) vs 1.0±0.15 (cort), p=0.51, Student’s t-test n=6 in each group).

These data suggest MR activation is required for maintenance of tonic levels of NCC protein. They further show that glucocorticoids can regulate NCC function by acutely increasing NCC phosphorylation through GR activation. This enhanced NCC activity has implications for blood pressure control, particularly in individuals with inappropriately elevated glucocorticoids, such as in Cushing’s syndrome, metabolic syndrome, or during chronic stress.

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PCA128

Caustic oesophageal burn injury in rats is alleviated by the antifibrotic drug halofuginone

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Background: Accidental ingestion of corrosive substances in children, leading to oesophageal strictures, is still an important health issue in developing countries. We have previously shown that halofuginone, a specific inhibitor of collagen type 1 synthesis, is beneficial in ameliorating oxidative damage in different rat models. The aim of this study is to evaluate the anti-inflammatory and antifibrotic effects of halofuginone in caustic oesophageal burn injury in rats.

Materials and Methods: Under ketamine (100 mg kg−1, I.P.) and chloropromazine (0.75mg kg−1, I.P.) anaesthesia, caustic oesophageal burn injury (EBI) was produced in male Wistar albino rats by the application of 37.5% NaOH onto the distal oesophagus (n=40), while only 0.09% NaCl solution was instilled in the control group (n=8). Until the rats were decapitated on the 3rd day (early group) or on the 28th day (late group) of EBI induction, rats were treated intraperitoneally with saline or halofuginone (100 mg/kg/day) on each day and during chronic stress. Nitric oxide (NO), peroxynitrite (ONOO-), nuclear factor (NF)-kβ, caspase-3 and luminol- and lucigenin-enhanced chemiluminescence (CL) levels were measured in the oesophageal tissues. Tissue samples were prepared for histopathological evaluation. Statistical analysis was performed by ANOVA and Student’s t-tests. Results: NFkB and caspase-3 levels were not different among groups. Microscopic damage scores were elevated in both early and late EBI groups (p<0.001), while halofuginone treatment reduced the microscopic damage scores in both groups. NO, ONOO- and CL levels, which were elevated in the saline-treated early EBI group (p<0.05-0.001), were suppressed by halofuginone treatment (p<0.05). EBI-induced elevations in NO and ONOO- levels were reduced in the late period of saline-treated group, while these levels were increased by halofuginone (p<0.001). EBI-induced high CL levels were not changed in the late groups treated with either saline or halofuginone.
Conclusion: In the early period, halofuginone alleviated injury of the caustic oesophagus by reducing the release of oxygen/nitrogen-derived free radicals. Although halofuginone was still efficient in reducing EBI in the chronic phase, its oxidant scavenging effect was replaced by enhanced production of nitrogen radicals, suggesting the contribution of other anti-inflammatory mechanisms.


Karadeniz CK et al. (2013). J Pediatr Urol 9, 174-83

Karayoyun B et al. (2010). Dig Dis Sci 55, 607-16

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**PCA129**

Placental uptake and metabolism of vitamin D

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Low vitamin D levels are common in pregnancy and are linked to suboptimal fetal growth (Harvey et al., 2008). This is important as poor fetal growth is associated with increased risk of chronic disease in adulthood (Barker, 1998). Supplementation with inactive 25-hydroxyvitamin D (25(OH)D) is often recommended. However, it is unclear whether 25(OH)D or the active 1,25-dihydroxyvitamin D (1,25(OH)2D) are both taken up into the placenta. In addition, serum vitamin D is bound to carrier proteins, vitamin D binding protein (DBP) and albumin (Chun et al., 2014), but their role in vitamin D uptake is uncertain. We aimed to establish whether both 25(OH)D and 1,25(OH)2D are taken up into the human placenta, and whether the presence of carrier proteins impacts uptake.

Term human placentas were collected within 30 min of delivery with ethical approval and informed consent. To investigate placental uptake of vitamin D, placental villous fragments were cultured for 8 h in Tyrodes buffer containing 20 µM 25(OH)D (n=6), 25(OH)D + 0.7 mM albumin (n=6), 50 nM 1,25(OH)2D (n=11), 1,25(OH)2D + 0.7 mM albumin (n=11) or 1,25(OH)2D + 5 µM DBP (n=6). Controls were incubated with vehicle and albumin. Endocytic processes for 1,25(OH)2D uptake were investigated by adding 5 mM 1,25(OH)2D incubated with vehicle and albumin. Endocytic processes (n=11) or 1,25(OH)2D + 5 mM 1,25(OH)2D (n=11), 1,25(OH)2D + 0.7 mM albumin (n=6).氨or with endocytic blockers (n=3) for ≤1 h at 4 and 37°C. Samples were fixed, stained with lectins, and viewed on the confocal microscope. Data were analysed by one- and two-way ANOVA. CYP24A1 mRNA expression increased with 25(OH)D (p<0.001) compared to controls indicating 25(OH)D uptake and conversion to 1,25(OH)2D within the placenta. The magnitude of the effect was greater with 25(OH)D and albumin (p<0.01), suggesting it facilitates vitamin D uptake. Incubation with 1,25(OH)2D increased CYP24A1 mRNA expression (p<0.001) compared to controls, which did not increase further with albumin (p=0.16) or DBP (p=0.88). Uptake of FITC-albumin increased proportionately with time (p=0.08) at 37°C but not at 4°C (p=0.004). Dynasore did not alter CYP24A1 expression compared to 1,25(OH)2D with (p=0.9) or without albumin (p=1). Amiloride significantly reduced CYP24A1 mRNA expression compared to 1,25(OH)2D with (p<0.001) and without albumin (p=0.006) and also reduced FITC-albumin uptake (p=0.03). These data suggest that 25(OH)D and 1,25(OH)2D are taken up into the placenta and can induce vitamin D dependent gene expression, implying the placenta converts 25(OH)D to 1,25(OH)2D. Furthermore uptake of 25(OH)D may be enhanced by albumin, while amiloride inhibited both albumin uptake and 1,25(OH)2D stimulated CYP24A1 expression.


Gerald Kerkut Trust, RMC.

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**PCA130**

The correlation between microbiota metabolic activity & mucosal homeostasis after ceftriaxone treatment

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It is well established that compositional changes in the intestinal microbiota can lead to severe dysregulation of the physiological and immunological intestinal homeostasis with adverse consequences for the host, but mechanisms of this interaction are not fully understood. Recent studies suggest that products of microbial metabolism in the gut act as signaling molecules and influence host energy homeostasis (1, 2). According to epidemiological studies increasing exposure to antibiotics is associated with increased risk of developing multiple inflammatory disorders (3). Here we investigated whether and how antibiotic-induced disturbance in microbiota composition and its metabolic profile may affect the functional state of colon mucosa cells. Methods: Male Wistar rats (n=16, 140-160 g) were treated for 14 days with broad-spectrum antibiotic ceftriaxone (CF) (300 mg/kg, i.m.) or vehicle; euthanized by CO2 inhalation followed by cervical dislocation next day after CF withdrawal. The study was approved by the bioethical committee of Taras Shevchenko National University of Kyiv (Protocol No 8 issued by Nov 2, 2015). The parietal microbiota was analyzed by bacteriological culture methods; faecal short chain fatty acids (SCFA) - by gas chromatography; colonic localization and levels of FFAs – by immunohistochemistry, levels of Erk1/2, p38, Egr-1, Sp-1 and Hif-1α proteins – by Western blot analysis, superoxide dismutase (SOD) activity - by zymography method; catalase activity – colorimetrically. Results: CF injection leads to increase the level of Propionibacterium and Bacteroidetes 1.6, 2.7-fold (p=0.05) respectively. Despite that the absolute
amount of SCFA, levels of butyrate, propionate, acetate were decreased by 5.1, 9.3, 15.0, 2.7-fold (p<0.05), respectively. FFA2 receptors were localized on the goblet cells and surface enterocytes; FFA3 receptor – on surface enterocytes and in myenteric ganglia in the colon; MCT1 and MCT4 transporters - on the epithelial basolateral membrane surface and crypts enterocytes, SMCT1 - in the brush border of enterocytes. Cf administration decreased the immunoreactivity for FFA2 & FFA3 receptors and SMCT1 transporter but increase the MCT1 and MCT4 transporters. These changes were accompanied by the increase of Erk1/2 and decrease p38 MAP kinases activity in colon mucosa. Cf administration also decreased the activity of SOD and catalase antioxidant enzymes 1.4-fold (p<0.05) and increased levels of redox-sensitive transcriptional factors Egr-1, Sp-1 – 1.3-fold & Hif1α - 2-fold (p<0.05). Conclusions: Antibiotic-induced changes in SCFA amount and number of specific receptors and transporters to them evoked a signifi- cant shift in colonic mucosal homeostasis.

Shaw SY et al. (2011). Am J Gastroenterol. 106, 2133-42

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA131

A macrophage shuttle for transendothelial stem cell delivery
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Microbial modification of macrophages to arrest phagosome maturation is generally considered an undesirable outcome in terms of host defence. However, repurposing of this process may provide a new avenue for stem cell delivery in regenerative medicine. We investigated the feasibility of creating a "macrophage shuttle" – where a modified macrophage would ingest, but not digest, one or more stem cells and then deliver these to a tissue site requiring regeneration. After obtaining ethical clearance from the Human Research Ethics Committee of Stellenbosch University, peripheral monocytes were isolated from human donor whole blood using density centrifugation. Primary monocyte cultures were maintained under normal cell culture conditions, but exposed to 50ng/ml E.coli LPS and 20ng/ml IFN-γ for 6 days to pre-differentiate them into type M1 macrophages, which have known ability to cross endothelial barriers. M1 macrophages were then treated with Wortmannin, Concanamycin A and Chloroquine to achieve phagosome maturation arrest and thus prevent digestion of stem cells after phagocytosis. For ethical reasons, blue fluorescent latex beads (diameter 6 μm) were used to simulate stem cells. These beads were opsonised by coating them with (red) Alexa Fluor 647-labelled goat anti-human IgG. This also allowed for visualisation of phagosomal digestion. Phagosomal degradation of red antibody signal and phagosome acidification (using pHrodo as indicator) was assessed visually over a period of 2 hours using confocal microscopy, as well as quantitatively by flow cytometry at 2 hours. All experiments were carried out in triplicate and repeated on three separate occasions (n=3). Results showed that phagosome maturation arrest was indeed succesful: firstly, the pH within phagosomes of modified cells was less likely to become acidic when compared to controls (40±35% vs. 92±3% of cells, t-test P<0.05). Secondly, the coated antibody was digested from latex beads in control cells to a greater extent that in modified cells (complete digestion in 7.5±3.1% vs. 1.5±1.1% of cells, P<0.05). Thirdly, macrophage modification did not compromise phagocytic capacity (successful phagocytosis in 61±16% of control cells vs. 65±13% of modified cells). Evidence of transendothelial transport of C2C12 skeletal muscle stem cells by modified macrophages will also be presented. Variability in results for phagocytic capacity and antibody digestion was similar for control and modified cells, but the extent of inhibition of phagosome acidification varied more. This variability may be ascribed to the fact that a different human monocyte donor was used for each repeat experiment and highlights the requirement for individualised medicine in this context. In conclusion, although more developmental work is required, this system presents an exciting new avenue for investigation in the fields of regenerative medicine and inflammation.

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PCA132

Epithelial regeneration after gastric ulceration causes prolonged altered cell types and weakened defenses in mice
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The peptic ulcer heals through a complex process, although the ulcer relapse often occurs several years later after healing even in H. pylori eradicated or non-NSAIDs treated patients. Our hypothesis is that even after visual evidence of healing of gastric ulceration, the regenerated epithelium is aberrant for an extended interval, increasing susceptibility of the regenerated epithelium to damage and further diseases. C57BL/6j (Wild-type), TFF2 KO and NHE2 KO mice were used in the present study. Under isoflurane anesthesia, the abdomen was incised and the intact stomach exposed. A microcapillary tube (0.7 mm in diameter) filled with acetic acid (99%) was placed in contact with the exterior surface of the stomach corpus region and left in place for 25 s. A single gavage of 10⁶ H. pylori (Sydney strain 1) was performed 30 days after ulcer induction. Gastric corpus ulcers induced by acetic acid visually healed in a time dependent manner, and they cannot be identified macroscopically at 30 days after ulceration. However, Day 30 regenerated epithelial architecture was poor. The gene profile of regenerated tissue was abnormal, indicating increased stem/progenitor cells, deficient differentiated gastric cell types, and deranged cell homeostasis. Despite upregulation of PDX1 in the regenerated epithelium, no mature antral cell type, such as gastrin-expressing cell, was observed. Using RT-PCR or immunostaining, the regenerated epithelium at 30 days and 4 months is shown to completely lack parietal cells (H-K ATPase) and Na/H exchanger 2 (NHE2: a TFF2 effector in gastric healing) while the trefoil factor 2 (TFF2) co-expressed Gsii (Griffonia Simplicifolia lectin II), DCLK1 (Tuft cell marker: doublecortin like kinase), SOX9, or Ki67 are upregulated and widely distributed even deep in the gastric gland, suggesting that regenerated epithelium sustains a metaplastic condi- tion. Similar to ulcer-regenerated epithelium in the wild-type mouse stomach, gastric epithelium in NHE2 KO lacked parietal...
cells and upregulated TFF2/GSII and DCLK1, SOX9, and Ki67 in the base of gland, suggesting that NHE2 plays important roles in maturation of gastric epithelium. Gastric ulcer healing was strongly delayed in TFF2 knockout mice, and re-epithelialization was accompanied with upregulation of GSII. Following H. pylori inoculum 30 days after ulceration, we observed that the gastric ulcer selectively relapses at the same site where it was originally induced, although H. pylori did not cause gastric injury in other sites. Follow up at 8 months showed that the relapsed ulcer was not healed in H. pylori infected tissues. These findings reveal that this macroscopically regenerated epithelium has prolonged abnormal cell distribution and is differentially susceptible to subsequent damage by H. pylori.

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PCA133

Susceptibility to experimental type I diabetic nephropathy is influenced by multiple P2X7 isoforms

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The P2X7 receptor is an ATP-gated cation channel implicated in pro-inflammatory diseases including diabetic nephropathy. P2X7 is expressed on multiple cell types including pancreatic cells, endothelial cells, and macrophages; glomerular expression increases with the onset of kidney disease (1-3). Mice have five protein coding isoforms; the canonical transcript (P2X7), a full-length variant (P2X7k), and three truncated forms (P2X7Δc). Gene-targeted murine models expressing alternative P2X7 isoforms provide an opportunity to determine their functional importance in vivo. We investigated the role of P2X7 variants in pancreatic injury and diabetes using streptozotocin (STZ) injection (50mg/kg, IP) for five consecutive days following 4-6 hour fasting into mice expressing either all 5 isoforms (control), P2X7Δc only, or P2X7k only. Three weeks after STZ, control mice developed hyperglycaemia (control vehicle = 8.6±1.0 vs. control STZ = 22.8±7.0 mmol/l; P<0.05; mean±s.d.), however P2X7Δc mice were resistant to a rise in blood glucose (P2X7Δc vehicle = 8.5±1.0 vs. P2X7Δc STZ = 14.1±8.6 mmol/l; P=ns; mean±s.d.). P2X7Δc mice had better preservation of islet architecture, β-cell density and insulin staining (p=0.036 vs controls). One explanation for this may be that P2X7Δc mice lack the capacity to assist in the formation of pro-inflammatory membrane pores. P2X7k mice, by contrast, were equally susceptible to STZ induced hyperglycaemia when compared with STZ injected controls. At 3 weeks. We extended the protocol to 12-weeks following STZ injections. Resistance to hyperglycaemia was again confirmed in P2X7k mice. In the kidney, CD68+ macrophage accrual was 70% lower in P2X7k mice compared with controls (P<0.01). Following STZ treatment, macrophage accumulation increased (control vehicle = 0.14 ± 0.15 vs. control STZ = 0.87 ± 0.53 cells/glom; p<0.001; mean±s.d.) and type IV collagen deposition increased (control vehicle = 13.9 ± 1.5 vs. control STZ = 17.8 ± 2.2 % surface area; p<0.01) in control mice only. In conclusion, our investigations into P2X7Δc and P2X7k mice found abolished and reduced susceptibility to experimental diabetic nephropathy respectively.


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PCA134

UT-B1 urea transporter localisation in human ureter

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UT-B1 has been localized to bladder and ureter in both rat and mouse (1,2). Recently UT-B1 has been localized to bladder urothelium in humans (3), while its expression and localisation in human ureter have not yet been reported.

In this study, Western blotting of normal human ureter whole homogenate protein was performed. Immunoperoxidase staining was performed using 4μm formalin-fixed, paraffin-embedded normal human ureter tissue sections. Both methods employed a well-characterized hUT-B c19 antibody. A characteristic 40-45kDa band equating to glycosylated UT-B1 was detected in human ureter whole homogenate protein. Treatment with PNaseF gave a discrete 30kDa band as previously reported for human bladder tissue. This signal was completely ablated in de-glycosylated protein probed with primary antibody incubated with the immunizing peptide. In control experiments strong peroxidase staining was evident in human bladder urothelium. In contrast, minimal peroxidase staining, localized to the basal cell layer, was detected within ureter urothelium. Some strong staining was detected in sub-epithelial blood vessels of the ureter tissue. UT-B1 expression and localisation in human ureter urothelium differs markedly from that of human bladder urothelium. This may be reflective of the separate embryological lineages of each tissue and the varied efflux kinetics associated with the differing functional roles of each tissue. Further research is now required to investigate whether ureteral UT-B1 may be subject to more acute regulation.


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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Sec16A is critical for both conventional and unconventional secretion of CFTR

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The cystic fibrosis transmembrane conductance regulator (CFTR) is a transmembrane protein with anion channel activity that reaches the cell surface via the conventional Golgi-mediated secretion pathway under normal conditions. Interestingly, ER-to-Golgi blockade or ER stress induces alternative GRASP-mediated, Golgi-bypassing unconventional trafficking of wild-type CFTR and the disease-causing ΔF508-CFTR, which has folding and trafficking defects (Gee et al., 2011). However, the molecular mechanisms that underlie the unconventional secretion, and particularly, how the CFTR leaves the ER are unknown. Here, we show that Sec16A, the key regulator of conventional ER-to-Golgi transport, plays a critical role in the ER exit of protein cargos during unconventional secretion. In an initial gene-silencing assay, Sec16A knockdown abolished both the conventional cell-surface trafficking of the Golgi complex-glycosylated WT-CFTR (band C) and the unconventional cell-surface trafficking of the ER core-glycosylated WT-CFTR as well as ΔF508-CFTR (band B) induced by Arf1-Q71L or GRASP55 overexpression. The surface expressed CFTR relative to total lysates value was quantified. The results of multiple experiments are summarized and data are shown as mean ± SEM. Statistical analysis was performed using paired t-tests or with one-way ANOVA. Surface/lysates value of CFTR was reduced by Sec16A silencing (band C of WT-CFTR: 100 ± 10.79 i-Sec16A, p< 0.05; band B of WT-CFTR: 100±0.0 vs. 14.29±5.15 i-Sec16A, p< 0.001; Arf1-Q71L induced ΔF508-CFTR: 100±0.0 vs. 43.45±6.97 i-Sec16A, p< 0.001; GRASP55 overexpression induced ΔF508-CFTR: 100±0.0 vs. 38.31 ± 7.34 i-Sec16A, p< 0.001). Moreover, Sec16A colocalized with GRASP55 in mammalian cells, following stimuli that induce unconventional secretion, such as Arf1 mutant-induced ER-to-Golgi blockade or thapsigargin-induced ER stress. GRASP55 overexpression or ER-to-Golgi blockade relocalized the Sec16A puncta into >80% of the whole cellular area in HeLa cells, whereas only ~20% of the cellular area was Sec16A-positive in control cells. In contrast, subcellular localization of Sec31A, a representative subunit of COPII showed no significant alteration under the conditions of GRASP55 expression or ER-to-Golgi blockade. Collectively, the results suggest that the formation of GRASP-mediated unconventional vesicles is accompanied by the assistance of Sec16A, but is independent of the COPII proteins. In addition, IRE1α depletion reduced unconventional secretion of CFTR, while supplementation with Sec16A rescued the IRE1α depletion-induced reduction in unconventional secretion (ANOVA: p < 0.001), suggesting that IRE1α acts as an upstream signal for ER stress-associated unconventional secretion by modulating Sec16A. These findings highlight a novel function of Sec16A as an essential mediator of ER stress-associated unconventional secretion.


Cadmium (Cd) exposure is linked to breast cancer since Cd mimics the effects of estrogens in mammary epithelial cells (Johnson et al., 2003). However, Cd exposure leads to epithelial mammary cells transformation into a highly aggressive cancerous phenotype independently of hormonal receptors suggesting that Cd promotes cancer progression by different mechanisms (Benbrahim-Tallaa et al., 2009). Ion channels are involved in cancer progression by various mechanisms including membrane potential, volume and calcium signaling regulations. Among ion channels, the transient receptor potential melastatin related-7 (TRPM7) allows trace metals entry including Cd entry (Monteilh-Zoller et al., 2003). Importantly, TRPM7 overexpression predicts poor outcome in breast cancer patients (Middelbeek et al., 2012). Moreover, TRPM7 regulates breast cancer cell migration and invasion (Middelbeek et al., 2012; Guilbert et al., 2013), and metastasis formation in vivo (Middelbeek et al., 2012). The aim of this work is to assess the role of TRPM7 in transformed mammary epithelial cells induced by Cd exposure. MCF10A mammary epithelial cells were incubated with low Cd concentration (2.5 µM) during 40 weeks (Benbrahim-Tallaa et al., 2009). Invasive properties of transformed cells were studied by using Boyden chamber assays with and without Matrigel. Matrix Metallo-protease (MMP-2 and -9) secretion was assessed by gelatin zymography. Plasminogen activator secretion (tPA and uPA) was analyzed by gelatin-plasminogen zymography. Finally, activity of TRPM7 was studied by whole-cell patch-clamp. In MCF10A exposed to Cd, both cell migration and invasion were increased (+29.74 ± 3.22 % for migration (N=4), and +33.24 ± 0.03 % for invasion (N=3)) when compared to non-treated cells (p<0.001). Moreover, Cd exposure stimulated MMP-2 and MMP-9 as well as tPA and uPA secretions. TRPM7 current was also increased in MCF10A exposed to Cd (44.91 ± 3.89 pA/pF at +100 mV; n=15) when compared to the non-treated cells (27.67 ± 2.17 pA/pF at +100 mV; n=14; p<0.001). TRPM7 silencing decreased more migration in Cd-treated cells (-73.13 ± 1.92 %, N=4; p<0.001). Similarly, TRPM7 silencing decreased cell invasion by 55.64 ± 2.95 %, in non-treated cells and in a larger manner in Cd-treated cells (-82.57 ± 1.04 %; N=3; p<0.001), and also inhibited proteases secretion. Collectively, our results clearly show that TRPM7 regulates invasiveness in Cd-transformed mammary epithelial cells.

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PCA137

**STIM and Orai proteins regulate Ca^{2+} entry into isolated human eccrine sweat gland secretory coil cells**

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The production of sweat fluid by eccrine sweat glands in humans, in response to heat or exercise, is crucial to the regulation of body temperature. A key event in this process is a biphasic increase in the level of intracellular Ca^{2+} ([Ca^{2+}]_{i}) consisting of an initial agonist-induced release of Ca^{2+} from internal stores, followed by an influx of extracellular Ca^{2+}. We have shown previously in a cell line derived from human eccrine gland secretory cells (NCL-SG3) that the regulation of Ca^{2+} entry is initiated by the emptying of intracellular Ca^{2+} stores, and maintained through a process known as store-operated Ca^{2+} entry (SOCE) via the interaction of STIM and Orai proteins. However, it is unclear if the role of STIM-Orai in these cells truly depicts the events in human eccrine secretory coils. The aim of this study was to investigate SOCE in isolated eccrine sweat gland secretory coils. Sweat glands were freshly isolated from skin samples obtained from individuals with consent and ethics approval. The isolated glands underwent PCR, Western blot analysis and immunofluorescence in order to identify the presence and localisation of STIM and Orai proteins. The functionality of the SOCE pathway in the isolated glands was investigated using calcium-imaging techniques in conjunction with the ER Ca^{2+}-ATPase inhibitor thapsigargin (Tg), and a variety of known SOCE inhibitors. SiRNA knockdown of STIM and Orai proteins was used to determine relative contributions to the SOCE process. Expression for STIM1 and Orai’s 1 and 3 were confirmed by PCR and protein expression. Immunofluorescence studies showed that STIM1 was localized intracellularly (probably the ER), and Orai1 and 3 in the plasma membrane of the secretory coil cells. Fluorescence imaging experiments using the dye Fura-2, showed that the influx of extracellular Ca^{2+}, initiated by Tg-induced emptying of the ER stores, was reduced in a dose-dependent manner with an IC_{50} between 10^{-6} and 10^{-7}M by either diethylstilbestrol, carboxamidotriazole or BTP2, whilst the general inhibitor 2-APB was less efficacious (IC_{50} approx. 10^{-4}M). Calcium imaging of eccrine glands showed that Tg-induced SOCE was reduced ~50% in an Orai1 knockdown, whilst Orai3 knockdown had no effect on SOCE. This suggests that Orai3 can partially substitute for Orai1, and Orai1 can totally substitute for Orai3 during respective knockdowns. A double Orai1–Orai3 knockdown completely inhibited SOCE. This study demonstrates that STIM1 and Orai proteins are key components of the SOCE pathway in human eccrine sweat gland secretory cells, and are central to the activation of the membrane transport proteins that drive transepithelial chloride flux, and ultimately the production of sweat.

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PCA138

**Ibuprofen nanoparticles and its cytotoxicity on A549 and HaCaT cell lines**

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Ibuprofen (IBF) is an outstanding non-steroidal drug for analgesic and anti-inflammatory therapies but it exhibits poor solubility in water [1, 2]. Increased dosage administration has been linked to gastrointestinal and cardiovascular complications [3]. Many techniques have been employed to improve the solubility of NSAIDs [4]. In this study, the anti-solvent precipitation method was used to make Ibuprofen nanoparticles (IBF NPs). Optimised preparation parameters such as solvent (ethanol), raw drug concentration (400 mg), solvent/anti-solvent ratio (1:50) and surfactant concentration (0.25 mg/ml) have been studied to yield nanoparticles with a mean size of 58.8 nm, which is confirmed by dynamic light scattering and transmission electron microscopy. These IBF NPs possess increased aqueous solubility compared to the micro counterpart and maintain with chemical integrity indicated by high performance liquid chromatography and Fourier transform infrared spectroscopy.

In addition, in vitro cytotoxicity of IBF NPs has been studied on A549 and HaCat cell lines using MTT and LDH assays. Both cells were obtained from ATCC. The A549 cells were grown in a modification of Ham’s F-12, containing L-glutamine, called F-12K. The HaCat cells were grown in DMEM containing sodium pyruvate (110 mg/l). Normal cell culture and sub-culture were applied and the cells were used after around 45 passages [5]. The cell culture media containing 10^{5} cells/
ml were placed in a 96-well plate with addition of IBF NPs and Micro form at concentrations in the range of between 6 and 500 µg/ml by diluting them with DMEM and F-12K for use with the HaCaT and A549 cells respectively. After 24, 48 and 72h exposure, the MTT and LDH cytotoxicity assay were performed in triplicates and on three separate experiment cultures and the absorbance was recorded at 570 nm and 492nm respectively with Elisa micro plate reader. The cell viability (%) related to control (cells in culture medium without NPs) was calculated. A very good cytotoxicity profile was observed, indicating an in vitro cytocompatibility of the IBF NPs in these cell culture systems and no significant changes in cytotoxicity compared with Micro IBF.

We conclude that our IBF NPs have increased solubility, same chemical integrity and unchanged cytotoxicity compared to IBF Micro drug. Further work will concentrate on optimising more rigorous parameter to produce excellent quality NPs. More detailed characterisation of IBF NPs is to be tested, such as using PXRD and SEM to further corroborate particle shape and size. The range of no toxic in vitro concentrations is also to be further confirmed. Eventually scaled up preparation of IBF NPs will be developed without relinquishing NPs quality. This would improve the potential for in vitro / in vivo applications and clinical use of IBF NPs and NSAIDs in general.


RP, MZ, NB, QI, FM contribute equally, CAs: LS, XC

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PCA139

Cytotoxicity of in vitro exposure of polystyrene latex bead nanoparticles to human keratinocyte (HaCaT) cells and human cervical cancer (HeLa)cultures

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Nanoparticles are increasingly used in industry and medicine due to their unique physiochemical properties such as their small size, charge, shape, chemical architecture, large surface area, surface reactivity and media interactions, etc [1-5]. However, very little is still known on the interactions between nanoparticles and the biological system. This study aims to evaluate the cytotoxicity of polystyrene latex bead nanoparticles on HaCaT and HeLa cell lines. Carboxyl-modified 20 nm polystyrene NPs core labelled with fluorophore were from Invitrogen. We chose to use polystyrene NPs because this specific type of NP is being increasingly characterized for use in nanosensors and drug nanocarrier investigations. 1x 10^4 cells/100 µl of cell culture medium were plated into 96-well plates in triplicate, measuring activity post 24 hours at concentrations of 10, 50, 100 µg/ml of polystyrene NPs exposure. The extracellular lactate dehydrogenase release was measured by using a colorimetric CytoTox 96 nonradioactive assay kit from Promega and the absorbance were recorded at 450nm (FLUOSTar) with Elisa micro plate reader. The MTT assay was used to evaluate mitochondrial activity. This was performed by inserting a premixed optimized dye solution in the culture wells. The Absorbance was recorded at 570 nm, from the recorded absorbance is directly proportional to the number of live cells. The cell lines were kept in a plastic T-75cm² tissue culture flasks grown in DMEM.

We found that cytotoxicity of polystyrene NPs on both cells was concentration dependent. For Hela cells, with exposure of polystyrene NPs at concentrations of 10, 50, 100 µg/ml for 24 hrs, the percentage cytotoxicity of positive control for LDH assay was 35.9%, 49.5% and 73.4% respectively. With the MTT cell viability assay the percentage MTT reduction of negative control was 88.9%, 42.9% and 26.4% respectively. Cell toxicity increased with increasing polystyrene NPs concentration. For HaCaT cells, the cytotoxic effect is less significant than those on Hela cells. With MTT assay, when compared to HaCaT cells exposed to a negative control containing only PBS, the cell viability decreased as the concentrations of NPs increased. Cells exposed to 100µg/ml of polystyrene NPs for a period of 24 hours compared to those exposed to a positive control (100% cell viability) had an average cell viability of 49%, with those numbers decreasing from 59% for cells exposed to 10µg/ml of polystyrene NPs to 57% for cells exposed to 50µg/ml of polystyrene NPs.

Our results indicated that polystyrene NPs acted differently in two different cell types and that cautions should be taken about its cytotoxicity. Further understanding of the mechanism involving the ROS generation could provide more information on how polystyrene NPs increase cytotoxicity.


RP, MZ contribute equally

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PCA140

Relating ligand binding and channel gating in nicotinic acetylcholine receptors

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Nicotinic acetylcholine receptors (nAChRs) are expressed in different neuronal and non-neuronal cell types throughout the body, where they mediate fast synaptic cholinergic transmission. nAChR current responses are characterized by a rapid activation upon agonist binding followed by a complex desensitization in presence of the agonist. To contribute to the understanding of the reciprocal relationship between agonist binding and desensitization of the receptor, we applied electrophysiological as well as fluorescence techniques employing a fluorescently tagged ACh derivative, Cy3-ACh. Herein, we studied adult muscle-type nAChRs, composed of α1, β1, δ, and ε subunits in a ratio of 2:1:1:1, heterologously expressed in HEK293 cells. For electrophysiological recordings
as well as for fluorescence imaging, whole cells were lifted after obtaining the whole-cell configuration and positioned in front of a double-barreled application pipette. Fast solution exchanges were realized by a piezo device. Fluorescence recordings were performed with the confocal laser scanning microscope LSM710 (Zeiss, Jena, Germany). Cy3-ACh was excited with a 543nm HeNe laser line. Values are means ± S.E.M.

First, we characterized the functionality of Cy3-ACh on muscle-type nAChRs. We found that Cy3-ACh is as efficient as untagged ACh to open the channel (I_{max,Cy3-ACh}/I_{max,ACh}=0.97±0.01; n=3). The potency for Cy3-ACh (EC_{50}= 0.91 µM) was slightly higher than for untagged ACh (EC_{50}=2.4 µM). EC_{50} values were derived by fitting the Hill equation to averaged relative peak currents (I/I_{max}; n=5 for ACh, n=3 to 8 for Cy3-ACh). The desensitization kinetics was similar for both agonists over the whole concentration range tested (0.3 to 1000 µM). In imaging experiments, application of Cy3-ACh led to fluorescence signals, which were not observed when applied to non-transfected control cells. Agonist binding and unbinding appeared as bi-exponential time courses, whereby in both cases the first phase was too fast to be resolved. Interestingly, the second phase of fluorescence increase during agonist application was similar to the time course of desensitization. The second phase of fluorescence decrease during wash-off phase was similar to the time course of recovery from desensitization.

These results led us to the following conclusions: (1) Cy3-ACh is a suitable agonist to monitor binding and unbinding in adult muscle-type nAChRs as well as their respective consequences for channel gating, (2) Our direct macroscopic approach confirmed that desensitization leads to higher agonist affinity as suggested with single-channel approaches before (Akk and Auerbach, 1996. Biophys. J.70,2652-8), (3) Recovery from desensitization is rate-limited by ligand unbinding. Hereby, we conclude that Cy3-ACh derivative can be a potential tool to study relation between ligand binding and channel gating.

“inorganic, monovalent cations compete with agonists for the transmitter binding site of nicotinic acetylcholine receptors”

Akk and Auerbach, 1996. Biophys. J.70,2652-8

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**PCA141**

Bile acids regulate intestinal wound healing by FXR mediated inhibition of CFTR expression in human colonic epithelial cells

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Introduction: Epithelial restitution is an essential process for maintenance of intestinal barrier function. Increased levels of colonic bile acids have been proposed to be involved in the pathogenesis of inflammatory bowel disease (IBD) but their roles in regulating restitution are not yet known. Here, we investigated the effects of bile acids on epithelial restitution and molecular pathways involved in colonic epithelial healing.

Methods: T84 colonic epithelial cells, grown as monolayers on transparent permeable supports, were wounded by scratching with a pipette tip at T=0. Cells were treated with either the most abundant colonic bile acid, deoxycholic acid (DCA; 150 µM), the “therapeutic” bile acid, ursodeoxycholic acid (UDCA; 100 µM), a Farnesoid X Receptor agonist, GW4064 (5 µM), or a cystic fibrosis transmembrane conductance regulator (CFTR) channel blocker, CFTR(inh)-172 (10 µM). Restitution was measured as wound area after 48 h expressed as % T=0 wound area. HEK-293 cells were transfected with vector expressing luciferase gene under control of the CFTR promoter and vectors expressing FXR. Protein expression was assessed by western blotting and cell migration by Boyden chamber assay.

Results: After 48 h post-wounding, wound closure in untreated cells was 63.3 ± 13.5% of that at T=0, while in cells treated with DCA (150 µM) it was reduced to 24.5 ± 13.1% (n=5; p <0.001), whereas UDCA enhanced healing to 88 ± 4% (n=5; p <0.001). Furthermore, UDCA prevented inhibition of wound closure by DCA. The effects of DCA are mediated via a decrease in cell migration to 0.7 ± 0.1 fold (n = 5, p < 0.05) of that in untreated controls, rather than inhibition of cell growth. Furthermore, DCA decreased cell surface CFTR expression to 23 ± 5% of controls (n = 3, p < 0.001), while a CFTR inhibitor, CFTR(inh)-172 (10 µM), attenuated wound closure to 37 ± 2% (n = 5; p < 0.01), compared to control. Moreover, DCA decreased CFTR promoter activity, in a concentration-dependent manner that was also dependent on co-expression of FXR. Finally, GW4064 (5 µM), an agonist of FXR, mimicked DCA effects on wound healing and CFTR expression.

Conclusion: Our data suggest that colonic bile acids differentially regulate intestinal epithelial restitution and that UDCA promotes healing and protects against the detrimental effects of DCA. Thus, manipulation of the colonic bile acid pool may prove to be a useful approach for promoting intestinal barrier function in IBD.

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**PCA142**

Ergothioneine up-regulates anti-oxidant enzymes and thus protects from tobacco smoke-related damage

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Tobacco smoke is a major contributing factor for chronic obstructive pulmonary disease (COPD) and exposure leads to oxidative stress, which contributes to alveolar wall destruction, mucus hyper secretion, inflammation and defective tissue repair. Ergothioneine (ET), an amino acid transported across cell membranes by OCTN1 (SLC22A4), has been shown to exhibit antioxidant capacities. The objective of this study was to investigate the role of OCTN1-transported ET in tobacco smoke-induced oxidative stress.

For *in vitro* studies, human NCI-H441 cells were cultured for at least 5 days in the presence or absence ET and then exposed to cigarette smoke extract (CSE). Reactive oxygen species were measured using H$_2$DCFDA assay. Enzymes involved in oxidative stress defence (e.g. catalase, thioredoxin, sulfite-
doxin 1) and markers of xenobiotive defence (e.g. MRP1, BCRP and PXR) were studied by q-PCR and immunoblot. Moreover, Octn1 knock-out mice and wildtype controls were exposed for 3 and 6 months to second-hand cigarette smoke and changes in lung morphology were assessed in haematoxylin-eosin (HE) stained lung sections. In addition, total cell numbers and types in broncho-alveolar lavage fluid (BALF) were counted and inflammation markers quantified by q-PCR and ELISA.

q-PCR and immunoblot revealed elevated expression levels of catalase, thioredoxin and sufrideroxin 1 as well as PXR, MRP1 and BCRP following ET treatment of NCH441 cells. Moreover, lower levels of oxidative stress were observed in cells, which were cultured in the presence of ET prior to CSE exposure. When exposed to room air, knock-out mice showed little differences compared to wildtype animals. However, numbers of total cells and PNMs in BALF as well as increased alveolar damage and increased inflammatory markers were observed in knock-out animals compared to control mice, when exposed to second-hand smoke. These data suggest that ET can protect lung epithelial cells from oxidative damage and consequently, variants of OCTN1 might play a role in the pathogenesis of tobacco smoke-induced COPD by regulating ET transport.

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PCA143

P2X7 receptor activation induces pro-inflammatory and pro-apoptotic signalling in human urothelial cells after radiation

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Background: Radiation-induced bladder toxicity (RIBT) is an unavoidable consequence of pelvic radiation therapy leading to bladder dysfunction (1, 2). RIBT is due to direct irradiation of cells within the radiation field and also indirectly due to effects in non-irradiated, bystander cells which respond to signals released by irradiated cells (3). The current study aims to investigate the underlying molecular mechanisms in bystander urothelial cells that contribute to RIBT.

Methods: Immortalized human urothelial cells (SV-HUC) were cultured to subconfluence before being irradiated (2 Gy) to generate conditioned medium (CM). Non-irradiated SV-HUC were exposed to CM for 1 hour and subsequently studied with qPCR, Western blotting, cell survival assays, and patch-clamping. Data sets were analysed with student t-test and one-way analysis of variance, and results were presented as mean±S.E.M with P<0.05 considered as significant.

Results:

Application of CM to naïve cells decreased clonogenic cell survival (n=3, P<0.05) indicating the possibility of cell death. Protein analysis from these cells by Western blot showed enhanced expression of cleaved caspase-8, cleaved caspase-3 and cleaved PARP (n=3) consistent with activation of pro-apoptotic signalling. These cells also exhibited enhanced protein expression of cleaved caspase-1 and IL-1β, indicating activation and production of pro-inflammatory mediators. Urothelial cells released ATP into the medium, greater than basal levels after irradiation (luciferin-luciferase assays n=3, P<0.05). The possibility that ATP in the CM modulated induction of pro-apoptotic and pro-inflammatory signalling was confirmed by dose-dependent decrease in cell survival (n=3, P<0.05) by ATP and increased expression of pro-apoptotic and pro-inflammatory proteins (n=5, P<0.05) by ATP. Application of ATP (1mM) evoked inward currents when cells were held at -60mV (n=3) indicating the involvement of membrane purergic receptors/channels. Protein analysis of the purinergic receptor P2X7R, showed its enhanced expression by CM (n=3). Pre-treatment with P2X7R inhibitors 4A38079 (100µM) or AZ11645373 (0.5µM) attenuated the CM-evoked and ATP-evoked expression/activation of pro-apoptotic caspases-8, 3 and pro-inflammatory caspase-1 resulting in normalisation of cleaved PARP and IL-1β protein levels, respectively (n=3). Transient knockdown of P2X7R mimicked the effects of inhibitors, confirming the pathological role of P2X7R-mediated signalling after irradiation (n=2).

Conclusion: Urothelial cells respond to CM from irradiated cells with induction of pro-apoptotic and pro-inflammatory signalling. Our data indicates that ATP/P2X7R signalling contributes to this aspect of RIBT. Targeting P2X7R may therefore present a promising therapeutic strategy for treatment of RIBT.


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PCA144

TREK-1 and TREK-2 two-pore domain K+ channel subunits form functional heterodimers

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Usually K<sub>2p</sub> background K+ channels assemble as homodimers. Coexpression of TREK-1 and TREK-2 (TWIK-related K+ channel 1 and 2) K<sub>2p</sub> channel subunits in native cells raised the question whether they can form heterodimers, thus increasing the diversity of background K+ currents, leading to delicate regulation of the resting membrane potential and cell excitability. TREK-1/TREK-2 heterodimerization was enforced artificially by covalently linking the two subunits. Biophysical and pharmacological properties of this model construct were tested and compared with those of the homodimers. Then TREK-1 and TREK-2 were coexpressed and heterodimerization was evaluated by analyzing the appearance of properties charac-
teristic of the tandem construct. Finally, TREK-1/TREK-2 heterodimerization was detected in a native tissue.

Channels were expressed in Xenopus laevis oocytes. Currents were measured in whole cells and in excised membrane patches. Dorsal root ganglion (DRG) neurons were isolated from mice and used for single channel recording. Results are given as mean±SEM, statistical analysis was done using ANOVA.

Extracellular acidification (to pH 6.5) inhibited TREK-1 (by 49.7±4.3%, n=10 oocytes) and the tandem channel (by 32.7±1.4%, n=8), while TREK-2 was activated (243.3±17.5% of the control, n=6). TREK-1 was insensitive to 30 μM ruthe- nin red (RR) (9.6±2.3% inhibition, n=10), while TREK-2 was potently inhibited (85.9±2.3%, n=6). The tandem was moderately sensitive to RR (49.4±4.3% inhibition, n=8). Coexpression of TREK-1 and TREK-2 subunits resulted in a current with pH and RR sensitivity suggesting spontaneous heterodimeric assembly.

The tandem can also be distinguished from the homodimers (TREK-1: 39.2±2.7 pS, n=6; TREK-2: 148.8±4.2 pS, n=7 patches) on the basis of its single channel conductance (83.5±5.0 pS, n=8, p<0.01). Coexpression of TREK-1 and TREK-2 resulted in channels with conductances characteristic of the heterodimer. The effects of RR and the selective TREK-1 blocker spadin (described as an endogenous antidepressant) were examined in outside-out oocyte membrane patches. The effect of RR on TREK channels was confirmed on the single channel level. TREK-1 was inhibited by 1 μM spadin (by 60±11.2%, n=4), while TREK-2 was resistant (101±14.4% of control, n=4). The tandem was also sensitive to spadin (85±12% inhibition, n=5).

Single channel currents were recorded in outside-out patches excised from DRG neurons. Nine out of 21 channels were sensitive to both RR and spadin, which is characteristic of the heterodimer.

TREK-1 and TREK-2 subunits form functional heterodimers when coexpressed in Xenopus oocytes and in DRG neurons. The heterodimer is inhibited by extracellular acidification and spadin. We propose that the heterodimer may play a role in the sensing of changes in extracellular pH and mediate the antidepressant effect of spadin.

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PCA145

A patient with multiple-organ failure carries a truncation mutation in human SLC12A2, the gene encoding the Na-K-2Cl cotransporter, NKCC1

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In 2010, an 8 year old female experiencing obstructive apnea, episodes of vomiting and dehydration, ketotic hypoglycemia with illness, fatigue, exercise intolerance, dilated cardiomyopathy (left ventricle dilation), and seizure-like episodes was referred to the NIH Undiagnosed Diseases Program. To identify potentially pathogenic DNA sequence variants coding mutations in the patient’s genome, exome sequencing was performed on the patient, on both unaffected parents, and on 3 unaffected siblings. A frameshift truncating variant in SLC12A2, the gene encoding the Na-K-2Cl cotransporter-1, was identified. As a result of the deleterious nature of the variant, the NKCC1 mutation received the highest priority. The mutant cDNA harboring an 11 bp deletion in exon 22 was created for expression in Xenopus laevis oocytes. In contrast to the wild-type cotransporter, which demonstrated a significant bumetanide-sensitive and hypertonic-stimulated K+ uptake, the mutant cotransporter was non-functional. Western blot analysis of oocytes expressing a cmyc tagged mutant cotransporter revealed a molecular size consistent with the formation of a dimer, while very little signal was observed at the size of a truncated monomer. This is in contrast to oocytes expressing wild-type cotransporters which expectedly showed monomer and dimer forms of the cotransporter. Co-expression of mutant NKCC1 with wild-type NKCC1 in oocytes did not result in a dominant-negative effect. K+ influx measurements in fibroblasts isolated from the patient revealed that 50% of the K+ influx was ouabain-sensitive or mediated by the Na+K+ pump, whereas 41% of the flux was bumetanide-sensitive or mediated by NKCC1. Addition of ouabain and bumetanide resulted in 87% reduction in K+ influx. When the cells were exposed to hypertonicity, there was no activation of NKCC1, neither in wild-type fibroblasts nor fibroblasts isolated from patient. The bumetanide-sensitivity, however, disappeared in mutant but not wild-type fibroblasts, indicating some deleterious effect of the mutant cotransporter. Western blot analysis of protein lysates from patient’s fibroblasts also showed a larger amount of dimer when compared to HEK293 cells which were used as controls. Expression of fluorescently-tagged mutant and wild-type transporters in Hela cells revealed that the wild-type transporter helps the mutant reach the plasma membrane. Altogether, these data indicate that the mutation leads to a non-functional allele without some deleterious effect on the function of the cotransporter (bumetanide insensitivity under hypertonicity). Whether the mutant protein exerts deleterious effects on other cellular functions still needs to be explored.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA146

Expression and regulation of GLUT12 in small intestine

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Background: The human facilitative glucose transporter GLUT12 was isolated from the breast cancer cell line MCF-7 (Rogers, S et al. 2002). GLUT12 is expressed in human crude membranes of adipose tissue, small intestine and skeletal muscle, where it seems to act as a secondary insulin-sensitive transporter. We have demonstrated in Xenopus laevis oocytes expression system that hGLUT12 can transport α-methyl-glucoside (αMG), a specific SGLT substrate, and that this transport is enhanced in the presence of Na+ (Pujol-Gimenez et al. 2015). Based on this information, the aim of the present work was to investigate the location and function of GLUT12 in small intestine.

Methods: GLUT12 location in human and rat small intestine was investigated by immunohistochemical methods. The samples were part of the department collection that had been
collected for previous studies and approved by the Ethics Committee of the University of Navarra. Samples were fixed in bouin and the antibody anti-glut12 used at 1:100. Expression of GLUT12 in brush border membrane vesicles (BBMV) of Caco-2 cells and its regulation by different sugars was studied by Western blot using the same antibody at 1:1000. Uptake experiments were performed in Caco-2 cells grown on 24 well plates. Cells were incubated for 15 min with 5 mM α-MG (with traces of the radiolabel sugar) in the presence and absence of Na⁺ and in the presence of the GLUT12 substrates fructose or 2-deoxy-glucose (2-DOG) at 50 mM. Regulation of GLUT12 activity by PKC and PKA was investigated measuring α-MG uptake (5 mM, 15 min) after 60 min pre-incubation of the cells with PMA (0.1 µM) or Forskolin (10 mM). Regulation of GLUT12 activity by the pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α) was investigated by Western blot and uptake assays after 1 hour of pre-incubation of the cells with 10 or 25 ng/ml TNF-α.

Results: Immunolabeling for GLUT12 appeared in the apical cytoplasm, below the brush border of rat and human enterocytes; in the perinuclear region of human enterocytes and in rat basolateral cytoplasm. Western blot analysis showed GLUT12 expression in the BBMV of Caco-2 cells. This expression was upregulated by incubation of the cells with glucose, galactose, fructose and α-MG. In the absence of Na⁺, α-MG uptake was inhibited by 2-DOG, a specific substrate of the GLUTs transporters. Transport of α-MG was up-regulated by PKC but not by PKA. TNF-α increased α-MG uptake by Caco-2 cells. This increase was accompanied by translocation of GLUT12 to the apical membrane.

Conclusion: These results demonstrate the expression, functional activity and regulation of GLUT12 in the apical membrane of enterocytes, opening new venues to investigate its role in physiological and pathophysiological conditions in the intestine.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

**PCA147**

*Resveratrol selectively kills cancer cells by modulation of cancer-specific mitochondrial Ca²⁺ homeostasis*

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Introduction: Mitochondria are supplying the cell with adenosine triphosphate (ATP) and are crucially involved in cell differentiation, cell cycle and intracellular signaling. Since they are essential for cell survival, their important role in human diseases and aging processes is under intensive investigation. The transfer of Ca²⁺ into the matrix of mitochondria is an important process that substantially contributes to multiple cellular functions. The Ca²⁺ concentration within mitochondria ([Ca²⁺]mito) controls, for instance, the metabolic activity of these organelles and can also trigger cell death pathways.

Methods: To investigate the role and possible mechanism(s) of cell death pathways triggered by mitochondrial Ca²⁺, we applied resveratrol and its derivative, piceatannol, which are known to cause apoptosis in certain cancer cells. Using genetically encoded, mitochondria or endoplasmic reticulum (ER) targeted, fluorescent Ca²⁺ and ATP probes as well as fluorescent dyes, we investigated how these compounds affect calcium and ATP homeostasis in HeLa and Ea.hy926 in comparison to freshly isolated human umbilical vein endothelial cells (HUEVs). Hence, we studied the effect of these compounds on cell viability by MTT assay in tumor and somatic cells.

Results: Our results indicated that chemical compounds such as resveratrol, piceatannol and oligomycin bind different subunits of the ATP synthase and reduce mitochondrial ATP production. The lack of sufficient ATP supply in the gap between mitochondria and the ER reduced SERCA activity. This resulted in slower refilling of ER Ca²⁺ stores, an increased intra-organelle Ca²⁺ concentration and, consequently, an increased mitochondrial Ca²⁺ uptake upon IP₃-mediated intracellular Ca²⁺ release. Such excessive mitochondrial Ca²⁺ uptake resulted in mitochondrial Ca²⁺ overload and ultimately cell death in cancer cells, while this effect was significantly reduced in somatic cells due to lower stability of mitochondria-associated ER membranes (MAMs).

Conclusion: Resveratrol and its derivative piceatannol act as new MitoCans by triggering cell death pathways via mitochondrial Ca²⁺ overload in cancer cells because of cancer-specific mitochondrial Ca²⁺ homeostasis with higher MAM stability than somatic cells. Moreover, this study reveals a crucial involvement of mitochondria in cancer cell survival. These organelles are therefore promising targets for future treatments.

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**PCA148**

*Rearrangement of MICU1 multimers for activation of MCU is solely controlled by cytosolic Ca²⁺*


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Introduction: The transport of the universal second messenger Ca²⁺ across the mitochondrial inner membrane plays a key role in numerous physiological processes such as cell cycle control, signal transduction and cell death pathways. The mitochondrial Ca²⁺ uptake machinery consists of several components amongst others the channel forming mitochondrial Ca²⁺ uniporter (MUC), its regulatory components including the dominant-negative pore-forming subunit MUCβ, the essential MCU regulator (EMRE), and mitochondrial calcium uptake 1 (MICU1). MICU1 acts as gatekeeper for MCU; under low Ca²⁺ conditions it forms hexamers that inhibit MCU activity while at high Ca²⁺ concentrations it disassembles to dimers that do not further prevent MCU activity.

Methods: In this study a Förster resonance energy transfer (FRET)-based live-cell approach was applied to dynamically monitor the arrangement of MICU1 multimers after Ca²⁺ mobilization by an IP₃-generating agonist. For this purpose...
Kir7.1 K+ channel: Expression and possible role in the developing mouse respiratory system

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Kir7.1 K+ channel, encoded by the Kcnj13 gene, is a member of the inwardly-rectifying Kir family of proteins expressed in epithelia such as the choroid plexus, retina and small intestine. Owing to its remarkable colocalisation with the Na+/K+ ATPase, Kir7.1 has been proposed to be responsible for K+ recycling needed to maintain high rates of ion and fluid transport across these epithelia. To study its role, we generated a Kir7.1 knockout (KO) mouse for Kir7.1 expressing the LacZ gene under the control of Kcnj13 promoter. Heterozygous (Het) mice were indistinguishable from their wild type (WT) littermates, but KOs presented growth retardation, craniofacial abnormalities, and did not survive beyond the first day of life (P0) (Villanueva et al., 2015). We detected Kir7.1 expression in the respiratory tree indirectly through β-galactosidase activity in cryopreserved sections. Tissues were obtained from adult mice euthanized by cervical dislocation. Expression was also evident in adult Het respiratory system. To corroborate that the addition of HA tag to the extracellular loop of the protein did not affect the subcellular localization of the Kir7.1 channel in the respiratory system.

Rearrangement of MICU1 is independent of mitochondrial membrane potential and expression levels of MCU/EMRE. To assess the distribution of Kir7.1 protein we used the CRISPR-Cas9 technology to generate a knockin mouse where the native Kir7.1 protein was replaced by a haemagglutinin-Kir7.1 (HA-Kir7.1) fusion protein, allowing us to determine the subcellular localization of the Kir7.1 channel in the respiratory system. To corroborate that the addition of HA tag to the extracellular loop of the protein did not affect the subcellular localization of Kir7.1, we immunodetected the knocked-in channel with an anti-HA antibody in choroid plexus of HA-Kir7.1+/− adult mice. As expected, HA-Kir7.1 was present at the apical membrane of choroid plexus epithelial cells thus confirming previous results. Immunodetection experiments in the airways showed that Kir7.1 channel is expressed in the basolateral side of the epithelial cells of the trachea, bronchi and bronchioles, and in type II pneumocytes of the lungs of adult mice. We hypothesise that Kir7.1 channel is necessary for proper lung development in mice possibly playing a role in the adequate production of lung fluid, one of the main signals for lung maturation. In adult mice Kir7.1 may be relevant in airway and alveolar ion homeostasis.

Bile acids conundrum: To stimulate or inhibit secretion?

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Bile acids (BAs) play a complex role in regulating colonic fluid secretion. We showed that chenodeoxycholic acid (CDCA), but not lithocholic acid (LCA), stimulates CFTR secretion via CAMP-PKA activation of CFTR in human colonic T84 cells (AJP 305:C447-56, 2013). Further, LCA inhibits forskolin-, CDCA- or 8-Br-cAMP-induced increases in short-circuit current, Isc, and forskolin-induced short-circuit current, Isc, and forskolin-induced [cAMP]i (Gastro. 146:5:S785, 2014). Here, we further delineate the processes underlying the contrasting actions of CDCA (500µM) and LCA (50µM) in T84 cells. LCA’s inhibition is rapid, specific to CAMP secretagogues, and slowly reversible (2h: 50% recovery), while that of CDCA is rapidly reversible (min). In nystatin-permeabilized cells, LCA stimulates an apical CFTR CFTR current and inhibits a basolateral K+ current. LCA (1-24h) has no effects on transepithelial resistance (TER), dilution potentials (cation selectivity), permeability (measured by dextran flux), or occludin localization. In contrast, CDCA (1-24h) decreases TER, decreases cation selectivity, increases permeability, induces IL-8 release, and redistributes occludin. Proinflammatory cytokines (PIC: TNFa+IFNy+IL1β) also increase permeability, TER and IL-8; PIC and CDCA effects are additive. LCA reverses CDCA or PIC-induced changes in permeability and IL-8 release but not those of TER. BA actions did not involve the BA receptors, FXR or TGR5, or muncaricin M3 receptors. The EGFR inhibitor, AG1478, did not reverse LCA’s attenuation of CFTR secretion. However, both AG1478 and EGFR siRNA attenuate (>67%) CDCA’s action. Interestingly, while both LCA and CDCA...
increase ERK1/2 phosphorylation >2-fold, that was blocked by the MEK inhibitor PD98059, the latter did not inhibit CDCA or LCA-dependent ΔIsc. Although LCA and CDCA increase [Ca²⁺], only CDCA action was blocked by the chelator BAPTA. CDCA increases [IP₃] and requires IP₃ receptor activation. Since CDCA action involves PKA and [Ca²⁺], further exploration revealed that inhibition of the exchange protein activated by cAMP (Epac) with ES109 (10μM) reduced CDCA’s secretory response (ΔIsc; μA/cm²); CDCA: 17±2; CDCA+ES109: 5±1; n=6; p<0.05). Inhibition of both PKA (H89, 30μM) and Epac blocks CDCA response by 97% (n=5). H89 and ES109 decrease CDCA-induced EGFR phosphorylation (n=2), implying that cAMP mediates CDCA’s transactivation of EGFR. Downstream effector of Epac, Ras-related protein 2, but not Rap1A and Rho kinase, may be involved in CDCA action. In summary, CDCA and LCA have opposite effects in T84 cells: CDCA stimulates Cl⁻ secretion via complex signaling (PKA+Epac+Ca²⁺+EGFR), causes barrier dysfunction and releases IL-8, while LCA attenuates cAMP-stimulated Cl⁻ secretion, restores barrier integrity and suppresses IL-8 release. The interplay of these two BAIs in regulating colonic function in health and inflammatory diseases could provide useful insights to novel therapeutic strategies.

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PCA151

Phosphatidylinositol (4, 5)-bisphosphate (Pi(4,5)P₂) as a regulator of the Kᵥ2p background K⁺ channel TASK-2

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Regulation of K⁺ channels by phosphoinositides (PIPs) is a characteristic of many of the members of this large superfamily of proteins, but whether the same applies to those belonging to the two-P domain in tandem Kᵥ2p clade, has been controversial. TASK-2 is a member of the Kᵥ2p channel family with important functions in the kidney and chemosensitive neurons (López-Cayuqueo et al., 2015). Here we have investigated the possible regulation of TASK-2 activity by membrane PIPs. K⁺ currents in HEK-293 cells expressing recombinant TASK-2 were measured in whole cell or inside-out isolated patches. Whole-cell currents are stable in the presence of intracellular ATP but rapidly rundown in its absence, upon replacement with a non-hydrolysable ATP analogue or in the presence of polycation scavengers of PIPs such as neomycin. Activation of a coexpressed voltage-sensitive phosphatase from Danio rerio (DrVSP), that removes 5-phosphate groups from PI(4,5)P₂, also led to a marked inhibition of TASK-2 current. This was reversible but only in the presence of Mg²⁺ suggesting an endogenous kinase activity involvement. Activation of Gq-coupled receptors is accompanied by membrane PI(4,5)P₂ depletion. Consistent with a sensitivity to PI(4,5)P₂, TASK-2 channels expressed with angiostatin II AT1 receptors were markedly inhibited by receptor stimulation. Diacylglycerol addition had only a minor effect, suggesting it is not the main drive behind AT1-dependent inhibition of TASK-2. TASK-2 rundown in excised membrane patches in the absence of ATP could be restored by intracellular addition of PI(4,5)P₂ or other PIPs tested in their soluble DiC8 form. Largest effect was obtained with PI(3,4,5)P₃ while that of PI(4)P was quite small. EC₅₀ values ranked in the order PI(3,4,5)P₃ < PI(4,5)P₂ < PI(3,4)P₂ < PI(4)P. The interaction between PIPs and the proteins they regulate have been shown to be electrostatic and occur through interactions with basic residues. TASK-2 positively charged residues facing intracellularly were systematically replaced one at a time by cysteines to disable interaction and inhibit activity. Cysteines can be charged positively using small methanethiosulfonate reagents such as (2-trimethylammonium)ethyl methanethiosulfonate (MTSET) thus restoring interaction. Of the 45 mutants, only two, K254C and K297C, were significantly activated after MTSET treatment suggesting they are involved in the regulation of TASK-2 by PIPs. Screening the same mutants using the kinetics of inhibition of TASK-2 by DrVSP revealed a third residue, R438, that might also be involved in the interaction.

Our results suggest that TASK-2 is under the regulation of PIPs, of which the main plasma membrane representative is PI(4,5)P₂, through an interaction with basic residues of its C-terminus. This modulation might be of relevance to the physiological roles of TASK-2.


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PCA152

Benefits of omega-3 fatty acid against bone changes in salt-loaded rats. Possible role of kidney

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There is evidence that dietary fats are important components contributing in bone health and that bone mineral density is inversely related to sodium intake. Salt loading is also known to impose negative effects on renal function. The present study aimed to determine the effect of the polysaturated fatty acid omega-3 on bone changes imposed by salt loading, highlighting the role of kidney as a potential mechanism involved in this effect. Rats were anaesthetized with thiopental sodium (40 mg/kg b.w. i.p.). Male Wistar rats were divided into 3 groups: I: Control group (n=8) consisting of rats consuming tap water. II: Salt-loaded group (n=8) consisting of rats consuming 2% NaCl solution as drinking water for the 8 weeks of the experiment. III: Omega-3 fatty acid-treated salt-loaded group (n=9) consisting of rats receiving omega-3 fatty acid daily at a dose of 1 g/kg b.w. orally by gavage, with consumption of 2% NaCl solution as drinking water for 8 weeks. Systolic (SBP), diastolic (DBP) and mean (MAP) arterial pressures and heart rate (HR) were recorded. Plasma levels of sodium, potassium, calcium, inorganic phosphorus (Pi), alkaline phosphatase (ALP), creatinine, urea, 1,25-dihydroxyvitamin D [1,25(OH)₂D₃] and transforming growth factor-beta1 (TGF-β1) were measured. The right tibia and kidney were removed for histologic examination and renal immunohistochemical analysis for endothelial nitric oxide synthase (eNOS) was performed.
The results revealed that high salt intake increased SBP, DBP and MAP and plasma levels of sodium, potassium, Pi, creatinine, urea and TGF-β1, but decreased plasma levels of calcium, ALP and 1,25(OH)₂D₃ as well as renal eNOS. This was associated with thinning of trabecular and cortical bones, many irregular resorption areas on bone surface together with increased number of osteoclasts and decreased new bone formation. The renal structure revealed a significant increase in glomerular hypercellularity, renal tubular affection, congested blood vessels, thickened arterioles and interstitial cellular infiltration. Omega-3 administration resulted in reduction of SBP, DBP and MAP and plasma levels of sodium, potassium, Pi, creatinine, urea and TGF-β1, but increased plasma levels of calcium, ALP and 1,25(OH)₂D₃ as well as renal eNOS. Omega-3 increased cortical and trabecular bone thickness, decreased osteoclast number and increased newly-formed osteoid bone. Renal morphology was found preserved.

In conclusion, omega-3 alleviates the disturbed bone status imposed by salt loading. This osteoprotective effect is possibly mediated by attenuation of alterations in Ca²⁺, Pi and ALP, and improvement of renal function and arterial blood pressure. Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA153

Effects of the abstinence length on sperm physiology
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According to the prescribed guidelines of the World Health Organization, subjects must remain abstinent for a minimum period of 48 hours, but not longer than 7 days before collecting a semen sample for standard analysis. However, the basis for this recommendation remains uncertain. Various studies have investigated the typical time after which human semen samples should be collected for standardized analysis and have shown contradictory results. This study aimed to investigate the effect of short and long term abstinence on sperm kinematics in young healthy individuals.

Semen samples (n=100) were collected from potentially fertile, healthy males (20 to 30 years). Donors abstained for 4 days prior to the first sample collection, while the second sample was collected from the same donor after 4 hours of abstinence. Sperm motility parameters were quantified by the Sperm Class Analyser (SCA) (Microptic, S.L., Barcelona, Spain). Motility and Kinematics parameters included total motility, progressive motility, curvilinear velocity (VCL), straight-line velocity (VSL), linearity (LIN), and amplitude of lateral head displacement (ALH). Semen pH was assessed with pH indicator paper (MERCK) with graduated colours indicating pH from 6.4 to 8.0. Sperm superoxide (O₂⁻) levels were assessed by flow cytometry, using dihydroethidium (DHE).

Data were analysed using paired Student’s t-tests on Graph Pad Prism™ software and presented as Mean ± S.E.M, calculated using Graph Pad Prism™ software.

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PCA154

Physical exercise versus natural estrogen supplement in treatment of metabolic syndrome in aged postmenopausal Wistar rats
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Postmenopausal state could be associated with increased risk of the metabolic syndrome, lifestyle intervention, either with diet modification or exercise training, could alleviate such metabolic effects. This study evaluated the effects of either a low intensity swim- exercise program or low soybean flour supplementation on metabolic syndrome in aged postmenopausal rats. Fifty five rats, weighing 260-350 gm, were randomly allocated into: Control group (C), Metabolic Syndrome group (MS), Metabolic syndrome 6-week –swim exercised group (MSE), and Metabolic syndrome 6 week –soybean flour supplemented group (MSS). All rats were followed weekly for assessment of body weight and arterial blood pressure which was found to be significantly increased in all metabolic syndrome groups (MS, MSE and MSS) compared to C group after 8 weeks. At the end of the experimental period, all rats were subjected to measurement of arterial blood pressure. Under anesthesia with pentobarbital 40 mg/kg B.W, biochemical analysis including plasma levels of insulin lipid profile and fibrinogen was performed, then HOMA-IR and atherogenic index (AI) were calculated. Values are in Mean ± SEM, compared by ANOVA. MS rats exhibited significant increase in their body weight gain (BWG), visceral fat (VF), visceral fat/body weight ratio (VF/BW Ratio), arterial blood pressure values (ABP), as well as, plasma triglyceride level (TG) and AI compared to their controls (57.5 ± 12.3 vs 30 ± 6.9, 0.9 ± 0.008 vs 0.07 ± 0.008, 153.58 ± 0.95 vs 133.76 ± 0.32, 91.92 ± 1.2 vs 80.5 ± 0.48, 112.47 ± 0.98 vs 98.28 ± 0.51, 120.9 ± 13.86 vs 47.1 ± 4.8, 6.69 ± 2.15 vs 2.28 ± 0.41, P<0.05, <0.05, <0.001 in ABP, <0.05, respectively). MSE group exhibited significant decrease in their BWG, VF, VF/BW Ratio and AI compared to MS group (27 ± 10.01 vs 30 ± 6.9, 16.7 ± 1.59 vs 29.29 ± 3.19, P = 0.0110) was observed in the second sample compared to the first sample. No significant differences were observed in ALH and O₂⁻.

The beneficial effect of a short abstinence period (4hr) on sperm movement characteristics was accompanied by increased pH, but appeared to be independent of O₂⁻ levels. The clinical significance of this finding would be that short abstinence prior to sample collection may improve the outcomes of ART and fertility preservation.


requirements. described here conform with the Physiological Society ethical
regards hypertriglyceridemia and hypertension in metabolic
values. Low dietary intake of soybean flour or low intensity
plasma insulin level in addition to significant decrease in ABP
pared to the MS group. MSE group showed normalization of
120.9 ± 13.86, 2.64 ± 0.60 vs 6.69 ± 2.15). Plasma insulin and
total cholesterol were significantly higher in MSS group com-
pared to the MS group. MSE group showed normalization of the
values of their BWG, VF, VF/BW Ratio. MSS group showed
normalization of their BMI, VF and VF/BW Ratio. Compared to
the MSE group, MSS had a significant increase in plasma insulin level in addition to significant decrease in ABP
values. Low dietary intake of soybean flour or low intensity
swim-exercise program for 6 weeks were equally effective in
normalizing visceral adiposity, but were partially effective as
regards hypertriglyceridemia and hypertension in metabolic
syndrome aged rats.

Where applicable, the authors confirm that the experiments
described here conform with the Physiological Society ethical
requirements.

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**PCA155**

Evaluating the potential beneficial effects of short-term physical activity on human pain: A study using quantitative sensory testing and contact heat evoked potentials (CHEPS)

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Many studies report a beneficial effect of physical activity on chronic pain and exercise is a recognised non-medical treat-
However more data is needed to inform an evidence-based approach to the concept of ‘exercise as a prescription for
pain’. In this preliminary study, we have utilised quantitative sensory testing (QST) and the recording of contact
heat-evoked potentials (CHEPS), to evaluate the effect of a short-term exercise programme on pain perception in healthy
control (HC) participants and participants self-reporting chronic lower back pain (CLBP).

All protocols had local ethical approval. HC (n=4) and CLBP (n=4) participants undertook a short-term exercise pro-
gramme (2 weeks; total daily steps increased by 25% rela-
tive to 1 week control baseline average). Pain perception was assessed using a QST protocol for measurement of hot
pain threshold and CHEPS were recorded using a standard EEG methodology (Brain Vision, UK) in response to thermal stimuli (49-51°C) applied to the distal v oral forearm surface
(Pathway System, MEDOC, Israel). A 1-10 numerical rating scale (NRS) was used to report subjective pain intensity. Sub-
jects were tested in week 1 prior to the exercise regime and again in week 3 after completion of the exercise programme.
Values are means ± S.E.M. and paired Student’s t-tests used for statistical analyses.

For HC subjects, the amplitudes of CHEPS elicited by painful
thermal stimuli (49 - 51°C) in the post-exercise period (16.70 ± 1.85 µV), were significantly lower compared to pre-exercise
values (19.94 ± 2.47 µV; P<0.05). A similar effect was observed in the CLBP group with post-exercise CHEPS amplitude val-
ues (21.35 ± 3.16 µV) significantly lower than pre-exercise
depth velocities were reduced; HC
group pre-exercise values were 7.13 ± 0.48 compared to 5.13 ± 0.83 post-exercise (P<0.05). For the CLBP group, the corre-
sponding NRS values were 5.88 ± 0.44 vs 5.00 ± 0.46 (n.s.). For HC subjects post-exercise, there was a significant increase in
the hot pain threshold temperature (from 46.87 ± 1.65 to 49.56 ± 0.97°C, P<0.05). For CLBP subjects, pre- and post-ex-
ercise pain thresholds for nociceptive heat were unchanged
(respective values of 45.10 ± 5.99 versus 45.47 ± 6.38°C, n.s.).
Overall, these preliminary data support the view that short-duration, low intensity physical activity can modify an
individual’s pain experience, as measured by QST and CHEPS,
but extended studies are required to determine if such regimes
may be beneficial to those with musculo-skeletal chronic pain.

Where applicable, the authors confirm that the experiments
described here conform with the Physiological Society ethical
requirements.

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**PCA156**

Association between physical activity and serum bilirubin levels and its potential modulating effect in trained and untrained adult males

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While studies over the last two decades had shown low lev-
els of serum bilirubin to be associated with high risk for vari-
eties of systemic diseases in human, we propose that one
potential modifiable behavior to increase bilirubin levels is
physical activity. The purpose of this study was to examine
the association between physical activity and serum bilirubin
levels and its potential modulating effect among trained and
untrained adult males. Employing purposeful sampling tech-
nique, 20 trained and 20 untrained adult males were recruited
for this study following inclusion and exclusion criteria. The
University institutional review board (ABUTH/HREC/TRG/36)
gave approval for all procedures in accordance with the Decl-
aration of Helsinki. Blood samples were taken to measured
serum total bilirubin and leukocyte counts respectively from
all subjects at rest. The VO2Max was estimated from a stan-
dard regression equation. The independent student’s t-test
was used to compare values between the two groups. Lin-
ear regression analysis was also used for prediction of any
association. The level of significance was set at P< 0.05. Our
result showed that VO2 max exhibited significant differences
between the trained and untrained, leukocyte counts in the
untrained group (140.10±1.65 x 50 mm3) was significantly
(p<0.05) higher compared to the trained group (134.50±2.46
x 50 mm3). On the other hand, serum total bilirubin in the
trained group (11.35±2.6 mmol/l) was significantly (p<0.05)
higher compared to the untrained group (134.50±2.46
x 50 mm3). The VO2Max, correlated positively with serum total bilirubin
(p<0.0001), in both the untrained group (R²= 0.002, +0.045)
and in the trained group (R²= 0.088, +0.297) respectively.
In addition, Leukocyte counts correlated negatively with serum
total bilirubin (p<0.0001), in the untrained group (R²= 0.162,
-0.403) and correlated positively in the trained group (R²= 
0.032, +0.178; p<0.0001). With the present results revealed,
physical activity was positively associated with bilirubin among
the trained males in an increasing trend. This means that an
increased physical activity might increase hemooxyginase-1
activity which is the enzyme responsible for the conversion of biliverdin to bilirubin. In addition, leukocyte counts in the untrained was higher compared to the trained which means the potential modulating effect of the bilirubin might be on inflammation process. This study is novel, as, to our knowledge, no studies to date have examined this association. This finding has implications for sports physicians, diagnosis or applied exercise physiology.


The authors would like to thank the study participants that took part in this study for their patients and cooperation with dedication. The present study was self-sponsored

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA157

Aerobic capacity and efficiency: does the addition of heat stress induce greater improvements than exercise alone?

M. Black, B.G. Perry, D. Cochrane and T. Mundel

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The extra strain caused by the heat during exercise has been extensively documented by previous literature. Regular training in hot environments induces cardiovascular, haematological, sudomotor, and metabolic adaptations. These are characterised by reduced cardiovascular strain and core body temperature, increased sweat rate and improved fluid balance. Collectively, these adaptations are termed heat acclimation and improve exercise performance in hot environments. Due to these adjustments it is possible that heat acclimation may improve performance in moderate environments, however, previous studies have not included an adequate control group and have only compared highly trained athletes. The purpose of this study was to investigate whether heat stress-induced acclimation improves submaximal and maximal exercise in moderate environments. In a randomised, matched control group study, eighteen healthy males completed maximal and submaximal aerobic tests, followed by an 11-day training protocol, consisting of 60 minutes of incline walking each day on a treadmill at 50% of VO2max in either a hot (35°C, 45% RH) or moderate (18°C, 53% RH) environment. Maximal VO2, submaximal VO2, heart rate and lactate were measured to monitor changes pre and post training. Rectal temperature, heart rate, plasma volume, forearm blood flow, whole body sweat rate, local sweat rate, and perceptual measures were taken throughout the 60 minutes of walking over the 11-day training period, in combination with resting heart rate and blood pressure. The exercise protocol improved maximal aerobic capacity in both conditions (Δ+4±2 and Δ+5±2 mL/kg/min for control and heat group, respectively p<0.001), although, additional heat stress did not improve maximal aerobic capacity above exercise alone. The exercise protocol, in both environments, lowered submaximal heart rate (Δ-6±11 and Δ-6±6 beats/min at 50% of VO2max, p=0.008) but not submaximal blood lactate (p = 0.2). Training lowered resting heart rate (Δ-4±5 and Δ-2±6 beats/min, p<0.001) and core temperature (Δ-0.1±0.2°C and Δ-0.3±0.2°C, p=0.04). Compared with the first training bout, perceived exertion after 60 minutes of exercise was reduced (Δ-0.9±0.7 and Δ-1.1±1.3 aU, p<0.001), but forearm blood flow was increased (Δ+1.4±1.5 and Δ+1.5±4.3 mL/100 mL tissue/min p=0.05). Additionally, only the heat group demonstrated an increased whole body sweat rate (Δ+0.1±0.1 L/hour, p=0.01), and improved thermal comfort (Δ+0.4±0.5 aU, p=0.02). Eleven days of exercise at 50% of VO2max, regardless of environment, can improve maximal and submaximal performance in a moderate environment and induce positive cardiovascular adjustments, although eleven days of exercise in 35°C can induce heat acclimation, illustrated through an increase in whole body sweat rate, and a reduction in core temperature.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Pressor response to isometric exercise and correlation of body mass index, total body fat, visceral fat and body age in obese and non-obese female medical students

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Higher magnitude of adiposity, abdominal obesity and a lower muscle mass vis-à-vis body mass index (BMI) as compared to white Caucasians and significant positive association of BMI and body fat with blood pressure are reported in Indians (1). Studies exploring baseline blood pressure and pressor response to isometric exercise in Indian (Muslim) girls with restricted outdoor activities and physical exercise are scanty.

Ninety female Muslim medical students (18-22 yrs) of identical ethnicity and socio-economic status were administered a detailed questionnaire and signed informed consent was obtained as per Helsinki declaration. They were divided into non-obese group (BMI<25,n=66), overweight (BMI=25-30) and obese (BMI>30) groups, clubbed together(n=24). After recording baseline diastolic (DBP) and systolic blood pressures (SBP), they performed isometric exercise (Hand grip dynamometer test, HGT) at 30% of T max value, and SBP and DBP were recorded at 1, 2 and 3 minutes of exercise. Our results indicated higher incidence of obesity (25%) and hypertension (12.5%) than reported [25% (2,3) and 12% (2,4) respectively]. Statistical analysis by student’s T-test at 95% confidence level (paired and independent sample comparisons, P<0.05) revealed the baseline DBP, SBP and SBP at 2 and 3 minutes of HGT to be significantly higher in the obese group (P<0.002,0.003, <0.04 and <0.007 respectively) that correlated positively with BMI(Pearson correlation, r=0.4,0.4,0.32 and 0.35 respectively, P<0.001). Using OMRON body fat analyser (HBF-362 Karada scan, based on bioelectrical impedance analysis), total body fat (TBF%), visceral fat (VF, number) and body/biological age (BA) were obtained. All the values are means ± S.E.M., compared by ANOVA. Significant increases (P<0.001) in body age (41.0±1.17vs23.39±0.68), TBF% (36.2±0.7vs27.0±0.5) and VF (9.58±1.1 vs 2.48±0.15) were observed in obese subjects. Significant (P<0.01) positive correlation between TBF% vs body age (r=0.9), VF vs body age (r=0.88) and TBF% vs visceral fat (r=0.8) and significant linear relationship with BMI (linear regression analysis, P<0.01, R²=0.95,0.89 and 0.95 respectively) were observed. Surprisingly, in 50% of the control group with normal BMI(<25,n=33), having total body fat% greater than normal (≥28%), the body age was significantly higher (27.6±0.8vs19.18±0.36,P<0.03) than rest of the controls. Biological age defines how time and lifestyle have affected organs and cells and includes changes in the physical structure of the body, performance of motor skills and sensory awareness as compared to other people of similar chronological age (5). Higher body age and total body fat% can lead to cardiovascular ailments and morbidity even if BMI is within normal limits and hence should be considered while classifying obesity.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Effect of Ascorbic acid on fatigue of skeletal muscle fibers in long term cold exposed Sprague Dawley rats
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Background: On exposure to prolonged cold temperature, the body responds for effective heat production both by shivering and nonshivering thermogenesis. Cold exposure increases the production of reactive oxygen species which influence the SR Ca++ release from the skeletal muscles and affect their contractile properties. The role of ascorbic acid supplementation on the property of fatigue of cold exposed skeletal muscles was evaluated in this study.

Method: The study was conducted in Physiology Department of Islamic International Medical College, Rawalpindi, National Institute of Health, Islamabad and Railway hospital Rawalpindi, from January to December 2009. Ninety Sprague Dawley rats were randomly divided into three groups of control, cold exposed and cold exposed along with ascorbic acid supplementation (Vitamin C Ascorbic acid MERCK, research grade Cat No. 500074) in a dose of 500mg/L mixed in drinking water. They were exposed to cold environment between 8-14°C for 1h/day for one month by keeping the cages in ice-filled tubs and recording the temperature by thermometer.

Results: After one month of study, the Extensor Digitorum Longus muscle (EDL) was dissected out. Muscle was placed on the muscle holder of the Power Lab. Muscle fatigue was determined by stimulating the muscle for 30 seconds with supramaximal velocity having 5 seconds of rest for 5 minutes. The property of fatigue in the skeletal muscle fibers was analyzed on computerized data acquisition system. Data was analyzed by SPSS version 16.0. Mean and standard deviation was calculated for the decline of force during muscle fatigue in all the three groups.

Conclusions: The cold exposed group showed a significant decline in the contractile properties of skeletal muscle fibers as compared to the control group. In the third group, the duration of fatigue were reduced.

Key words

Comparison of force fatigue (decline in force) between control group and cold exposed group

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control group</th>
<th>Cold-exposed group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Force of Fatigue</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Fatigue 0.5 min</td>
<td>0.2557</td>
<td>0.0209</td>
</tr>
<tr>
<td>Fatigue 1.0 min</td>
<td>0.1699</td>
<td>0.0186</td>
</tr>
<tr>
<td>Fatigue 2.0 min</td>
<td>0.1212</td>
<td>0.0164</td>
</tr>
<tr>
<td>Fatigue 3.0 min</td>
<td>0.1060</td>
<td>0.0164</td>
</tr>
<tr>
<td>Fatigue 4.0 min</td>
<td>0.0937</td>
<td>0.0222</td>
</tr>
<tr>
<td>Fatigue 5.0 min</td>
<td>0.0849</td>
<td>0.0171</td>
</tr>
</tbody>
</table>

** P value < 0.01 is taken as highly significant


Arruda AP, Ketzer LA, Nigro M, Galina A, Carvalho DP, deMeis L. Cold tolerance in hypothyroid rabbits: Role of skeletal muscle mitochondria and sarcoplasmic reticulum Ca2+ATPase isoform 1 heat production Endocrinology 2008;149:6262-71.


I am grateful to The Physiological Society, UK. I am grateful to my supervisors Brig. amjad hameed and Dr. Umar ali Khan for their guidance and support. I also acknowledge the cooperation of the staff of NIH, Islamabad for their animal house and in animal handling.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA161

The dynamics between heart rate physiology and performance under stress in a realistic shooting task
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Performing under the threat of injury or death is a significant source of stress for military operators; despite these risks maintaining a high level of performance is imperative. Understanding predictive mechanisms underlying performance during high stress is critical for developing adaptive training paradigms and technologies to enhance performance. In our study, trained operators (n = 15) performed a shooting task in a simulator in which they had to discriminate enemy vs. friendly targets and decide to shoot or refrain, respectively. Decision accuracy was measured as the performance outcome. Three levels of stress were induced by manipulating performance feedback on incorrect trials: low (LO; none), medium (MD; visually displayed), high (HI; electric shock). Shock was delivered using a ThreatFire™ belt with a 200ms, 50µA pulse for incorrect decisions. The shock was administered no sooner than every 30s. The voluntary, fully informed consent of the participants was obtained in adherence with the Declaration of Helsinki. EKG and respiratory signals were used to extract features to relate to performance. Four features used were derived in overlapping 35s windows: low frequency heart rate variability, high frequency heart rate variability, and total power Normalized 4s windows.
rate variability (LF-HRV), high frequency heart rate variability (HF-HRV), respiratory rate (RR), and heart rate (HR). Each of these features (and their interactions) were related to performance at various time delays using a generalized linear mixed effects model. The final step was a backward selection procedure to generate the most parsimonious model. Results, reported as Odds Ratio (OR), revealed that in the HI stress condition, performance was worse than in LO and MD stress conditions (OR = 0.82, 95% C.I. 0.72-0.93, p = 0.002). HR alone was inversely related to performance at 2 distinct time periods: 1) coincident with behavior (OR = 0.92, 95% C.I. 0.88-0.98, p = 0.003) and 2) 50-90s prior to behavior (OR = 0.88, 95% C.I. 0.80-0.96, p = 0.003). The interactions between HR 50-90s prior to behavior in MD and HI stress conditions were positively related to accuracy (MD, OR = 1.16, 95% C.I. 1.01-1.33, p = 0.03), (HI, OR = 1.15, 95% C.I. 1.01-1.32, p = 0.03). Further analysis demonstrated that HR was positively related to LF-HRV in all conditions, however, HF-HRV was positively related to HR only in the MD and HI stress conditions F(1,592) = 5.8, p = 0.02; F(1,599) = 26.5; p = 0, respectively (Figure 1).

Figure 1. HF-HRV (parasympathetic) and LF-HRV (sympathetic) activity as it relates to HR. GLMs were performed in each condition and the coefficients of the models are plotted. In each condition sympathetic activity is related to heart rate, but only in medium and high stress conditions are parasympathetic activity related.

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PCA162

Possible blood glucose-lowering mechanisms of Cnidoscolus aconitifolius (chaya) medicinal leaves

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Persistent hyperglycaemia is increasing in humans due to globally ravaging metabolic disorders and diseases like prediabetes and diabetes. Cnidoscolus aconitifolius leaves (CA) (Euphorbiaceae) are commonly consumed as vegetable and are also used in various traditional medical systems as herbs against diabetes. Yet, the physio-pharmacological mechanisms of CA are still under-investigated. In this study, the hydro-methanolic (1:4; v/v) Soxhlet extract of air-dried powdered CA leaves was authenticated for its blood glucose-lowering activity in alloxan-induced diabetic albino Wistar rats (Iwuji et al., 2014); in compliance with the Institution’s research ethics. In Phase 1 experimentation, six groups of diabetic rats (n=5) orally received 5-200mg/Kg of the extract. In Phase 2, four groups (n =5) received 50 - 100mg/Kg/CA and 0.1 - 0.5mg/Kg of glibenclamide daily for seven days. All the animals were fed ad libitum and the two Control groups were kept on normal saline only. Prestige Glucometer with glucose test strips (code No. 22) was used to estimate the pre- and post-treatment fasting blood glucose (FBG) immediately after tail venepuncture. Paired t-tests were carried out with the SPSS (version 15) descriptive statistics. The data were expressed as mean ±SD and p<0.05. Data were also evaluated for in vivo glucose-lowering effect using the percentage difference. Nuclear Magnetic Resonance (NMR) analysis of the chloroform fraction of CA extract was used to identify and quantify the major and minor phytochemicals responsible for its activities (Iwuji and Nwafor, 2015). The results showed that the CA extract caused a dose-dependent reduction of blood glucose comparable with glibenclamide. 100-200mg/Kg CA caused significant reduction of blood glucose level in the diabetic rats. All the doses of CA (5-200mg/Kg) were found to reduce blood glucose concentration by 6.25% to 73.46% in the study. Paired t-test (p=0.05) showed that the optimal effect of 100mg/Kg CA was more potent than 50% effect of 0.1mg/Kg Glibenclamide. In addition, the weight gain associated with glibenclamide was 200% greater than that with the extract. NMR analysis revealed the presence of the bioactive derivatives of flavonoids: Eupafolin, Hissipedin, Oleandine acid, β-sitosterol, Campesterol and Isoquercitrin which are reported to have various anti-oxidant, anti-inflammatory and modified glucose metabolic mechanisms. The study suggests further physio-pharmacological studies on these minor flavonoids to favour the development of safer drugs.


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PCA163

Effects of an exercise intervention program on cardiovascular disease risk factors in the United Arab Emirates

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Cardiovascular disease (CVD) is a leading cause of death worldwide (WHO, 2010). The statistics in the United Arab Emirates (UAE) are especially alarming with one in four deaths attributable to cardiovascular disease (Al-Sarraj et al, 2010). Research indicates that although the propensity for
CVD increases with age, risk factors appear much earlier in life (McGill et al, 2002). This reflects the need for reduction of these risk factors at an early age in order to lessen the chances of CVD in later life. The aim of this study was to obtain the first data set on the cardiorespiratory health, body composition, and blood profiles of a young UAE university population, and subsequently determine the effects of a physical activity intervention on cardiorespiratory fitness. Forty-four male and female students, with a mean age of 21 ± 1 years old, participated in an 8-week exercise intervention program, where they engaged in moderate-high intensity exercise at least 3 times a week for 60 minutes. Participants were then divided into a control and experimental exercise intervention group based on BMI values. Individuals in the experimental group (BMI ≥ 25) had higher waist to hip ratios (WHR), total cholesterol, LDL-cholesterol and triglycerides, and lower values of HDL-cholesterol compared to the control group (BMI <25). The cardiorespiratory fitness of both groups was generally poor, as determined using a beep test, with average predicted VO2 max values of 27 ± 6 ml/kg/min. Post intervention, there were statistical differences in WHR in both groups (decrease, P = 0.03), and an increase in the VO2 max of both groups to 31 ± 6 ml/kg/min showing a change from poor to average cardiorespiratory fitness. In addition, HDL-cholesterol in both groups increased (P= 0.001). Although a larger sample set is needed within this population, our data demonstrates that an 8-week exercise program can positively change cardiorespiratory health and fitness of a relatively young UAE population.

This data highlights the need for such targeted interventions in order to reduce the risks of CVD in this highly susceptible population.


The authors would like to acknowledge the American University of Sharjah for funding this research. We would also like to thank Solmaz Karimi and Omar Guessoum for their assistance in data collection as well as all the participants who dedicated themselves to the study for 8 weeks.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=44)</th>
<th>Experimental (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.4 ± 1.6</td>
<td>21.3 ± 1.5</td>
</tr>
<tr>
<td>Gender</td>
<td>22 (50%)</td>
<td>20 (50%)</td>
</tr>
<tr>
<td>Metropolitan Area</td>
<td>20/24</td>
<td>20/20</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.4 ± 4.5</td>
<td>28.4 ± 4.5</td>
</tr>
<tr>
<td>WHR</td>
<td>0.92 ± 0.4</td>
<td>0.92 ± 0.4</td>
</tr>
<tr>
<td>Physical Activity</td>
<td>25.9 ± 9.6</td>
<td>26.5 ± 9.5</td>
</tr>
<tr>
<td>VO2max (ml/kg/min)</td>
<td>31.4 ± 6.1</td>
<td>31.4 ± 6.1</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>46.2 ± 14.2</td>
<td>46.2 ± 14.2</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>129 ± 44.6</td>
<td>129 ± 44.6</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>132 ± 108.1</td>
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<td>132 ± 108.1</td>
</tr>
</tbody>
</table>

Table 2. Baseline indices of control and experimental groups at the baseline and following 8 weeks of physical activity. Parameters marked with * are statistically significant.

<table>
<thead>
<tr>
<th>Treatment (n=44)</th>
<th>Control (n=44)</th>
<th>Experimental (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic Activity</td>
<td>25.9 ± 9.6</td>
<td>26.5 ± 9.5</td>
</tr>
<tr>
<td>VO2max (ml/kg/min)</td>
<td>31.4 ± 6.1</td>
<td>31.4 ± 6.1</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>46.2 ± 14.2</td>
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<td>Triglycerides</td>
<td>132 ± 108.1</td>
<td>132 ± 108.1</td>
</tr>
</tbody>
</table>

Temporal response of muscle mRNA expression to endurance training in chronic obstructive pulmonary disease (COPD)

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Compared to healthy age-matched controls (HC), patients with COPD have impaired muscle metabolism and function, which contributes to exercise intolerance. We aimed to determine whether the temporal transcriptional response of muscle to endurance exercise training in COPD was different to HC, and if so whether any differences could be associated with the magnitude of physiological training adaptation (VO2 PEAK). Nineteen patients with COPD and 10 HC (Table 1), none of whom exercised regularly, performed symptom limited incremental cycle ergometry, lean mass assessment (DEXA), physical activity monitoring (tri-axial accelerometry) and underwent resting (fasted state) vastus lateralis muscle biopsies at baseline and after 1, 4 and 8 weeks of supervised cycle exercise training (30 min 65% peak power, 3 x week). mRNA was extracted from vastus lateralis tissue and the expression of 94 genes, selected because of their known responsiveness to exercise intervention, was assessed using quantitative RT-PCR. Gene expression data were analysed using Ingenuity Pathway Analysis (IPA; Qiagen) to identify significantly changed biological functions.

Altered biological functions were apparent at weeks 1, 4 and 8 in both groups (Figure 1). A similar response was observed in both groups for networks representing energy production and fuel selection; muscle and connective tissue development and function; and free radical scavenging and inflammation. VO2 PEAK increased 15% in the HC group (p <0.01) but was unaltered in the COPD group (p = 0.62) following training. Both HC and COPD groups experienced similar temporal transcriptional responses to endurance exercise training. Furthermore, functions of gene networks found to be significantly altered were similar. Changes in mRNA expression were not however associated with increases in whole-body VO2 PEAK in response to training, with COPD patients showing a clear transcriptional response, but no increase in VO2 PEAK. These data support the contention that the responsiveness of skeletal muscle to exercise training in COPD is not blunted, at least at the level of mRNA expression.

Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Gender (Male-Female)</th>
<th>HC (n=29, IQR)</th>
<th>COPD (n=11, IQR)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>71 (65-76)</td>
<td>69 (65-76)</td>
<td>1.15</td>
<td>0.33</td>
</tr>
<tr>
<td>FEV1 (% predicted)</td>
<td>82 (65-85)</td>
<td>81 (65-65)</td>
<td>0.63</td>
<td>0.52</td>
</tr>
<tr>
<td>VO2max (l/min)</td>
<td>28.9 (28.7-31.4)</td>
<td>22.9 (18.6-27.7)</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

FEV1, forced expiratory volume in 1 s; VO2 PEAK, oxygen uptake at peak exercise in incremental cycle ergometry test.
Aim: To investigate the effects of consuming instant coffee on the fitness level of medical students in Islamic University of Indonesia (IUI) using Mc Ardle step test.

Methods: This research is an experimental study using post-test control group design. The research protocol had been approved by the Ethical Committee of Faculty of Medicine, IUI no. 24/Ka. Kom.Et/70/KE/II/2016. The subjects were 51 female medical students in IUI, who were suitable with the inclusion and exclusion criteria and were randomly classified into three groups. Group 1 was the control group, consisting of students who consumed 200 cc solution containing mocca essence and 1 sachet low calorie sugar. Group 2 consisted of students who consumed 200 cc solution containing full-dose instant coffee (2 spoonful or 7.45 grams coffee powder) with 158 mg caffeine and 1 sachet of low calorie sugar. Group 3 involved students consuming 200 cc solution containing half-dose instant coffee with 79 mg caffeine and 1 sachet of low calorie sugar. The fitness level of the subjects was measured using Mc Ardle step test 60 minutes after treatment. Pulse rate was measured twice, i.e. before treatment and after fitness test. The results were mean±SEM analyzed using one way ANOVA test. The analysis of average pulse rate before treatment showed non significant difference among the groups (p=0.41). The result means that the pre-condition of one group was equal with others. The analysis of pulse rate after Mc Ardle step test showed that there was significant difference between the groups (p=0.00). The result implied that post-test-physiological-adaptation of the group consuming coffee was better than the control group. Meanwhile, the analysis of average fitness index after treatment showed that there was significant difference among the groups (p=0.00). The best index was gained by group 2 (40.08+0.70), followed by group 3 (36.43+1.32), and group 1 (33+1.10). It means that the students consuming coffee had better fitness level than those of the control group.

Results: In conclusion, instant coffee consumption has significant effect in raising the index of fitness level based on Mc-Ardle step test.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Effect of temperature on handgrip maximum voluntary force

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Handgrip is a complex functional movement which involves co-activation of many forearm flexor and extensor muscles (Johanson et al. 1998), with a small contribution from intrinsic hand muscles (Long et al. 1970). Several studies have shown that immersing the hand and forearm in cold water reduces handgrip maximum voluntary force (MVF) (e.g. Chi et al. 2012). However, in cold environments the forearm is often thermally protected with only the hand exposed when performing manual tasks. To our knowledge only one study has investigated the effects of cooling the hand alone on handgrip, which observed declines in MVF (Vincent & Tipton 1988). This result is surprising given the relatively small contribution of intrinsic hand muscles.

Vincent and Tipton did not compare the effects of cooling and warming the hand alone vs. both hand and forearm upon handgrip MVF in the same individuals, which was the aim of the present study. The hypothesis was that handgrip MVF would decrease to a greater extent with both hand and forearm cooling.

Twenty-four healthy subjects (15 male, 14 female, 18-70 years), attended twice when either their right hand alone or hand and forearm was cooled and warmed using a Cryocuff™ containing iced water at 6°C, or warm water at 45°C. The subject’s hand, or hand and arm was removed from the Cryocuff at 4-minute intervals and two handgrip MVF measurements obtained using a handgrip dynamometer (Camry Model EH101) after which the Cryocuff was re-applied. Skin temperature over first dorsal interosseous was monitored throughout the experiment and recorded immediately before each set of MVF measurements. The reported MVF is the maximum force exerted at each temperature point. Data were analysed using R (v3.2.3) and the LME4 package (Bates et al. 2015), using a mixed model approach. Temperature, gender and condition were fixed effects, with subject as a random effect, and by-subject random slopes for the effect of temperature.

Cooling resulted in a decrease in handgrip MVF when both hand and forearm and hand alone were cooled (p<0.0001 for both conditions) although the decrease was significantly greater when hand and forearm were cooled (p<0.05). Whilst the greatest reduction in handgrip MVF was observed with hand and forearm cooling, significant decrements from hand only cooling MVF suggest that mechanisms other than, or in addition to, direct muscle cooling are involved. This has implications for manual work at cold temperatures. Furthermore, if cooling the foot results in analogous declines in maximal ankle dorsi- and plantar-flexion forces, this may contribute to the increase in indoor fall rates observed in older frail people in winter.


ESPRC for PhD funding. Tony Christopher and Lindsey Marjoram for invaluable technical support.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

The effect of hypoxia on thermoregulation during exercise heat stress in man

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The thermoregulatory responses to exercise heat stress under hypoxic conditions are unclear due to variations in methods of prescribing exercise workloads in both sea-level and hypoxic conditions (Kacin et al., 2007) and in the measurement of cutaneous vascular responses to exercise at a fixed metabolic heat production (Cramer & Jay, 2014) in combined heat and hypoxia stress. Eight healthy, unacclimatised males (23.5±2.5 years, 77±10.23 kg, 1.81±0.08 m, sea-level VO₂peak 3.5±0.5 L.min⁻¹ and hypoxia VO₂peak 2.8±0.4 L.min⁻¹) underwent 40 minutes of steady state cycling at a fixed metabolic heat production (592±33 Watts, 7.7±0.7 Watts.kg⁻¹, VO₂; 2.1±0.1 L.min⁻¹, Power Output; 144±18 Watts) at 28°C (40% relative humidity) under 2 different inspired fractions of oxygen; (1) Sea-level and (2) Hypoxia (13 % FIO₂). Local skin blood flow (SkBF; laser Doppler flowmetry; expressed as cutaneous vascular conductance; CVC) and local sweat rate (SR; capacitance photoplethysmography) on the forearm and whole-body SR were recorded. Core (TCORE; intestinal pill) and local skin temperatures (TSK) blood pressure (BP; automated sphygmomanometry and digital photoplethysmography), heart rate (HR; ECG) oxygen saturation (SpO₂; pulse oximetry) were also monitored. Mean body temperature (TBODY; product of TCORE and TSK) thresholds for heat stress-induced elevations in SkBF and SR, as well as the SkBF/SR:TBODY slopes were calculated. Data are presented as Means±SD. During exercise in Hypoxia SpO₂ was lower (97±1 vs. 81±5 %, P<0.001) and HR was higher (129±23 vs. 148±27 beats.min⁻¹, P<0.001). both TCORE (37.5±0.4 vs 37.7±0.4 °C, P<0.01) and TSK (33.6±1.0 vs 34.4±0.7 °C, P<0.01) were higher in Hypoxia. Whole-body SR was higher during exercise in Hypoxia (0.87±0.49 vs. 1.38±0.87 L.hr⁻¹.m⁻², P<0.07). During exercise the forearm SR TBODY threshold (36.7±0.2 vs. 36.8±0.2 °C, P=0.18) was not different between trials whereas the forearm SR TBODY slope (1.7±1.0 vs. 2.1±1.0 mg.cm⁻².min⁻¹ °C, P=0.03) was increased during Hypoxia. The forearm SkBF TBODY threshold (36.7±0.3 vs. 36.9±0.1 °C, P=0.22) was not different between trials nor was the forearm SkBF TSBODY slope affected during Hypoxia (63±47 vs. 110±65 CVC %Maximum units•°C, P=0.23). Thermal discomfort (6±1 vs. 7±1 units, P<0.01) and ratings of perceived exertion (13±1 vs. 14±2, P<0.01) were higher during exercise in Hypoxia. During exercise-heat stress performed at a fixed metabolic heat production under hypoxic conditions core temperature and thermal discomfort are increased relative to sea-level. These findings do not appear to be caused by impaired vasodilatory and sweating thresholds or local vasodilatory sensitivity, rather, whole-body and local sweating sensitivity are increased during hypoxia; possibly via a higher skin temperature.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA168

Effects of physiological predictors on endurance performance in recreational runners

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Anaerobic threshold (AT) and VO2max have been used as endurance predictors (1). It is unclear that metabolic stress (MS) as indicated by ‘AT’ reflects more towards physiological fatigue or muscular fatigue. This is because of the contradictory research findings between lactate (La) and muscle fatigue (2,3) and ‘La’ kinetics with O2 transport system (4). Due to this complexity, this study will have three purposes.

1) To investigate if endurance time (ET) at individual’s anaerobic speed (vAT) will be similar between participants during a discontinuous constant speed and a continuous constant speed protocol. 2) To evaluate anaerobic fitness (%VO2max) at anaerobic threshold speed (vAT) to aerobic fitness (%VO2max) as indicated by ‘AT’ reflects more towards physiological fatigue or muscular fatigue. This is because of the contradictory research findings between lactate (La) and muscle fatigue (2,3) and ‘La’ kinetics with O2 transport system (4). Due to this complexity, this study will have three purposes.

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Table 1: Descriptive statistics of variables causing termination of discontinuous and continuous treadmill protocol against volitional exhaustion factors

<table>
<thead>
<tr>
<th>Protocols</th>
<th>Variables</th>
<th>continuous ET</th>
<th>discontinuous ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session 2</td>
<td>Lactate (m mol/L)</td>
<td>5.74±1.13</td>
<td>1.42±2.74</td>
</tr>
<tr>
<td></td>
<td>VO2 (mL/kg/min)</td>
<td>56.74±3.41</td>
<td>49.93±4.64</td>
</tr>
<tr>
<td></td>
<td>VO2max (mL/kg/min) max</td>
<td>10.48±1.85</td>
<td>11.37±2.09</td>
</tr>
<tr>
<td></td>
<td>Speed (KPH)</td>
<td>15.45±1.34</td>
<td>13.39±0.71</td>
</tr>
<tr>
<td></td>
<td>%VO2max</td>
<td>61.45±3.52</td>
<td>80.10±5.31</td>
</tr>
<tr>
<td>Session 3</td>
<td>Speed (KPH)</td>
<td>15.45±1.34</td>
<td>13.39±0.71</td>
</tr>
<tr>
<td></td>
<td>%VO2max</td>
<td>61.45±3.52</td>
<td>80.10±5.31</td>
</tr>
<tr>
<td></td>
<td>Lactate (m mol/L)</td>
<td>7.31±2.74</td>
<td>5.70±1.60</td>
</tr>
</tbody>
</table>

Figure 1: Oxygen reserve (ΔVO2) relationship with endurance time (ET) and anaerobic fitness (%VO2max) at anaerobic threshold speed (vAT) to exhaustion


Gupta, N., et al. Determination and Validation of Maximal Aerobic Speed. In MEDICINE AND SCIENCE IN SPORTS AND EXERCISE. 2012. LIPPINCOTT WILLIAMS & WILKINS 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA.

This Study was approved by ethical committee “Institutional Review Board”, NTU, Singapore.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Progressive resistance exercise training (RE-T) is known to enhance muscle mass and function in both young and older individuals (1); thereby providing a myriad of health benefits, such as decreasing the risk of diabetes, cardiovascular diseases and/or sarcopenia (2). However, achieving compliance with regular RE-T can be difficult due to perceived and real socio-economic, psychological and environmental factors e.g. access to specialised equipment (3). Therefore, there is significant need for the development of alternative RE-T programmes to try and alleviate these barriers to exercise, in order to provide a widely-accessible intervention that can deliver the associated health benefits of RE-T to a wider population (4). This study aimed to assess the efficacy of 4-weeks home-based RE-T, fully integrated into activities of daily living, on leg lean mass and physical function. Twelve healthy older volunteers (63±3y, 60:40 M:F, BMI: 29±1 kg.m⁻² (mean±S.E.M.)) were recruited to this study. Before and after RE-T all volunteers performed a battery of physical function tests including: leg extension 1-RM (one repetition-maximum), a short physical performance battery test (SPPBT; assessing balance, gait speed and time to rise), lower limb power (via Nottingham power rig), handgrip strength and maximal voluntary contraction (MVC) for leg extension. Ultrasound measures were made before and after RE-T to assess quadriceps cross sectional area (CSA). For the RE-T, volunteers were provided with instructions for 8 different home-based RE-T exercises that could be incorporated into habitual activities (e.g. “toothbrush squats”, “hoover lunges”, “cooking bicep curls”). Volunteers were instructed to perform 3x12 repetitions of each exercise, every day for 4-weeks. After RE-T, despite no significant increase in 1-RM, handgrip strength or SPPBT, there was a significant (p<0.05) increase in quadriceps CSA (1.5±0.8 cm², (mean±S.E.M., paired t-test)), MVC (14.6±7.7 Nm) and muscle power (46.8±19.8 Watts). In conclusion, this study demonstrates that RE-T fully integrated into activities of daily living has potential benefit for the maintenance of muscle mass and physical function. Home-based RE-T, such as this, i.e. an exercise intervention without the requirement of finances or access to specialised equipment, could benefit not only older individuals, and those with co-morbidities associated with loss of muscle mass (e.g. diabetes, COPD or sarcopenia/ frail elderly (5)); but may help alleviate psycho-social barriers to physical activity such as, one of the most commonly cited; “lack of time” (3).


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The role of miR-378 in sarcopenia
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Sarcopenia is the loss of muscle mass and function with age and it affects all individuals from approximately the 4th decade of life. The mechanisms behind sarcopenia are unclear, however they are likely to be multifactorial. MicroRNAs (miRNAs, miRs) are small non-coding RNAs that post-transcriptionally regulate gene expression. microRNAs have been shown to be play key roles in muscle development and disease. We and others demonstrated a decrease in miR-378 expression in muscle during ageing in both rodents and humans. We manipulated the expression and function of miRNA-378 in muscle of mice using microRNA mimic and antagomiR delivered via tail vein injection. Inhibition of miR-378 expression resulted in decreased muscle mass/myofibre size and function (force generation). In contrast, overexpression of miR-378 resulted in an increased muscle mass, myofibre size and function in both adult and old mice. These data suggest miR-378 plays a role in sarcopenia and may be a potential therapeutic target against sarcopenia. Further work will focus on the validation on targets of miR-378.

The authors would like to thank University of Liverpool, Wellcome Trust, MRC-Arthritis Research-UK CIMA and BBSRC for funding.

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The studying of colors' influence on internal time flow speed and adaptive opportunities of the organism
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Internal time flow speed (IT) of a human is an important factor that reflects the ratio of the basic processes in the CNS. The aim of the research was to study the dependence of the IT flow speed on colors. Materials and methods. With the help of a specially developed computer program the assessment of the individual minute duration (IMD) was performed, using. During the process of a time countdown the testees were looking at the screen of the display with the set color. The research was conducted in the conditions of common blackout in order to create light and color dominance of glowing screen of the display. 48 students with an accelerated IT flow who have volunteered to take part in the experiment were studied. Each of them carried a series of 10 measurements for every time of the main background colors (red, orange, yellow, green, blue, dark blue, purple).

Results and its discussion. The findings indicate that the color significantly affects the IT flow speed. The most significant changes are observed under the impact of extreme spectral colors: red and blue-purple. Red color caused much more acceleration of IT, purple color, on the contrary, caused its slowdown. Green and related to it medial spectral colors caused less significant multidirectional and unreliable changes of the IT flow speed. The findings show that the most distinct positive effect on the IT flow speed was done by dark blue and especially purple colors. It was found that the determination of the IMD in terms of using dark blue and purple colors of 10 minutes duration is accompanied by the improvement of the IT flow speed and other studying indicators. The duration of physical activity was increased to 2.5 min in average, along with this the more adequate reaction of the cardiovascular and respiratory systems to physical activity was observed. The recovery of deviation indicators in restorative period flew faster. These data indicate to decreasing of the "value" of adaptation. At the assessment of intellectual productivity evaluation it was found that purple color reduces the number of errors that the students made while having the correction test. The decreasing of anxiety and frustration of mental status under the influence of purple color is determined. Under the influence of red color of background there is a clear increase of the IT flow speed, the clear prevalence of the excitation process over inhibition one. The result of these changes was reducing the physical and intellectual productivity, increasing of aggressiveness and anxiousness, the rapid worsening of the vegetative indices.

Conclusions. Thus, the color is a factor that significantly affects the IT flow speed and the condition of the main processes in the CNS. It allows suggesting that certain colors can be used as artificial modulators of IT speed of a human to optimize its adaptive opportunities.


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Exercise-induced plasma steroid hormone responses in men: the development of a new tool to highlight hormonal alterations during overreaching to reduce the incidence of the overtraining syndrome
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Athletes commonly progressively overload the body to improve physical performance, however, this can lead to detrimental states of overreaching – functional (FOR) or non-functional (NFOR) - or the overtraining syndrome (OTS). Recovery from these states may take weeks to years (Meeusen et al., 2013). Exercise-induced responses of salivary testosterone
and cortisol are suggested as overreaching markers with blunted exercise-induced (cycling exercise) responses found following an intensified-training period (Hough et al., 2013). This study develops a 30-min running bout to induce reproducible plasma cortisol and testosterone elevations in healthy, physically active males when in a normal-trained state (not suffering from overreaching/OTS). This bout may become a tool to highlight blunted exercise-induced hormonal responses when overreached.

10 males [mean age 22, S.D. = 2 years; body mass 77.7, SD = 13.4 kg, maximal oxygen uptake (VO₂max) 56.8, S.D. = 7.3 ml kg⁻¹ min⁻¹] performed 7 main trials, 6 exercise trials [30-min continuous treadmill run composed of alternating blocks of either (a) 1 min at 50% and 4 min at 70% of velocity at VO₂max (νVO₂max) (50/70); or (b) 1 min at a rating of perceived exertion of 11 (fairly light) and 4 min at 15 (hard) on the 6-20 Borg scale (RPEtreadmill)], and 1 resting/control trial (CTL). Each participant completed 3 trials structure (a) and 3 trials following structure (b). Blood samples were collected pre, 1 min and 30 min post-exercise. Plasma cortisol and testosterone concentrations were assessed using enzyme-linked immunosorbent assay (ELISA) kits, according to manufacturer’s instructions.

Data is reported as mean ± S.D., compared by ANOVA. Heart rate (155 ± 10 bpm) and speed (9.6 ± 1.7 km·h⁻¹) lower was found in the 50/70 compared with the RPEtreadmill (168 ± 10 bpm & 11.2 ± 1.8 km·h⁻¹) (P < 0.05). Speed was consistent across all trials in the RPEtreadmill (P > 0.05). Cortisol and testosterone did not change during CTL (P > 0.05). Cortisol did not significantly elevate in any exercise trial (P > 0.05), but exercise-induced mean % -increase was 29% and 50% in response to 50/70 and RPEtreadmill, respectively. Testosterone elevated from pre- to post-exercise in both the 50/70 and the RPEtreadmill (P < 0.01). Cortisol (ICC=0.964; ICC=0.947) and testosterone (ICC=0.839; ICC=0.868) showed excellent reproducibility (Atkinson et al., 1999) to the 50/70 and RPEtreadmill, respectively. The RPEtreadmill provoked greater hormonal elevations compared with 50/70 and will not require a test to be used, suggesting this test may be a good tool to indicate any overreaching-related alterations. Therefore, this exercise test could be a useful tool when tracking individuals at risk of suffering from NFOR, aiming to reduce the incidence of OTS.

Changes in arterial shear stress can induce functional and structural adaptations in the vasculature. Studies using in vitro endothelial cell (EC) cultures and isolated vessels from animals have shown that increases in shear stress (similar to what is observed during exercise) can lead to increased expression of total endothelial nitric oxide synthase (eNOS) (1,2) and/or phosphorylated eNOS at serine 1177 (P-eNOS1177) (3), the primary activation site on eNOS. However, it is unclear whether results obtained in vitro can be extrapolated to ECs in a human artery that is subjected to an increase in blood flow in vivo. Therefore, we aimed to determine if local increases in arterial shear during repetitive muscle contractions induce acute changes in eNOS and P-eNOS1177 expression in humans. Seven young males (25±1 yr) performed 20 separate bouts (3 min each) of rhythmic forearm exercise at 20% of max over a 2-hr period. Each bout of exercise was separated by 3 min of rest. The switching between exercise and rest allowed subjects to complete the entire protocol without fatiguing and promoted brachial artery shear to remain elevated above baseline throughout the entire 2-hr protocol. An additional six male subjects (24±1 yr) served as time controls (no exercise). ECs were freshly isolated from the brachial artery using sterile J-wires shortly after catheter placement at baseline and again following the 2-hr exercise or time control period. The ECs were incubated with antibodies against Von Willebrand factor and DAPI (to identify ECs with intact nuclei) along with antibodies for eNOS or P-eNOS1177, followed by fluorescent secondary antibodies to assess protein expression via fluorescence microscopy. Fluorescence intensity for each subject sample was normalized to the intensity of cultured human aortic ECs (HAECs) and expressed as ratios of subject EC protein expression/HAEC. Brachial artery mean shear rate was elevated compared to baseline throughout the course of the 2-hr exercise protocol (Fig. 1, P<0.001). Total eNOS expression did not change in either the exercise (0.13±0.04 vs. 0.12±0.03 a.u.) or time control (0.12±0.03 vs. 0.11±0.03 a.u.) group following each respective trial (P>0.05 for both). However, P-eNOS1177 was increased in the exercise group (Fig. 2, P<0.02) with no change observed in the time control group (P=0.72). Moreover, there was a moderate yet non-significant correlation between the relative changes in mean shear and P-eNOS1177 in the seven subjects that performed forearm exercise (r=0.65, P=0.11). Our novel results suggest that elevations in brachial artery shear in response to forearm exercise increase eNOS1177 phosphorylation in ECs of young healthy males. Additionally, our data may provide insight into the beneficial effects of exercise on endothelial function in humans.
The more established blood flow measurement technique of
The aim of this study was to test the validity of VOP against
evidence exits pertaining to its accuracy in an exercise setting.
non-invasive blood flow measurement technique suitable to
measuring whole limb blood flow, however, conflicting evi-
dence exits pertaining to its accuracy in an exercise setting.
The aim of this study was to test the validity of VOP against
the more established blood flow measurement technique of
Doppler ultrasound (DU) to quantify the dynamic response of
leg blood flow (LBF) during exercise. Ten healthy young male
participants performed 6 bouts of 6 min intermittent (3-s duty
cycle: 1-s contraction, 2-s relaxation) plantar-flexion exercise
of the right calf muscle in the prone position on a custom-built
calf ergometer at 30, 50 and 70% of maximum voluntary con-
tractions (MVC). Simultaneous VOP and DU measurements
of LBF were recorded and repeated on two separate days so
that in total, 4 bouts were performed at 30 and 50% MVC
and 2 bouts at 70% MVC. Two empirical models (triphasic
or quadraphasic) were fitted to averaged LBF data of all the
individual time series of LBF using a weighted least-squares
non-linear regression procedure. A quadraphasic model
was fitted in participants in whom a second or slow ‘decay’
in blood flow was apparent (50% of the cases), but only the
first and second ‘growth’ phases and the first ‘decay’ phase
(which followed the first growth phase and was apparent
in most cases) were compared across measurement techniques.
Responses (shown as means ± SD) were compared using a Stud-
ent’s T-test or Wilcoxon Signed Rank test. The time constants
of the second growth phase of the LBF kinetic response were not
different between measurement techniques (30% MVC: 20.4 ± 10.5 vs. 27.2 ± 19.7, 50% MVC: 19.0 ± 11.9 vs.
16.1 ± 7.3, 70% MVC: 9.1 ± 4.9 vs. 11.0 ± 3.9). Similarly, the
end-amplitudes (mL min⁻¹) at 30 and 50% MVC were not dif-
f erent between VOP and DU (30% MVC: 353 ± 109 vs. 359 ±
95; 50% MVC: 535 ± 171 vs. 588 ± 182) but they were signifi-
cantly lower for VOP than DU at 70% MVC (667 ± 230 vs. 798 ±
261). In addition, resting LBF estimates were also significantly
lower for VOP than DU. The rest of the kinetic parameters
were not affected by measuring technique. The current study
supports the validity of using VOP as an accurate technique
to measure the dynamic response of lower limb blood flow
during exercise. However, VOP tends to underestimate
the total amplitude of the LBF response at high intensities (i.e.
70% MVC) most likely given that arterial infl ow into the limb
is progressively reduced as the venous volume and pressure
rises, with the possibility of venous outflow.

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Coupling between internal carotid artery and vertebral
venous flow during orthostatic stress in humans
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Institute of Physical Fitness, Japan Women’s College of Physical
Education, Tokyo, Japan
In a supine position the main drainage from the brain is
through the internal jugular vein (IJV) but the vertebral veins
(VV) become important during orthostatic stress when the
IJV is “collapsed”. To indicate whether this shift in venous
drainage from the brain during orthostatic stress reflects
that flow from the part of the brain served by the internal
Association between cerebrovascular resistance and ageing in men and women

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Understanding age-related changes in cerebral perfusion and regulation of cerebral blood flow is crucial for determining neurological pathology. Previous studies have shown that total cerebral blood flow (CBF) is inversely associated with ageing in men and women (1); however, not all studies demonstrate an age-related reduction in CBF, specifically when comparing young and middle-aged adults (2). In addition, sex differences in both CBF and cerebrovascular resistance (CVR) have been reported with women having greater total CBF and lower CVR in young and middle-aged adults (2). In contrast, CVR was not different between young and middle-aged adults (63±2 vs. 62±2 mL/100mL tissue/min; p=0.68) and not associated with age (r=−0.01; p=0.94). Finally, we evaluated potential interactions between sex and age. There were no significant interactions for CBF; however, CVR was higher in middle-aged women compared with young women (1.50±0.05 vs. 1.32±0.07 mL/100mL tissue/mmHg/min; p<0.05), and the age-related difference in men did not reach significance (1.42±0.04 vs. 1.57±0.09 mL/100mL tissue/mmHg/min, in young compared with middle-age; p=0.13). MAP was greater in young men compared with young women (90±1 mmHg vs. 80±2 mmHg; p<0.001). MAP was also higher in middle-aged women compared with young women (92±2 mmHg vs. 80±2 mmHg; p<0.001). Our results demonstrated higher CVR in middle-aged adults compared with young adults, despite no age-related differences in CBF. This suggests that elevated CVR in normotensive middle-aged adults may be linked to age-related increases in blood pressure typically seen in older adults. Furthermore, it is possible that the higher CVR is driving an increase in arterial pressure to maintain adequate cerebral perfusion.

Funding-BHF


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PCA177

Effects of ageing upon muscle functional and inflammatory responses to eccentric exercise

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Ageing has been purported to impair day-to-day muscle regeneration with muscle tissue inflammation being a putative culprit. Nonetheless, the impact of ageing on muscle function and inflammation at rest and following exercise in young (Y) vs. older (O) volunteers remains poorly defined. Here we investigated muscle functional and inflammatory responses to unilateral eccentric exercise (ECC) as a “muscle damage” stimulus in both Y and O men. Eight Y (22±1 y, mean±SD) and eight O (70±1 y) healthy, exercise naïve men performed a single bout of unilateral ECC (7×10 repetitions at 80% of ECC one-repetition maximum). We assessed perceived leg muscle soreness and sensitivity to pain, in addition to peak muscle torque, power, plasma creatine kinase (CK) and muscle tumor necrosis factor alpha (TNF-a) and phospho NF-kappaB protein levels (p-NFkB) proteins at baseline (BL), immediately (0), 5, 24, 72 and 168h after exercise. As expected, Y exhibited greater BL peak torque compared to O (Y: 253±21 vs. O: 166±15Nm; p<0.05, two-way ANOVA). Declines in peak torque (compared to BL) were observed in Y and O at 0h (Y: 171±15.3, P<0.01; O: 128.5±11, P<0.05), which persisted until 24h in O (113±13, P<0.01) and 72h in the Y (201±24, P<0.01). No BL age-related differences were observed for power (Y: 228±32 vs. 179±26W), which declined immediately following ECC in Y (169±16, P<0.05), and persisted for 24h (150±24, P<0.01) but...
did not change in O. Sensitivity to pain heightened in Y and O at 5h post-ECC (Y: 11±2; O: 7±1lbs, P<0.05) compared to BL (Y: 13±1; O: 10±1) but remained heightened for 72h (11±1, P<0.05) in Y only. Muscle soreness was elevated immediately after ECC (6±1 cm, P<0.01, one-way ANOVA) and persisted for 72h (4.9±0.8, P<0.01) in Y, and was elevated at 24h in O (6±1, P<0.01). Plasma CK was elevated at 24 and 72h in Y, and at 72h in O (P<0.05, Friedman’s one-way ANOVA) - confirming “muscle damage”. Interestingly, O displayed higher BL muscle TNF-α compared to Y (P<0.05, two-way ANOVA). Nonetheless, while TNF-α did not change post-ECC in O, it increased at 72h and remained elevated at 168h in Y (comparable to BL, P<0.01).

A similar, non-significant pattern was observed for p-NFKb. We report that ageing per se is associated with increased muscle TNF-α and reductions in muscle function, as shown previously (1). However, the data does not necessarily support the notion of a heightened induction of muscle inflammation and delayed recovery in response to “damaging” exercise in O, since Y exhibited a more sustained deficit in muscle function than O in tandem to the induction of muscle TNF-α. The absence of muscle mass and activation measures precluded investigation into differences in specific forces which could explain the apparent heightened susceptibility to ECC-induced muscle dysfunction in Y vs. O i.e. if Y lifted relatively more than O, heightening the stimulus.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA179

Prediction of maximal oxygen uptake at high altitude
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The maximal oxygen uptake (VO2max) at high altitude is a key quantity in alpine medicine but has not been explained yet in terms of physiological parameters – ventilation, cardiac frequency, PVO2, and inhaled gas O2 pressure. In this study, a novel theoretical approach (Kang et al., 2015) is used to predict the altitude dependence of VO2max from the above parameters values. By solving interactively the equations for O2 convection-diffusion in airways and O2 saturation in the pulmonary capillaries, the method yields the corresponding values of VO2max.

Using a quadratic fit of ventilation and perfusion data from literature (see the figure inset) and under the condition PVO2 = 20 mmHg, VO2max at different altitudes is computed. In the figure, it is given as a percentage of sea level value. The predicted VO2max, shown in red, has a curvilinear decrease with increasing altitude, which exhibit very good agreement with experimental data. Both prediction and experiments gives around 80% decrease in VO2max at Mt. Everest altitude. Further investigation shows that the influence of ventilation on VO2 becomes more significant at high altitude. This explains why hyperventilation is the most important feature of acclimatization to altitude (West, 2006).


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Impact of time of physical exercise workout on nesfatin-1 and irisin levels in trained and untrained male subjects
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Increased physical activity is a cornerstone for the regulation of energy homeostasis. Despite general acceptance of beneficial effect of exercise on energy homeostasis, the optimum time of exercise workout remain unclear. The main issue of question is the effects of exercise time of day on nesfatin-1 and irisin, which are involved in the control of food intake and increased energy expenditure. This have a critical importance considering the best time available for leisure activity during busy modern daily life. This study was aimed to investigate possible effect of acute exercise, performed at different times of day, on nesfatin-1 and irisin levels in relation to subjects’ training status.

Trained (n=14, 18.3±0.1 yr, 61.4±2.3 kg, 175±2.6 cm) and untrained (n=14, 18.6±0.1 yr, 63.2±2.4 kg, 176±1.4 cm) young male subjects performed in three soccer match (60 min) in field (30 m wide vs 50 m length) in the morning (M), afternoon (A) and night (N) on separate days (i.e72 hours between each match). The study protocol was approved by the Firat University Ethics Committee. Venous blood samples were taken at the onset and at the end of the match. Serum nesfatin-1 and irisin levels were analysed in a double-blind condition using ELISA methods. Values are expressed as means±S.E.M., compared by Wilcoxon-signed rank test and Mann-Whitney U test. p < 0.05 was accepted as statistically significant.

Baseline nesfatin-1 levels being significantly higher in untrained subjects compared to trained subjects (138.9±12 pg/ml vs 97.6±9 pg/ml M, 136.2±7 pg/ml vs 107.3±9 pg/ml A, p<0.05). The baseline irisin level was not different between trained and untrained subjects, in both morning, afternoon and night (p>0.05).

In trained and untrained subjects, serum nesfatin-1 and irisin levels showed a significant increase at the end of the soccer match in both morning, afternoon and night (p<0.05).

Poster Communications

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Impact of time of physical exercise workout on nesfatin-1 and irisin levels in trained and untrained male subjects
S. Algul1 and O. Ozcelik2
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Baseline nesfatin-1 levels being significantly higher in untrained subjects compared to trained subjects (138.9±12 pg/ml vs 97.6±9 pg/ml M, 136.2±7 pg/ml vs 107.3±9 pg/ml A, p<0.05). The baseline irisin level was not different between trained and untrained subjects, in both morning, afternoon and night (p>0.05).

In trained and untrained subjects, serum nesfatin-1 and irisin levels showed a significant increase at the end of the soccer match in both morning, afternoon and night (p<0.05).

Poster Communications

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A. 131.7±6 pg/ml vs 91.5±6 pg/ml, respectively, p<0.05). Baseline irisin levels being significantly higher in trained group compared to untrained group (235.3±5 ng/ml vs 112.7±2 ng/ml M, 249.7±4 ng/ml vs 112.6±2 ng/ml A, 262.3±5 ng/ml vs 113.8±2 ng/ml N, respectively, p<0.05). Following all exercise workouts utilized, irisin levels were increased significantly in trained and untrained groups (17.9% and 14.4% M, 20.3% and 9.3% A, 17.8% and 13.6% N, respectively, p<0.05). Nesfatin-1 levels were also increased after exercise workout, but the increase was statistically significant only for night exercises in trained and untrained groups (28.3% and 20.9% N, respectively, p<0.05).

This study documents that timing of exercise workout have different effects on irisin and nesfatin-1 levels, irrespective of subjects’ training status. The reason/role of the increases in these hormones in this experimental setting is not clear, but considering the role of these two hormones in regulation of energy expenditure and food intake our results stands in favour of night exercise.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

**PCA181**

**Arterial stiffness is decreased in estrogen deficient physically active women with functional hypothalamic amenorrhea**

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Estrogen deficient physically active women with functional hypothalamic amenorrhea (FHA) demonstrate elevated vascular resistance and blunted endothelial function. To test the hypothesis that arterial stiffness is also altered in these women, two age-matched (pooled mean, 24±1 years; mean±SEM) groups of physically active women were studied: those with FHA (ExFHA; n=11) or eumenorrheic menstrual cycles (ExOv; n=12). Radial artery tonometry was used to assess arterial stiffness (augmentation index [AIx, %]), Alx adjusted for heart rate [Alx75], and augmentation pressure [AP, mmHg]), and central mean arterial pressure (MAPc, mmHg). Doppler ultrasound measures of cardiac output (CO, L/min), stroke volume (SV, ml) and total peripheral resistance (TPR, dynes/sec/cm5) were calculated. All measures were recorded before and one hour after 45 minutes of moderate intensity dynamic exercise. Compared with ExOv, ExFHA demonstrated lower baseline (p<0.05) heart rate (48±2 vs. 54±1, beats/min), MAPc (66±2 vs. 72±2, CO (3.4±0.1 vs. 4.1±0.1), Alx75 (-10.6±2.8 vs. -6±3.3), and higher (p<0.05) TPR (1607±69 vs. 1400±47). Post-exercise, heart rate and CO were increased (p<0.05) and MAPc decreased (p=0.05) in both groups, yet values remained lower (p<0.05) in ExFHA. TPR decreased (p<0.05) in both groups, but remained higher (p<0.05) in ExFHA. Alx and Alx75 were decreased (p<0.05) in ExOv (2.5±3.5; -10.4±2.3; respectively), but were unaltered (p<0.05) in ExFHA. SV did not differ (p>0.05) pre- versus post-exercise within- or between-groups. Alx and AP correlated positively (p<0.05) with TPR and inversely (p<0.05) with CO both pre- and post-exercise in ExFHA only. In conclusion, ExFHA women demonstrate low resting and post-exercise arterial stiffness in association with high vascular resistance and low CO. Although the role of estrogen deficiency per se is unclear, these findings suggest elevated vascular resistance may serve to defend low CO rather than increase arterial stiffness in ExFHA.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

**PCA182**

**Nutritional ketosis: A metabolic replacement for glucose in exercise performance?**

P.J. Cox, F. Cvetko, O. Faull, T. Kirk, B.J. Stubbs and K. Clarke

Department of Physiology Anatomy and Genetics, Oxford University, Oxford, UK

Introduction: High performance endurance exercise demands extreme physical loads with carbohydrate intake long accepted as the gold standard for fuelling exercise performance[1]. However, recently we have shown that nutritional ketosis decreases glycolysis[2], increases fat oxidation[3], and remains an oxidisable fuel substrate even at high exercise intensities[4]. Thus we sought to determine whether nutritional ketosis could match improvements elicited by carbohydrate in endurance cycling performance.

Methods: Following informed consent, 12 endurance-trained volunteers (n=8 males, VO2 Max 5.4 L/min; n=4 females, VO2 Max 3.8 L/min; age 28.1 ± 1 years; mean ± SEM) completed 3 maximal 1h time trials on a bicycle ergometer following an overnight fast, in a 3-way single-blind crossover design. Before each trial isocaloric quantities of either carbohydrate (dextrose; CHO) or ketone ester (725 mg/kg; (R)-3-hydroxybutyl (R)-3-hydroxybutyrate; KE), or a calorie-free control drink and vitamin B3 (1000 mg; B3) were given. Serial blood samples were obtained via an IV catheter and analysed for glucose, free fatty acids (FFA), D-3-hydroxybutyrate, lactate, glycerol, insulin and acetoacetate. Muscle biopsies (vastus lateralis) were obtained from n=7 athletes pre- and immediately post- exercise. Samples were cryo-sectioned, stained, and analysed for glycogen+ intramuscular tri-glyceride (IMTAG) using confocal microscopy. 3-way repeated measures ANOVA with post-Hoc Tukey corrections were used to determine statistical significance (p<0.05).

Results: Power output during the time trial was improved with both CHO and KE supplementation compared to vitamin B3 (B3 284±6 W; CHO 295±5 W p=0.01; KE 292±6 W p=0.01), with no difference observed between CHO and KE. Blood D-3-hydroxybutyrate reached a mean of 3.2± 0.2 mM prior to the time trial following KE ingestion remaining elevated throughout exercise. There was a significant increase in blood glucose and insulin in CHO vs. KE and B3, while lactate production was lower during KE vs. both CHO and B3 (p<0.05). Plasma FFA were similar during exercise on B3 and KE, while a significant increase was observed in the latter half of exercise with CHO supplementation (p<0.05). IMTAG and glycogen were not significantly different between conditions at baseline, however IMTAG fell by 8% on KE but increased 9% on CHO (p<0.05) after 1 h of exercise. IMTAG was unchanged on B3. Glycogen levels were significantly greater after exercise on KE vs. CHO and B3 (p<0.05) indicative of glycogen preservation by ketosis.

Conclusion: In this study we have shown how nutritional ketosis enables equivalent physiological function and cycling performance to that of glucose, but via very different metabolic
Nutritional ketosis regulates BCAA metabolism in exercise

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Introduction

Nutritional ketosis has been shown to significantly alter oxidative metabolism during exercise (1-3), restoring metabolic flexibility, and reducing the reliance on glycolysis to sustain high intensity exercise. Limited carbohydrate availability can result in muscle protein breakdown for gluconeogenesis in energetic stress, such as starvation or endurance exercise (4). Therefore, we tested whether nutritional ketosis could reduce muscle proteolysis in exercise, by determining changes in intramuscular concentration of branched-chain amino acids (BCAA) and their relationship to glycolysis.

Methods

After providing informed consent, and following an overnight fast, 10 male endurance-trained volunteers (VO₂max 5.4±0.2 L/min; age 27.6±1.6 y) completed 3 trials of 1h fixed-intensity cycling at 75% W_max in a single-blind crossover design. Before each trial, participants drank taste-matched isocaloric drinks containing ≥96% of calories from carbohydrate (maltodextrin:fructose, 5:1; CHO), ketone ester (573 mg/kg BW; (R)-3-hydroxybutyl (R)-3-hydroxybutyrate; KE), or fat (FAT). Muscle biopsies were taken immediately pre- and post-exercise from vastus lateralis muscle. Muscle metabolites were extracted via the Folch method and analysed for intramuscular D(3)-hydroxybutyrate (D(HB)), pyruvate, leucine + isoleucine, valine, glucose and glycolytic intermediates using a triple quadrupolar mass spectrometer (Waters, UK). Serial blood samples were obtained via an IV catheter and analysed for D(HB) and lactate. Athletes maintained a standard diet for 24h prior to the CHO and KE trials, and an isocaloric high-fat (~70%), low-carbohydrate (~5%) diet prior to the FAT trial. 3-way repeated measures ANOVA with post-Hoc Tukey corrections were used to determine statistical significance (considered as p<0.05).

Results

Intramuscular leucine + isoleucine was reduced by ~50% following exercise with KE supplementation, compared to CHO or FAT (p<0.05). No exercise differences in valine were seen. After 1h of exercise at 75% W_max, intramuscular leucine + isoleucine positively correlated with pyruvate (r=0.74, p<0.01) and lactate (r=0.61, p<0.05), while D(HB) positively correlated with intramuscular glucose (r=0.55, p<0.05) and negatively correlated with pyruvate (r=0.46, p<0.05), glycolytic intermediates (r=0.52, p<0.05) and leucine/isoleucine (r=0.51, p<0.05).

Conclusion

In this work we have shown that nutritional ketosis reduces the rise in intramuscular BCAA levels during exercise, supporting previous evidence that ketosis tightly regulates glycolysis (and therefore pyruvate), ultimately reducing the requirement for muscle deamination (5). Such metabolic effects have a sound evolutionary basis, limiting the catabolism of carbohydrates and skeletal muscle protein for gluconeogenesis in starvation. These findings may also provide a key to reducing muscle catabolism, or enhancing recovery following sustained exercise.


The role of muscle fibre type in skeletal muscle substrate oxidation during nutritional ketosis

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Introduction

Ketone bodies act as both substrates and signals to conserve carbohydrates for cerebral oxidation during calorie deprivation [1]. Administration of exogenous ketones alters skeletal muscle substrate competition for respiration in exercise [2, 3], however the magnitude of this effect in relation to skeletal muscle fibre type composition is unknown.

Methods

After providing informed consent, and after an overnight fast, n=7 trained male volunteers (age 29.7 y, VO₂max 4.8 L/min) drank 1.14 g/kg BW of ketone ester (R)-3-hydroxybutyl (R)-3-hydroxybutyrate; KE) and dextrose, or isocaloric carbohydrates (CHO), in a randomised, blinded, crossover design. After 30 min, athletes performed 2 h of bicycle exercise at a fixed intensity of 70% VO₂_max. Blood samples were obtained via an intravenous catheter at regular intervals during
between glycogen use in exercise and IMTAG oxidation (levels. Overall there was a curvilinear reciprocal relationship storage during exercise, and a reduction in muscle glycogen in type II muscle fibre content associated with higher lipid opposite relationship was observed on CHO with an increase p

\[ r < 0.05 \] and commensurate reduction fibre content \( (r < 0.01) \) after 2 h of exercise. There was a direct rela-

\[ \text{relationship between IMTAG oxidation and } \% \text{ of slow type muscle} \]

\[ p < 0.01) \). \text{Results to date suggests that while ACTN3 polymorphism may alter SR Ca}^{2+} \text{ content it does not significantly change SR Ca}^{2+} \text{ content. The Journal of General}

\[ \text{results are not significantly different between conditions at baseline, however IMTAG fell by 24\% on KE vs. 1\% on CHO (p<0.01) after 2 h of exercise. There was a direct rela-

\[ \text{tion between IMTAG oxidation and } \% \text{ of slow type muscle fibre content} \]

\[ r = 0.65, p<0.05 \) and commensurate reduction in glycolysis \( (p<0.05) \) during ketosis. However the direct opposite relationship was observed on CHO with an increase in type II muscle fibre content associated with higher lipid storage during exercise, and a reduction in muscle glycogen levels. Overall there was a curvilinear reciprocal relationship between glycogen use in exercise and IMTAG oxidation \( (r = 0.8 p<0.01) \).

Conclusion

\[ \text{Nutritional ketosis is able to harness the innate metabolic response to starvation, increasing IMTAG oxidation during exercise in the presence of normal muscle glycogen and co-ingested carbohydrate. These data suggest that the metabolic effects of glycogen preservation and increased IMTAG oxidation during ketosis are accentuated in athletes with greater (oxidative) slow twitch muscle fibre content.} \]


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**PCA185**

**Calcium transients in single muscle fibres isolated from the flexor digitorum brevis of $\alpha$-actinin-3-deficient mice**

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Worldwide around 1.5 billion people lack the skeletal muscle fast-twitch fibre protein $\alpha$-actinin-3 due to homozygosity for a common null polymorphism (R577X) in the ACTN3 gene. In the human genome, it is very difficult to find single-gene loss-of-function variants that bear signatures of positive selection, yet intriguingly, the ACTN3 null variant has undergone strong positive selection during recent evolution in humans. We have previously demonstrated Head et al. (2015) that $\alpha$-actinin-3 deficiency in the Actn3 KO mouse results in (i) an increased rate of decay of the Ca$^{2+}$ twitch transient; (ii) a fourfold increase in the rate of SR Ca$^{2+}$ leak; (iii) a threefold increase in the rate of SR Ca$^{2+}$ pumping; and (iv) enhanced maintenance of tetanic Ca$^{2+}$ during fatigue. The SR Ca$^{2+}$ pump, SERCA1, and the Ca$^{2+}$-binding proteins, calsequestrin and sarcalumenin, showed markedly increased expression in muscles of KO mice. Together, these changes in Ca$^{2+}$ handling in the absence of $\alpha$-actinin-3 are consistent with cold acclimatisation and thermogenesis and offer one possible explanation for the positive selection of the ACTN3 577X null allele in humans. Here we investigate amplitude of Ca$^{2+}$ transients, the relationship of Ca$^{2+}$ release to stimulation frequency and releasable SR Ca$^{2+}$ content in single FDB fast-twitch fibres from ACTN3 KO mice. An intracellular microelectrode was used to ionophorese the free acid form of fura-2 or low affinity fura-ff. Fibres were electrically stimulated. In some cases fibres were immobilized with BTS. We utilised a published deconvolution formula to correct peak values of individual twitch Ca$^{2+}$ transients for the high affinity of fura-2 Bakker et al. (1997). Ca$^{2+}$-frequency curves were obtained using stimulation frequencies of 2 to 100 Hz. SR Ca$^{2+}$ content was assessed using the method described in Loy et al. (2011). The SR was completely emptied of Ca$^{2+}$ using a mixture comprised of 10 $\mu$M ionomycin, 30 $\mu$M cyclosporine acid and 100 $\mu$M EGTA. In FDB fibres of $\alpha$-actinin-3-deficient KO mice twitch Ca$^{2+}$ transients displayed a lower peak of Ca$^{2+}$ compared to fibres from WT. Peak Ca$^{2+}$ was 865nM $\pm 22.57$, n=31 fibres in KO and 1580nM $\pm 34.77$, n=36 fibres in WT \( (P<0.0001) \). The rate of SR Ca$^{2+}$ release was higher in KO \( (1.82 \pm 0.19 \text{ ratio units/s in KO, n=6 fibres}, \text{ versus } 1.01 \pm 0.19 \text{ ratio units/s in WT, n=6 fibres}) \). However there does not appear to be a difference in releasable Ca$^{2+}$ \( (445 \pm 101 \text{ ratio units in KO, n=6 fibres}, \text{ versus } 357 \pm 110 \text{ ratio units in WT, n=6 fibres}) \). Ca$^{2+}$-frequency curves showed no significant differences between fibres from KO and WT mice. Results to date suggests that while ACTN3 polymorphism may alter SR Ca$^{2+}$ content it does not significantly change SR Ca$^{2+}$ release parameters.


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*Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.*
Cartilage joint degeneration in the pig and the effect of cytokines on porcine cartilage explant

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Osteoarthritis (OA) is a musculoskeletal degenerative joint disorder and a leading cause of pain and disability\(^(1)\). In attempting to identify drug targets for the development of therapeutics that can prevent the progression of early OA preclinical models are critical.

Currently, rodent preclinical models are used for this purpose. However, OA joint damage in these models is predominantly induced through chemical or surgical means, poorly reflecting human OA. Furthermore, these models provide limited tissue for ex vivo studies. Therefore, in this study we assessed whether the commercial pig might present an alternative model of early OA. To this end, we examined whether cartilage joint degeneration occurred spontaneously in female pigs (chondropathy scoring; 2), and determined the effect of the cytokines IL-1β, leptin, resistin and visfatin (3) on mediating the production of IL-6 (ELISA) and proteoglycan loss on porcine cartilage explant tissue (DMMB sGAG assay). All values represent means ± S.E.M (n=6), compared by one-way ANOVA.

Chondropathy scoring carried out using the Collin’s grading and revised Systeme Francaise D’Arthroscopie (SFA) scoring systems was used to establish the severity of joint damage. Pigs as young as 17 wks show some signs of spontaneous osteoarthritis (Collin’s grade: 2:1.67 ± 0.3073, Revised SFA: 20.15 ± 4.004, n=6), with lesions developing on both the medial and lateral articular surfaces of the knee joint.

Stimulation of porcine cartilage explant for 24 h with recombinant porcine IL-1β (0.1, 0.3, 1, 3 or 10 ng/ml) significantly induced proteoglycan degradation (Control: 238.9 ± 37.16 p=0.0096, 0.1ng/ml: 380.6 ± 33.64 p<0.0001, 0.3ng/ml: 538.3 ± 34.91 p<0.0001, 1ng/ml: 597.2 ± 31.97 p<0.0001, 3ng/ml: 626.2 ± 22.99 p<0.0001, 10ng/ml: 693.8 ± 25.54 p<0.0001) and IL-6 secretion (Control: 238.9 ± 37.16 p=0.0096, 0.1ng/ml: 22020 ± 4650 p<0.0001, 0.3ng/ml: 597.2 ± 31.97 p<0.0001, 1ng/ml: 597.2 ± 31.97 p<0.0001, 3ng/ml: 626.2 ± 22.99 p<0.0001, 10ng/ml: 693.8 ± 25.54 p<0.0001) and IL-6 secretion (Control: 118.1 ± 56.71, 0.1ng/ml: 9068 ± 1563 p=0.7475, 0.3ng/ml: 22020 ± 2922 p<0.0075, 1ng/ml: 46276 ± 7588 p<0.00013ng/ml: 92503 ± 7658 p<0.0001 10ng/ml: 73229 ± 8650 p<0.0001). Similarly, stimulation of porcine cartilage for 24 h with recombinant human visfatin (500ng/ml) induced significant proteoglycan degradation (Control: 238.9 ± 37.16, Visfatin: 366.0 ± 46.05, p=0.0426) and IL-6 secretion (Control: 1296 ± 528.5, Visfatin: 20876 ± 4157, p<0.0001). In both cases, the induction was similar to that observed with human OA cartilage explant tissue. In contrast, we observed no significant effect of either leptin (100 or 300ng/ml) or resistin (30 or 100ng/ml) on either proteoglycan degradation or IL-6 secretion.

In summary, commercial pigs spontaneously develop signs of OA-like cartilage lesions by the age of 17 weeks. Furthermore, stimulation of porcine cartilage explants with either IL-1β or visfatin promotes proteoglycan degradation and IL-6 secretion, similar to that observed in human OA cartilage obtained from joint replacement procedures.

Poster Communications

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"Responder status" for muscle hypertrophy is not predicted by acute anabolic signaling or muscle protein synthesis either before or after 20-weeks resistance exercise training

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Resistance exercise training (RET) is a safe and effective way of enhancing or maintaining muscle mass in catabolic conditions (e.g. ageing, cancer, immobilization etc.). However, the heterogeneity of hypertrophic responses to RET is startling, with coefficients of variation often >100% (1-2). One possible explanation for this could be a [cumulative] blunting of muscle protein synthesis (MPS) after each bout of RE. Yet, there was no correlation between the degree of stimulation of anabolic signals or MPS after the first bout of RE and ensuing hypertrophy in younger individuals exposed to 16 weeks of RET (3). Nonetheless, whether bouts of RE beyond the first (i.e. where responses may be influenced by “muscle damage”) or the age of subjects impact this relationship remains unknown. To test this we recruited 44 individuals aged 18-65y (51±2.7y; body mass index (BMI) 25.4±0.7 kg/m²). All subjects were screened, with exclusion for muscle wasting, metabolic, respiratory or cardiovascular disorders or history of ill health. Subjects were habitually active but did not participate in aerobic exercise and none had participated in RET in the previous 2y. Before and after RET (20-wks, 3x/wk, supervised, whole-body, 70% 1-VM) subjects underwent dual-energy X-ray absorptiometry (DXA; Lunar Prodigy II, GE Medical Systems) to quantify lean leg mass. Stable-isotope tracers (1,2,13C\(_3\) Leucine) (4) were I.V infused to quantify MPS (incorporation into myofibrillar proteins isolated from vastus lateralis biopsies) 2.5h after an acute bout of RE under fed conditions (Fortisip 4.25x basal metabolic rate); immunoblotting was used to quantify the phosphorylation of proteins controlling MPS (5). Data are presented as means±SEM analyzed by ANOVA and Pearson’s correlations with P<0.05 considered significant. The mean cohort increase in lean mass was (5.1±0.8%, P<0.01; range -3 to +27%). However, there were no relationships between hypertrophy and acute increases in MPS before (r=0.30, P=0.1) or after (r=0.01, P=0.7) RET; the same was true for AKT (before r=0.13, P=0.4; after r=0.17, P=0.3) and 4EBP1 phosphorylation (before r=0.05, P=0.8; after r=0.12, P=0.5). Upon splitting the cohort into age groupings of: young (18-40y, n=11), middle-aged (40-60y, n=16) and older (60-80y, n=17), we again failed to identify significant relationships.
Likewise, dividing individuals by "responder status" for hypertrophy yielded no differences across quartiles. We conclude that while 20-wks RET was effective at inducing hypertrophy on average, there was significant heterogeneity unexplained by age or acute MPS/anabolic signaling—either before or after RET. Thus, metabolic and molecular responses to acute exercise are not predictive of hypertrophy and "responder status"; other approaches are needed to predict this.


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PCA188

The role of IL-15 in human skeletal muscle growth and differentiation

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Inflammation-mediated skeletal muscle atrophy is a feature of cachexic clinical conditions such as cancer, chronic obstructive pulmonary disease (COPD) and chronic kidney disease. It is now also considered to be one aetiological feature of the age-related loss of skeletal muscle mass and quality – sarcopenia. Such loss of lean body mass is associated with poor morbidity and mortality outcomes (1). Studies in murine cell lines and in mouse models suggest that IL-15 promotes myogenesis and may protect against inflammation-mediated skeletal muscle atrophy (2-4). However, the effects of IL-15 on human skeletal muscle growth and development remain largely uncharacterised.

In this study, primary human myoblasts were cultured from skeletal muscle biopsies obtained from young (age 18-30), healthy subjects. Differentiating primary human myoblasts were stimulated with human recombinant IL-15 (rIL-15) for 8 d, with or without the addition of 1 ng/mL recombinant TNFα (rTNFα). Differentiated myotubes were immunofluorescence stained for desmin, counterstained with DAPI and imaged on an epifluorescence microscope. Myoblast nuclear fusion and myotube thickness were quantified using ImageJ software. Accompanying changes in myogenic gene expression were quantified by SYBR green RT-qPCR. All data are presented as mean ± SEM. n=3 biological replications (technical replications are detailed in the figure legend) and were analysed by one-way ANOVA with post-hoc Bonferroni correction unless otherwise indicated.

rIL-15 (100 ng/mL) increased the thickness of differentiated myotubes by 22 ± 5 % (p < 0.01 by Mann-Whitney U test with post-hoc Holm’s sequential Bonferroni adjustment), compared to unstimulated control myotubes (see figure). rIL-15 at either 25 or 100 ng/mL also enhanced the nuclear fusion of myoblasts (35 ± 4 %, p < 0.0001; 45 ± 7 %, p < 0.0001), compared to unstimulated controls (see figure). Stimulation of confluent myoblasts with 25 ng/mL rIL-15 induced a small (1.29 fold) but highly significant (p < 0.0001) increase in the expression of myomaker, a cell membrane protein essential for myoblast fusion. rTNFα (1 ng/mL) induced a 30 ± 5 % decrease in myotube thickness (p < 0.0001) compared to an unstimulated control. Co-incubation of differentiating myoblasts with rIL-15 and rTNFα partially reversed this effect, limiting the reduction in myotube thickness to 11 ± 6 % of the control, a significant improvement compared to the rTNFα condition (p = 0.013).

In summary, we have demonstrated that rIL-15 enhances myogenesis and that such stimulation can partially reverse the deleterious effects of rTNFα on human myotube development. IL-15 may be an effective therapeutic target for inflammation-mediated skeletal muscle atrophy.
Platelet dysfunction is associated with lack of repair in patients with gastroduodenal ulcer bleeding

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Background. There is a growing body of data describing molecular mechanisms of healing of peptic ulcers [1, 2]. However mechanisms of recurrent ulcer bleeding (UB) development are still unclear. It was shown that ulcer bleeding is associated with platelet dysfunction and inflammatory reaction affecting thrombogenesis [3, 4]. Despite the fact that platelets are considered as an important moderator of ulcer healing [5], there is no data on relationship between platelets and ulcer healing after bleeding. Aim of this study was to assess platelets’ activity and peptic ulcer healing among patients with bleeding. Methods. 32 patients (males, 56±3 year-old) with gastric and duodenal peptic ulcers complicated with acute bleeding (Forrest II class) were enrolled in the investigation. According to the outcome of UB all patients were subdivided into two groups – with sustained hemostasis (1st group, n=18) and rebleeding (2nd group, n=14). All the recruited patients provided informed consent. Platelet aggregation induced by adenosine diphosphate (ADP, 5 µM) and collagen (1 µM) was measured alone and after co-incubation with inhibitors of protein kinase A and C (PKA and PKC; tolbutamide and neomycin respectively, 200 µM). To assess healing we counted CD31 and Ki-67 positive cells in biopsies of ulcer margin, taken within 24 hours of UB symptoms onset. RESULTS. There were no relationships between platelet aggregation and such factors as gender, age, comorbidity and severity of hemorrhage. Collagen- and ADP-induced aggregation of platelets among patients with UB was significantly lower comparing with control (p=0,01). We fixed the predominance of reversible pattern of the platelet aggregation in 2nd group. Both control group and 1st group demonstrated the decrease of platelet aggregation after inhibition of Pka. However in 2nd group tolbutamide did not change aggregation induced by ADP. Non-selective inhibitor of PKC significantly declined ADP-induced platelet aggregation in 1st group and increased platelets aggregation in 2nd group (p=0,02). However, this did not provide stabilization of ADP-induced platelet aggregation. These changes were accompanied with different repair pattern in 1st and 2nd groups. We found the decline of myofibroblasts number (p<0,001) and angiogenesis (p<0,01) among rebleeders, but there were no differences in Ki-67 positive cells with regard to outcome. Conclusion. Abnormal signalling in platelets among patients with UB was associated with decrease of platelets aggregation and lack of its stabilization. These changes could be caused by alteration of degranulation and lead to un-sustained hemostasis, violation of ulcer healing and rebleeding development. Szabo S et al. (2000). J Physiol Paris 94(2), 77-81. Arakawa T et al. (2012). World J Gastroenterol 18, 4811-4822. Barinov E et al. (2013). Clin Exp Gastroenterol 6, 139-148. Sulaieva O et al. (2015). Pathophysiology 22, 175-182. Wallace JL et al. (2006). Br J Pharmacol. 148: 274–278.

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XB1 promotes luminal breast cancer and resistance to anti-estrogen therapy by transcriptional regulation of NCOA3, an oncogenic nuclear receptor co-activator

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Background: The Cancer Genome Atlas (TCGA) consortium reported that most dominant feature of Luminal/ER-positive breast cancers is increased mRNA and protein levels of ESR1, GATA3, FOXA1, XB1P and MYB. Most notably ESR1 and XB1P were highly expressed and infrequently mutated. XB1P is a multitasking transcription factor and a key component of the unfolded protein response (UPR). Despite the wealth of knowledge about the role of XB1P-S in luminal/ER-positive breast cancer not much is known about the molecular effectors of XB1P-S in context of estrogen signalling.

Nuclear receptor coactivator 3 (NCOA3/SRC-3/AIB1/ACTR/pCIP/RAC3) is a member of p160 family of coactivators. NCOA3 not only functions to promote breast cancer development, it also participates in resistance to anti-hormonal therapy. NCOA3 was found to be overexpressed in >60% of primary breast tumours. Nonetheless, how NCOA3 becomes overexpressed in breast cancers is not well understood. In this study we demonstrate that expression of NCOA3 is regulated by XB1P-S during the conditions of UPR, as well as estrogen stimulation in human breast cancer cells.

Materials and Methods: We have used ER-positive human breast cancer cell lines and tumour samples from breast cancer patients to study the regulation of NCOA3 expression during conditions of ER stress and estrogen signalling. We have used combination of qRT-PCR, western blotting, promoter reporter assays cell proliferation and cell death assays for this work.

Results: We observed increased expression of NCOA3 during conditions of UPR and estrogen (E2) stimulation. Further investigations revealed that XB1P-S regulates the expression of NCOA3 via the XB1P-S binding sites in the promoter of NCOA3 during UPR and estrogen signalling. We identify a novel role for NCOA3 in activation of PERK-ATF4 axis during UPR where knockdown of NCOA3 compromised the optimal activation of PERK-ATF4 pathway. Further we show that NCOA3 is required for induction of XB1P-S upon E2-stimulation but not during the conditions of ER stress. Furthermore upregulated NCOA3 was required for XB1P-S-mediated resistance to anti-hormonal agents. Increased expression of NCOA3 was associated with poor prognosis and higher levels of XB1P-S in breast cancer tissues.

Conclusions: Our results showing the increased expression of NCOA3 during UPR provides a mechanism for overexpression of NCOA3 in human cancers. Our results uncover a novel steroid hormone independent role for NCOA3 in UPR signalling. Further we identify a positive feedback regulatory loop consisting of XB1P and NCOA3 that maintains high levels of NCOA3 and XB1P expression in breast cancer tissues. Taken together our data identify XB1P-NCOA3 axis that regulates cell fate decisions in ER-positive breast cancer cells.

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Matrix metalloproteinase -9 (MMP-9) and disease activity in rheumatoid arthritis patients

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Graphical abstract. Stressful conditions of the tumour microenvironmnet activate an adaptive mechanism called the unfolded protein response (UPR). X-box binding protein-1 (XBP1) is a critical transcriptional activator that is induced by the UPR. In oestrogen receptor-positive (ER+) breast cancer cells XBP1 is rapidly induced in response to E2 stimulation. In luminal breast cancers oestrogen signalling and UPR induce the expression of XBP1s. Here we show that XBP1s upregulate the transcriptional activation of NCOA3 during oestrogen signalling and UPR. NCOA3 is required for the induction of oestrogen-responsive and PERK-ATF4-responsive genes. Our results suggest that XBP1s regulates growth and proliferation of ER-positive breast cancer cells, in part, by transcriptional activation of NCOA3.

IRE1-XBP1 regulates the expression of NCOA3 in breast cancer. Ananya Gupta, Mosaraf Hossain, Mark Webber, Nicola Miller, Grace Callagy and Sanjeev Gupta. Manuscript accepted in Oncogene. 29th February 2016.

Where applicable, the authors confirm that the experiments described here confrom with the Physiological Society ethical requirements.

PCA192

Effects of opioid (tramadol) treatment on testicular functions in adult male rats: The role of nitric oxide and oxidative stress

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Nowadays, tramadol hydrochloride is frequently used as a pain reliever, and for the treatment of premature ejaculation. Decreased semen quality was noted in chronic tramadol users. The present study aimed to elucidate the effects of tramadol on the testicular functions of adult male rats. The methods: A total of 40 albino adult male rats were divided into control and tramadol groups, with 20 rats for each group. Rats of the tramadol group were subcutaneously injected with 40 mg/kg three times per week for 8 weeks. The control group received normal saline 0.9%. Blood samples from each animal were obtained. Then, all animals were killed under anesthesia with sodium pentobarbital. Plasma levels of different biochemical substances were determined. Nitric oxide was measured in testicular tissue samples. Those samples together with epidydimal tissue samples were processed for histopathological examination. Data were expressed as (mean ± SD (standard deviation)) for all parameters. Results: Tramadol significantly reduced plasma levels of luteinizing hormone, follicle-stimulating hormone, testosterone and the expression of specific members of the MMP family, including MMP-3, MMP-8, and MMP-9. However, to the best of our knowledge, there is no combined study that focuses on MMP-9, anti-Cyclic Citrullinated Peptide (anti-CCP), rheumatoid factor immunoglobulin M (RFIgM) and disease activity in RA patients. So, the present study reports the relationship between anti-Cyclic Citrullinated Peptide (anti-CCP), rheumatoid factor immunoglobulin M (RFIgM) and MMP-9, with respect to clinical activity of disease in patients with RA. A total of 85 frozen serum samples, 75 of them belonged to patients with RA, and 10 sample belonged to healthy people (HS), were enrolled prospectively. We used DAS-28 to evaluate disease activity. And then, selected patients were divided into four groups based on their DAS28 scores: remission group (RG), 16 patients (DAS28 < 2.6); low disease activity group (LDAG), 16 patients (DAS28 > 2.6–3.2); moderate disease activity (MDAG), 28 patients (DAS28 > 3.2–5.1); high disease activity group (HDAG), 15 patients (DAS28 > 5.1). The following clinical data gathered from the original patients’ charts. Serum MMP-9 levels and Anti-Cyclic Citrullinated Peptide (anti-CCP) levels were measured using an enzyme linked immunosorbent assay (ELISA; Abbott Diagnostics, USA). Rheumatoid factor (RF) was measured by nephelometry (Beckman Coulter IMMAGE® 800, USA). Our results showed that, patients with RA had significantly higher RF values (P < 0.001) than HS. Anti-CCP levels of RG compared to MDAG were different, but not statistically significant (P = 0.071). Anti-CCP levels were significantly different (P = 0.008) in HS compared to patient groups. Serum levels of MMP-9 in all RA groups were significantly different from HS (P < 0.055). In this regard, MMP-9 and other parameters contribute to pathological condition of RA. Where applicable, the authors confirm that the experiments described here confrom with the Physiological Society ethical requirements.
and total cholesterol, but elevated prolactin and estradiol levels compared with the control group \( p < 0.001 \). In addition, tramadol increased the testicular levels of nitric oxide and lipid peroxidation, and decreased the antioxidant enzymes activities significantly compared with the control group \( p < 0.001 \). The tramadol group showed decreased sperm count and motility, and numbers of primary spermatocytes, rounded spermatid and Leydig cells compared to control group \( p < 0.001 \) Immunohistochemical examinations showed that tramadol increased the expression of endothelial nitric oxide synthase in testicular tissues. Conclusion: The present study showed that tramadol treatment affects the testicular function of adult male rats, and these effects might be through the overproduction of nitric oxide and oxidative stress induced by this drug.

Figure 1: Immunohistochemical staining of sections from rats’ testes (X400). A] A photomicrograph of the seminiferous tubules of a control rat is showing a positive endothelial nitric oxide synthase activity (eNOS) in the cytoplasm of myoid cells (arrow heads). Germinal epithelial cells show no activity with exception of a mild reaction in few primary spermatocytes (arrow).

B] The seminiferous tubules of a tramadol-treated rat are showing a strong positive endothelial nitric oxide synthase activity (eNOS) in the cytoplasm of myoid cells (arrow head), primary spermatocytes (thick arrow), apoptotic cells (thin arrow) and Leydig cells (curved arrow). Bar = 50 \( \mu \)m.

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Background and Aim: Testicular torsion, as a surgical emergency, could affect the endocrine and exocrine testicular functions through oxidative stress and apoptosis that could be reduced by Gingko biloba administration. This study demonstrates histopathological and physiological effects of ischemia/perfusion (I/R) injury to testis, and the possible protective effects of Gingko biloba treatment.

Materials and Methods: Fifty adult male Wistar rats, weighing 180-200 grams were randomly divided into sham-operated group, I/R group and Gingko biloba treated I/R group. Overnight fasted rats were anaesthetized by Pentobarbital (40 mg/kg B.W. i.p.). I/R was performed by left testis 720° rotation, 2 hours of ischemia was induced by a vascular clamp, thereafter, removed for 2 hours of reperfusion in I/R and treated I/R groups. Orchietomy was performed for histopathological studies and detection of mitochondrial NAD⁺. Blood samples were obtained from abdominal aorta and the separated plasma was used for subsequent determination of free testosterone, FSH, TNF-α, IL-1β.

Results: Values are in Mean ± SEM, compared by ANOVA. Plasma free testosterone was significantly decreased in I/R, and ischemia only groups compared to control group (30 ±11.55 vs 142.5 ±29.83, 52.5 ±10.31 vs 142.5 ±29.83, P<0.005, <0.01 respectively), and was significantly increased in Gingko biloba treated I/R group compared to I/R group (113.33 ±34.8 vs 30 ±11.55, P<0.05). Plasma FSH level was significantly elevated in I/R group compared to control group (2.12 ±0.17 vs 1.76 ±0.03, P<0.02). Plasma TNF-α, IL-1β and mitochondrial NAD⁺ were significantly increased in I/R group compared to control group (107.65 ±19.78 vs 35.15 ±0.51, 97.71 ±19.08 vs 59.39±0.53, 16.83±1.53 vs 6.5±1.09, P<0.001, <0.01, <0.001 respectively), and were significantly decreased in ischemia only and Gingko biloba treated I/R groups compared to I/R group (36.38 ±1.4 vs 107.65 ±19.78, 55.88 ±6.22 vs 107.65 ±19.78, 60.57 ±1.17 vs 97.71 ±19.08, 47.04 ±0.07 vs 97.71 ±19.08, 11.29 ±0.92 vs 16.83 ±1.53, 8.67 ±1.58 vs 16.83 ±1.53, P<0.01, <0.001, <0.02, <0.002, <0.01, <0.001 respectively). I/R caused marked testicular damage in addition to significant decrease in both germ and Leydig cells compared to control group, which were reversed by Gingko biloba treatment. Also, there was increased APAF-1 in the apoptotic cells in ischemia only and I/R groups.

Conclusion: I/R caused a state of subfertility induced by apoptosis and oxidative stress that could be reduced by Gingko biloba administration alone.

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Ginkgo biloba ameliorates subfertility induced by testicular ischemia/reperfusion injury in adult Wistar rats: A possible new mitochondrial mechanism

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Kisspeptin increases intracellular calcium concentration by protein kinase C-mediated signaling in cultured rat hippocampal neurons

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Kisspeptins are the natural ligands for the G protein-coupled receptor 54 (GPR54). Although the peptide and its receptor GPR54 are mainly abundant in the hypothalamus and have been suggested to be involved in the onset of puberty and other aspects of the reproductive system, there is much evidence that kisspeptin may also have important effects on hippocampal functions. Our previous study (Ozcan et al, 2011) showed that kisspeptin increases intracellular free Ca²⁺ levels ([Ca²⁺]) via protein kinase C (PKC) activation in GT1 cells. Therefore, in the present experiment the effects of kisspeptin on [Ca²⁺]², has been investigated in the hippocampal neurons to determine whether kisspeptin exerts its effects on hippocampus by the same mechanism. Hippocampal neurons were obtained from the brains of fetuses which were removed on embryonic day 17 from maternal rats under general anesthesia with xylazine (80 mg/kg)/ketamine (12 mg/kg) cocktail sacrificed by decapitation. Hippocampal cells were then dissociated mechanically by trituration through a flame-narrowed Pasteur pipette and cells were plated on laminin-poly-D-lysine-coated coverslips and maintained in Neurobasal medium (Invitrogen) supplemented with 2% B27 (Invitrogen), 1% Glutamax, penicillin (5000 IU/mL), streptomycin (5000 µg/mL). Then hippocampal neurons were maintained at 37 °C in 100% humidity and gassed with 95% air with 5% CO₂ and used 6 hours after plating. The effects of kisspeptin on [Ca²⁺]², in hippocampal neurons were investigated by using in vitro calcium imaging system. Hippocampal neurons were treated with calcium-sensitive dye fura-2/AM ester, and [Ca²⁺]², responses were quantified by the changes in 340/380 ratio. Data are given as mean±SD. Differences between the [Ca²⁺]² levels at baseline and after the doses of 10, 30 and 100 nM kisspeptin were compared by means of one-way analysis of variance (ANOVA) followed by a post-hoc Tukey HSD test. The effects of PKC inhibitor chelerythrine chloride on kisspeptin-induced [Ca²⁺]², transients was evaluated using unpaired Student’s t-test. For all analyses, P<0.05 was accepted as evidence of significance. Kisspeptin (10nM, 30nM and 100nM) caused [Ca²⁺]² transients in hippocampal neurons in a dose dependent manner. Calcium level was increased to 104.4±3.6 (n=31, p<0.01), 111.6±3.9 (n=34, p<0.01) and 141.2±4.1 (n=42, p<0.01) of baseline levels (100%) after application of kisspeptin 10nM, 30nM and 100nM concentrations, respectively. The change [Ca²⁺]² was prevented by pretreating the cells with the PKC inhibitor chelerythrine chloride. These results demonstrate that kisspeptin activates intracellular calcium signaling in hippocampal neurons through PKC-dependent pathway, which suggests new roles of kisspeptin in hippocampal functions.


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Correlation between nuclear envelope circularity and chromatin texture in spleen follicular cells

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It is known that various genes may influence the structure and function of nuclear envelope. However, the exact relationship between chromatin organization on the larger scale, and nuclear shape remains unclear. In our study, on a sample of spleen follicular cells, we demonstrate that parameters of chromatin texture are correlated with circularity of nuclear membrane.

Spleen tissue was obtained from 10 male healthy Swiss albino mice. The experiment was a part of study for which the approval had been obtained from the Ethical commission of University of Belgrade, Faculty of medicine. All procedures accorded with current national legislation/guidelines. Spleen tissue was fixed in Carnoy’s solution and embedded in paraffin. Tissue sections were stained using the modification of Giemsa method for chromatin visualization as described in previously published research. A total of 200 follicular cell nuclei (20 per animal) were analyzed using National Institutes of Health (NIH, Bethesda, MD) ImageJ software and its plugins. Circularity of nuclear envelope was calculated based on the nuclear area and perimeter. Textural parameters, such as entropy (measure of textural chaos and disorder), angular second moment (indirect indicator of textural uniformity) and textural contrast were calculated using the Gray level co-occurrence matrix (GLCM) mathematical algorithm.

Average entropy of chromatin was 5.12±0.18. Mean values of GLCM angular second moment and GLCM contrast were 0.051±0.007 and 391.5±31.1, respectively. Average circularity of the nuclear envelope was 0.93±0.01. There was a statistically highly significant (p<0.01) negative correlation between the circularity and angular second moment. No such correlation (p>0.05) was found between circularity and entropy or circularity and textural contrast.

To the best of our knowledge, this is the first study to test the relationship between nuclear shape and chromatin texture in spleen follicular cells. Also, this is one of the first applications of Gray level co-occurrence matrix algorithm in chromatin structural analysis in spleen tissue. The results could be explained by physiological processes taking place in nuclear lamina that influence chromatin structure and distribution.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

The attenuation of cell proliferative effect of Leontice leontopetalum extracts on diabetes-induced pancreatic beta cell line

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Aim: Type-1 which is named as insulin-dependent diabetes is tried recovery the pathologic effect and complication related with diabetes by drugs. However, the synthetic drugs have been reported to have side effects on the body (1). One of the alternative therapeutic methods is herbal medicine. Leontice leontopetalum (LL) belongs to Berberidaceae, and its extract has been shown to have antiepileptic and anti-spasm (2). However, there are no studies on the effect of LL on diabetes. The aim of the study was to investigate the proliferative effect of LL’s extract on β-cell-treated with STZ. Methods: Human pancreatic beta cell (1.1B4) line was used the current study. LL’s extracts (1, 10, 100, and 1000 µg/ml) were supplemented in media for 24 hours and/or after STZ treatment (20 mM). Therefore, totally four groups were created as Control, STZ, L. leontopetalum’s extract and STZ+LL. Cells proliferation were shown by using xCelligence. Insulin content was measured at 1.1, 8.4 and 16.7 mM glucose concentrations. Results: All LL’s concentration caused to decrease cell proliferation. However, 20 mM STZ significantly affected by cell proliferation. STZ+LL group also gave rise to decline cell proliferation. STZ and STZ+LL extract led to decrease insulin content at all glucose concentrations. Conclusion: STZ caused diabetes by decreasing of insulin content resulting from decline cell proliferation. Unfortunately, L. leontopetalum has no protective effect on diabetes. The project was financially supported by the Research Foundation of Adiyaman University (TIFBAP/2014-0006).

Keywords: Diabetes, Leontice leontopetalum, Insulin, Cell Proliferation


The project was financially supported by the Research Foundation of Adiyaman University (TIFBAP/2014-0006).

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The effects of tumor necrosis factor alpha inhibition on apoptotic cell death in β-cell with type-1 diabetes-induced by streptozotocin

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Background: Patients with rheumatoid arthritis (RA) suffer from co-morbidities because inflammation is important for the development of some disease, for example, diabetes mellitus (1). Type 1 diabetes is one of the most common chronic autoimmune diseases characterized by loss of insulin-producing beta cells (2). Tumor necrosis factor alpha (TNF-α) promotes the inflammation response and apoptotic cell death. The approach of targeting TNF-α has considerably improved the success in the treatment of RA (3). TNF inhibitors may improve the glycemic control in patients with concomitant RA and diabetes mellitus (4) Objectives: The aim of the study was to investigate whether TNF-α inhibition by adalimumab, etanercept or golimumab can be achieved to modulate beta cell loss by apoptosis or not.

Methods: The current study was used to human β-cell line. Total 5 groups were created as control (C) Diabetes (D), Diabetes+Adalimumab (10 µg/ml; DA), Diabetes+Etanercept (5 µg/ml; DE), Diabetes+Golimumab (10 µg/ml; DG). After diabetes had been created by using streptozotocin (20 mM), TNF-α inhibitors were incubated for twenty-four hours. Protein was isolated to measure some apoptotic proteins for Western blot analysis.

Results: Diabetes gave rise to increasing energetic stress by diminishing AMP-kinase (AMPK) protein level and initiate to apoptosis by alteration of p53 protein level, resulting in Smac/DIABLO efflux from mitochondria to cytosol that occurs downstream of cytochrome c release. Pharmacological inhibition of TNF-α could modulate the energetic stress in a beta cell by increasing AMPK protein level in DA, DE, DG groups. Hence, all TNF-α inhibition groups restored to cell loss through apoptosis by rising of p53 protein level compared to the diabetic group.

Conclusion: Although type-1 diabetes leads to losing β-cell by apoptosis, resulting from inflammation response, TNF-α inhibitions might prevent to β-cell by decreasing apoptotic proteins.

Keywords: Adalimumab, Apoptosis, Diabetes, Etanercept, Golimumab, TNF-α, Inflammation

Intermittent dietary restriction caused reduction in lipid ratios in young healthy adult Nigerians

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Nonpharmacologic therapy is a key strategy in the management of dyslipidemias. Lipid ratios that identify the pro-atherogenic and anti-atherogenic fractions perform better in predicting cardiovascular risk than individual lipid levels [1,2]. There is a dearth of studies on effect of dietary restriction on these ratios in healthy adult Nigerians. This study was designed to evaluate the effect of intermittent dietary restriction on lipid ratios in apparently healthy young adults using the Ramadan model [3]. Ethical approval was obtained from Lagos State University College of Medicine Research and Ethics Committee. Written informed consent was obtained from all the volunteers. Experiments were carried out one week before (1BF) and in the 4th week of, the Ramadan fast (4WF). After obtaining subjects’ height (m) and baseline body weight (kg), venous blood was collected about 12 hours after the early morning meal at 1BF. Subjects then had calorie and fluid restriction from sunrise to sunset [2] during the 30 days of Ramadan fasting. Repeat body weight measurement and collection of venous blood were carried out during the 4th week (4WF). The blood was analyzed for total cholesterol (TC), triglyceride cholesterol (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). Non-HDL cholesterol was calculated as the difference between TC and HDL-C. Body Mass Index (BMI) was calculated as weight/height² (kg/m²). Values are presented as mean ± S.E.M. Paired Student t test was used to compare within group while Pearson correlation was used to determine relationship between BMI and the lipid ratios. Differences were accepted as significant at 95% confidence interval. Fifty healthy adults (19 females and 31 males), mean age 23.30 ± 0.7y participated in the study. Mean BMI reduced significantly from 22.87 ± 0.44 kg/m² (1BF) to 22.23 ± 0.45 kg/m² (4WF), p < 0.0001. All lipid ratios were significantly reduced as follows: total cholesterol: high density lipoprotein (TC/HDL-C) from 4.17 ± 0.10 (1BF) to 3.54 ± 0.10 (4WF), p < 0.0001; triglyceride cholesterol/high density lipoprotein (TG/HDL-C) from 0.88 ± 0.06 (1BF) to 0.7 ± 0.01 (4WF), p = 0.002; low density lipoprotein/high density lipoprotein (LDL-C/HDL-C) from 2.77 ± 0.1 (1BF) to 2.22 ± 0.1 (4WF), p < 0.0001. There was no significant correlation between BMI and any of the lipid ratios; however, BMI was significantly correlated with Total cholesterol: Total cholesterol/HDL-C; r = -0.31, p = 0.03. We conclude that 4-weeks’ intermittent dietary restriction reduced BMI and lipid ratios and may be a useful nonpharmacologic therapy in the management of dyslipidemias and thus the control of cardiometabolic diseases.
Elevated plasma concentrations of soluble (pro)renin receptor in patients with obstructive sleep apnea syndrome

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(Pro)renin receptor ((P)RR) is a receptor for renin and prorenin (Nguyen et al., 2002). (P)RR is implicated in the pathophysiology of diabetes mellitus, hypertension and their complications (Nguyen 2011). Soluble (P)RR (s(P)RR) composed of extracellular domain of (P)RR is generated from (P)RR by furin, and exists in blood (Cousin et al. 2009). We have for the first time reported that plasma concentrations of s(P)RR were elevated in male patients with obstructive sleep apnea syndrome (OSAS) (Nishijima et al. 2014). The aim of the present study was to clarify the difference in plasma s(P)RR concentrations between male and female OSAS patients. Plasma s(P)RR concentrations were studied in 289 subjects (206 males and 83 females) consisting of 259 OSAS patients and 30 non-OSAS control subjects. The 259 OSAS patients were classified into mild (5% apnea hypopnea index (AHI) < 15 events/h), moderate (15% AHI < 30), and severe OSAS (AHI ≥ 30). Plasma s(P)RR levels were significantly elevated in all three OSAS groups compared to non-OSAS control subjects in the entire cohort and male subjects, whereas in female subjects, the significant elevation was found only in severe OSAS. Soluble (P)RR levels were significantly correlated with AHI in both sexes, with a higher r value found in male subjects (male r = 0.413, p < 0.0001; female r = 0.263, p < 0.05). The OSAS patients with type 2 diabetes mellitus or chronic kidney disease showed higher plasma s(P)RR levels in both sexes, particularly female OSAS patients with both these comorbidities (diabetic nephropathy). By contrast, the presence of hypertension had negligible effects on plasma s(P)RR levels in OSAS patients of both sexes. When 41 OSAS patients (26 males and 15 females) with AHI ≥ 20 were treated by continuous positive airway pressure treatment, plasma s(P)RR levels were significantly decreased. In conclusion, plasma s(P)RR levels were elevated in OSAS patients of both sexes, and a higher association with the disease severity was found in male OSAS patients.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA200

Association between thyroid dysfunction and metabolic Syndrome and role of high sensitivity C-reactive protein

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Thyroid disease and the metabolic syndrome (MetS) are both associated with cardiovascular disease. Therefore we explore the association between thyroid dysfunction and metabolic syndrome and role of high sensitivity C-reactive protein (hs-CRP). This cross-sectional study included 200 subjects with metabolic syndrome in the study group and 100 subjects without metabolic syndrome in the control group. Participants were examined at Saurashtra medical centre, India between 2011 and 2014. Metabolic syndrome was assessed according to the National cholesterol Education Program’s-Adult Treatment Panel III Criteria. Triiodothyronine (T3), Thyroxine (T4), and Thyroid stimulating hormone (TSH) were estimated by the electrochem-iluminescence immune assay. (TSH >4.2–10 µIU/ml and normal T3 and T4 were subclinical hypothyroidism, TSH >10 µIU/ml and low T3 and T4, clinical hypothyroid and TSH >4.2–10 µIU/ml and normal thyroid hormone, Subclinical hyperthyroidism). Fasting glucose, insulin, lipid profile, and serum high sensitivity C-reactive proteins levels were measured and homeostatic model assessment was used to assess insulin resistance. The Association between metabolic syndrome and thyroid dysfunctions were examined using multiple leaner regression model analysis after adjusting potential cofounders. The overall prevalence of thyroid dysfunction in patients with MetS was 41.5% with high prevalence of sub clinical hypothyroidism (27%). TSH was significantly higher in the study group than in control group (P <0.001). T3 and T4 values of study group were significantly lower than those of control group (P< 0.01). There were significant differences in the all components of metabolic syndrome (P <0.05), Insulin resistance (P <0.01) and hs-CRP (P <0.001) between four subgroups of thyroid dysfunction. TSH levels were significant positively independent associated to high sensitivity of C-reactive protein (β =.370; P<0.01), waist circumference (β =.289; P<0.05) and systolic blood pressure (β=.52; P<0.01), while T4 levels were significant negatively associated to systolic blood pressure (β =.284; P<0.05) in sub clinical hypothyroidism. TSH levels were significant positively associated to hs-CRP (β=.602; P<0.01) and triglycerides (β=.540; P<0.05) but significant negatively associated with insulin resistance (β=-.546; P<0.05). T3 levels were significant negatively associated with waist circumference (β=.496; P<0.05), fasting blood glucose (β=-.651; P<0.05) and insulin resistance (β=-.741; P<0.05) in clinical hypothyroidism. It is concluded that hypothyroidism is associated with components of metabolic syndrome and systemic inflammation therefore increased compound risk of cardiovascular diseases. Key words: Metabolic syndrome, thyroid stimulating hormone, high sensitivity C-reactive protein, Insulin resistance, Thyroid dysfunction.


Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA 2001; 285:2456

163P
Introduction: The incretin hormone glucagon like peptide-1 (GLP-1) is secreted from the L-cells of the colon in response to short chain free fatty acids (SCFA) which are generated by the fermentation of non-digestible carbohydrates by the gut microbiota. This effect is mediated at least in part by signalling through the free fatty acid receptor 2 (FFA2) (1). FFA2 is reported to couple to both G<sub>i</sub> and G<sub>q</sub> (2). This study aims to determine the signalling pathways which regulate GLP-1 secretion in the L-cells of the colon.

Methods: Colon from adult C57BL6/NTac mice were digested with collagenase into cell structures resembling colonic crypts as previously described (3). After overnight incubation, the crypts were incubated for two hours in physiological saline, containing 1 mM acetate, 1 mM propionate, or 1 mM butyrate, in the presence or absence of 100 nM PTX (overnight) or 100 nM FR900359 (30 min). Active GLP-1 was assayed by ELISA in the culture supernatant and cell lysates. Amount released (GLP-1) was expressed as a percentage of total GLP-1 content and was normalised to baseline secretion per mouse. Data are means ± SEM, statistical analyses are one-way or two-way ANOVA with normalised to baseline secretion per mouse. Data are means ± SEM, statistical analyses are one-way or two-way ANOVA with normalised to baseline secretion per mouse. Data are means ± SEM, statistical analyses are one-way or two-way ANOVA with normalised to baseline secretion per mouse. Data are means ± SEM, statistical analyses are one-way or two-way ANOVA with normalised to baseline secretion per mouse. Data are means ± SEM, statistical analyses are one-way or two-way ANOVA with normalised to baseline secretion per mouse. Data are means ± SEM, statistical analyses are one-way or two-way ANOVA with normalised to baseline secretion per mouse.

Results: Propionate significantly increased GLP-1 secretion, while acetate and butyrate showed similar trends. Propionate-elicited secretion was unaffected by concomitant PTX incubation, but was abolished by the G<sub>q</sub> inhibitor FR900359 (see table 1). In colonic crypts from Ffar2<sup>-/-</sup> mice, no increases in GLP-1 secretion were observed with acetate, butyrate or propionate. Conclusions: GLP-1 secretion in response to SCFAs appears to be downstream of FFAR2 coupling to G<sub>q</sub>, whereas G<sub>i</sub> appears not to be involved. Potentially, FFA2 may be entirely responsible for GLP-1 secretion, rather than FFAR3, which is believed to be expressed on the same cells. This study is the first time the new G<sub>q</sub> inhibitor FR900359 has been used to demonstrate the involvement of G<sub>q</sub> downstream of FFAR2, or indeed any GPCR in the L-cell.

Table 1. Fold increases in GLP-1 secretion over baseline (n=3).

<table>
<thead>
<tr>
<th>SCFA</th>
<th>Baseline</th>
<th>PTX</th>
<th>FR900359</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>1.47 ± 0.04</td>
<td>1.15 ± 0.09</td>
<td>1.47 ± 0.35</td>
</tr>
<tr>
<td>Propionate</td>
<td>2.817 ± 0.51</td>
<td>1.15 ± 0.16</td>
<td>2.00 ± 0.34</td>
</tr>
<tr>
<td>Butyrate</td>
<td>2.147 ± 0.50</td>
<td>1.19 ± 0.52</td>
<td>1.85 ± 0.40</td>
</tr>
</tbody>
</table>

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Garlic oil improves small intestinal motility in experimentally induced type II diabetes mellitus in Wistar rats

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Background and Aim: Diabetes mellitus impairs small intestinal motility. This study evaluated the beneficial effects of garlic oil.

Materials and Methods: Thirty six adult Wistar rats were allocated into: control (C), garlic oil supplemented non-diabetic (GO), diabetic (D), and garlic oil treated diabetic (DG) groups. At the end of the study, all rats were anaesthetized by i.p. Pentobarbitone (40 mg/kg B.W). Small intestinal pieces were used for motility parameters study and oxidative markers measurement. Nasoanal length, waist circumference, fasting blood glucose level (FBG), plasma insulin level were measured.

Results: Compared to C group, D group showed decreased jejunal average duration, average force of contraction and motility index in all pieces, and increased Lee index, waist circumference, FBG, HOMA-IR, malondialdehyde (MDA) level and catalase (CAT) activity of all pieces. GO group showed decreased average force of contraction and motility index in all pieces, and elevated Lee index and waist circumference. DG group showed decreased jejunal and ileal average force of contraction and ileal motility index, and elevated Lee index, waist circumference and FBG. When compared to the D group, the DG group showed increased average duration of contraction, average force of contraction and motility index in all pieces, and decreased duodenal and ileal MDA level, jejunal and in ileal CAT activity.

Conclusion: Decreased small intestinal motility occurs in DM, mostly by oxidative stress, and in normal rats supplemented with garlic oil. However, garlic oil treatment in DM results in an improvement in small intestinal motility and in a remarkable anti-hyperglycemic effect.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Episensors to monitor human epigenome changes due to modulations of the SAM’s pathway

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Translation of academic research into clinical practice is dependent on tools and strategies that allow the understanding of an extremely complex system. The liver has a unique role in the regulation and maintenance of mammalian metabolism (Finkelstein, 1998) and as a consequence a balanced state of the epigenome. Our group aims to facilitate the understanding of the modulations of the S-Adenosylmethionine metabolic pathway (SAM) in humans that in turn regulate modulations of the epigenome. Extensive published research reveals, a common etiology in metabolic disorders is a dysregulatory state of insulin secretion, adipokine signalling and a subsequent dysregulatory state of the epigenome.

This in vivo and in vitro study is compliant with ethics review, as an IRB approved clinical trial (GHS # 1207-27). Subjects enrolled were patients scheduled to undergo metabolic surgery for weight loss; all donors (n=43, and 42 controls) signed informed consent. This dual model includes 1) a clinical precision medicine model and 2) liver in vitro model, based on hepatogenesis of mesenchymal stem cells (MSCs) (Stock et al., 2010) isolated from the same donors were used to profile the episensor. Multiple in vitro cell model platforms are being tested (Eshc et al., 2011). Expression profiles of a combination of proprietary transcripts followed the MIQE guidelines (referred here as episensors), Plasma metabolites and the episensors were assessed in human plasma, in the case of the in vitro model surrogate blood was used. The patients (n=43) that had metabolic surgery lost an average of 47±12 % excess BMI after 12 weeks. All patients in this cohort resolved type 2 diabetes, however not all of them resolved insulin insensitivity, adipokine, and epigenetic dysregulatory states. Our data suggest the dysregulatory state can be assessed with the episensors, Furthermore, physical activity is a major contributor to the modulations of the SAM’s pathway. This research suggests the episensors are an extraordinary tool for monitoring changes in the SAM’s pathway in vivo and/or in vitro, to investigate the etiology, prevention or treatment of metabolic disorders associated with dysregulatory states of the epigenome.

The work described was supported by 1) the Cornell Center on the Microenvironment & Metastasis through Award Number U54CA143876 from the National Cancer Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health. 2) The Guthrie Foundation 3) epiWELL, LLC

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Regulation of oxytocin neurons in the hypothalamic supraoptic nucleus by palatable food gavage in the rat

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Alongside its classical physiological roles in maternal behaviour, oxytocin is now considered to be involved in the homeostatic control of food intake [1]. Its anorexigenic effects are thought to be evoked by peripheral satiety signals, including cholecystokinin and gastric distention [2]. Using c-Fos like immunoreactivity and in vivo electrophysiology, we have developed a novel in vivo gut-brain signalling model to study the activity of oxytocin-releasing SON neurons in response to a palatable food delivered directly into the stomach. Adult male Sprague-Dawley rats were fasted overnight, anaesthetised (pentobarbital, 60mg/kg; ip), tracheotomised and the femoral vein cannulated (for anaesthesia maintenance (18.6mg/kg/hr) and blood sampling). A gavage tube was inserted orally into
the stomach and 5ml of sweetened condensed milk (SCM) was infused (n=8). Blood was sampled and plasma collected before, during, and after gavage. Precisely 1h after gavage, rats were perfused-fixed and the brains processed for c-Fos- and oxytocin-like immunoreactivity. Two control groups were subjected to a sham gavage (n=8) and gastric distention (inflated balloon attached to gavage tube; n=9). For in vivo electrophysiology, the ventral surface of the brain was exposed by transtherapeutical surgery and a microelectrode inserted into the SON. Oxytocin cells were identified by their excitatory response to iv cholecystokinin (n=9). Extracellular recordings were made continuously during SCM gavage. After SCM gavage there was an increase in c-Fos expression in SON recordings in comparison to sham gavage and gastric distention (p=0.04 and p=0.02 respectively, one-way ANOVA). There was no difference in c-Fos expression in SON oxytocin neurons between sham gavage and gastric distention (p=0.9, t-test). Identified oxytocin SON neurons showed a progressive increase in firing rate starting ~10 min after gavage onset (p<0.05, Kruskal-Wallis test (p=0.002)). Plasma osmolarity did not change before, during or after SCM gavage (p=0.12, two-way ANOVA). SCM gavage results in increased c-Fos-like immunoreactivity and a sustained increase in the firing rate of SON oxytocin neurons. This increase in SON neuron activity does not appear to be due to a change in osmolarity. Instead, SON oxytocin neurons may be regulated by satiety-related peripheral signals released in response to food in the stomach, providing further evidence for the role of oxytocin in the homeostatic control of food intake. 


This research has received Funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreements 607310 (Nudge-it) and 245009 (NeuroFAST).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA207

Effects of whole body vibration on breast cancer bone metastasis and vascularization in mice

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Breast cancer has a propensity to metastasize to bone, leading to osteolytic bone destruction and, in some cases, abnormal new bone formation. Bone destruction in turn promotes the release of tumor growth factors and contributes to a vicious cycle of bone metastasis. In contrast, whole body vibration (WBV) downregulates the activity of osteoclastic bone resorption, possibly inhibiting bone metastasis. This therapeutic potential of WBV, however, could be counteracted by its proangiogenic effect, which may provide a more fertile environment for bone metastasis. Using a mouse model of breast cancer bone metastasis, we evaluated the effects of WBV on cancer-induced osteolytic/osteoblastic changes and vascularization. Female Balb/c 6-week-old mice received intratibial injection of 4T1 breast cancer cells (5x10⁴ cells) in the right leg under anesthesia with isoflurane inhalation. The mice were randomly assigned to four groups (n=10 each): C2, W2, C4, and W4. Starting on the postoperative day 3, mice in W2 and W4 were exposed to WBV (0.3g at 90Hz) for 20min every day, and mice in C2 and C4 received a non-vibrated sham treatment. After 2 weeks of treatment, mice in C2 and W2 were thoracotomized under isoflurane anesthesia and perfused with zirconia-based vascular contrast-casting agent (Zr-CA) from the left ventricle. The animals were then euthanized by pentobarbital overdose (i.p.) and immersed in ice-cold water for solidifying Zr-CA. Mice in C4 and W4 were subjected to vascular casting similarly after 4 weeks of treatment. The proximal metaphyseal region was CT-scanned with synchrotron X-rays below and above the zirconia k-edge (SPRing-8, Harima, Japan). Using k-edge subtraction, vascular and bone images were obtained separately (3.95-μm voxel resolution). Newly formed bone (new-B) were differentiated from pre-existent bone (pre-B) based on the degree of mineralization. A volume extending a distance of 2 mm distal to the growth plate defined the region for analysis. Values are means ± S.D., compared by Mann-Whitney U test. The volumes of pre-B and new-B were similar between W2 and C2 (pre-B: 0.38±0.12 vs. 0.40±0.14 mm³; new-B: 1.00±0.33 vs. 1.03±0.25 mm³). The pre-B volume decreased more in W4 than in C4 (0.10±0.06 vs. 0.17±0.08 mm³, p<0.05), while the new-B volume was similar between W4 and C4 (0.39±0.20 vs. 0.48±0.22 mm³). The vascular volume within 300 mm from pre-B regions was similar between W2 and C2 (0.0986±0.0157 and 0.0971±0.0302 mm³), which was lowered less in W4 than in C4 (0.0078±0.0025 vs. 0.0032±0.0067 mm³, p<0.05). The pooled data of W4 and C4 showed a negative correlation between the volumes of pre-B and its nearby vessels (p<0.05). These data suggest that WBV may reduce tumor vascular degeneration, thereby enhancing osteolytic bone destruction.

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PCA208

Properties of voltage-gated sodium channels in pancreatic β-cells

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A characteristic feature of voltage-gated Na⁺ (Nav) channels is their voltage-dependent activation and inactivation. The range of membrane potentials in which a channel undergoes activation and inactivation can dramatically effect cell excitability and important cellular processes such as secretion. Nav channels in pancreatic β-cells undergo inactivation at very negative membrane potentials, rendering most Nav channels inactive and thus unable to contribute to the electrical activity of the cell (Plant, 1988). β-cells express Nav1.3, Nav1.6 and Nav1.7. We hypothesised that these channels have different properties in β-cells compared to other cell types. The aim of the project was to characterise these channels in a β- and non-β-cell model and to identify potential modulators of Nav channel properties in β-cells.

The channels were transfected into a rat insulinoma cell line (INS1-832) and a human embryonic kidney (HEK) cell line to model a β- and non-β-cell environment, respectively. Using
whole cell patch clamp the activation and inactivation properties of the Na_\alpha channel were measured. Statistical significance was calculated using unpaired Student’s t test. Half-maximal inactivation (V_{0.5}) of Na\textsubscript{1.7}, Na\textsubscript{1.6} and Na\textsubscript{1.3} expressed in HEK cells was observed at -58 + 2 mV (n=5) and -51 + 3 mV (n=5) and -48 + 2 mV (n=5), respectively. However when expressed in INS1-832 cells the V_{0.5} of Na\textsubscript{1.7}, Na\textsubscript{1.6} and Na\textsubscript{1.3} displayed a significant hyperpolarising shift to -93 + 2 mV (p<0.001, n=8), -76 + 1 mV (p<0.001, n=17) and -86 + 2 mV (p<0.01, n=13), respectively. This effect could also be observed in Na\textsubscript{1.3} channels that are not usually expressed in ß-cells. For example the cardiac ß-subunit Na\textsubscript{1.5} also produced a significant hyperpolarising shift in V_{0.5} from -106 + 2 mV (n=9) to -77 + 2 mV (p<0.001, n=5), when expressed in INS1-832 and HEK cells, respectively. The shift in V_{0.5} of all the channels occurred without a significant change in the activation properties of the channel. 

Receptor tyrosine kinases (RTKs) have been previously reported to modulate Na\textsubscript{1.3} channel currents (Fanger et al. 1995). The insulin receptor is a RTK and it was hypothesised that insulin secreted from ß-cells may act via autocrine signalling to modulate Na\textsubscript{1.3} channel inactivation. However application of an insulin receptor antagonist had no effect. Furthermore modulation of downstream insulin receptor signalling molecules such as PIP\textsubscript{2}, which is known to alter the gating properties of the ATP-sensitive potassium channel, also did not affect Na\textsubscript{1.3} channel modulation. The results suggest a ß-cell-specific modulation of Na\textsubscript{1.3} inactivation properties. Identification of this cell specific modulator warrants further investigation as it alters the inactivation property of a broad spectrum of Na\textsubscript{1.3} channels and from a ß-cell perspective recruitment of the channels could provide a novel mechanism for increasing insulin secretion.


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**Poster Communications**

**PCA209**

**Novel method to study circadian regulation of insulin secretion in vitro**

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Insulin secretion from pancreatic islets is tightly controlled by a complex mechanism. There is an emerging evidence for the role of circadian system in these regulations. Circadian system in mammals consists of central light-entrainable oscillator located in the brain in suprachiasmatic nuclei (SCN) and peripheral oscillators in other cells in the body. Peripheral oscillators are entrained by the SCN and also by other cues (e.g. timing of food intake). The clock mechanism is based on molecular circuits involving so called clock genes, namely Bmal1, Clock, Per1, Per2 and Cry. Using ex vivo approach, it is possible to study intrinsic properties of peripheral oscillators in conditions when they are not under influence of the entraining signals. This method allows us to study mechanism of how circadian clock in pancreas contributes to regulation of insulin secretion in vitro. Long-term measurement of biological rhythmicity is essential for analysis of circadian rhythms which cannot be assessed based only on data from one cycle.

Therefore, we propose a novel method to study islets in organotypic explants from pancreas, because the currently available systems using isolated islets of Langerhans are not well suited for this prolonged incubation.

We prepared organotypic explants from pancreas of transgenic mice expressing clock gene Per2 fused with gene for luciferase enabling us to detect bioluminescence rhythms of the pancreatic clock. Pancreatic explants exhibited robust rhythms in Per2 production for more than 10 days. Islets within the organotypic explants were subjected to repeated stimulation by higher glucose concentrations in media to test their viability. Insulin content was then measured by ELISA. After 3 days of incubation in low glucose (5mM), the insulin production was steady (up to 0.4ng/ml per day). Upon the stimulation with higher glucose (20mM), the islets within pancreatic explants exhibited higher insulin excretion rate (up to 1ng/ml per day). We can estimate the number of viable islets from the amount of excreted insulin into media. We were able to detect roughly 2 medium sized islets. The stimulations by higher glucose content in media were repeated at least twice, interrupted with low glucose (3mM), and insulin content changed accordingly. The results provided evidence for viability of the islets within the explants.

The results allow us to propose a novel experimental approach for studying of long-term insulin secretion in pancreatic organotypic explants in vitro and evaluation of internal clock functions. Additionally, this novel method could be used in future for testing of drugs influencing the insulin excretion.

Supported by GAUK grant no. 198215

*Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.*

**PCA210**

**Carbon monoxide acts differently on 24-hour and 48-hour dehydration-induced hormone secretion**

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In the last three decades, evidences of the carbon monoxide (CO) gaseous molecule modulation on neuroendocrine and other homeostatic systems have been reported. Data from literature have indicated CO as a fine modulator of neuroendocrine response to challenges which disrupt hydrosaline balance such as water deprivation (WD) and salt loading. The aim of this present study is to evaluate whether central CO formation blockage or central CO donation in progressive WD leads to different patterns of hormonal secretion. To that, 250g Wistar male rats were submitted to a prior right lateral ventricle cannula implantation under ketamine-xylazine anaesthesia (60 mg e 8 mg/100 g of body weight, i.p., respectively; Coletti et al., 2015). After 7 days, the animals were submitted to 24-hour or 48-hour dehydration with free access to chow, in the experiment day they were intracerebroventricularly injected (4 µl) with CO formation inhibitor (ZnDPBG, 50 mM) or vehicle (Na\textsubscript{2}CO\textsubscript{3}, 50 mM), or CO donor (CORM-3, 100 µM) or vehicle (CORM-3\textsubscript{Inactivated}, 100 µM) then were euthanized by decapitation after 30 min and trunk blood collected. We observed that, in basal condition (euhydrationed animals), the central CO formation inhibition increased only corticosterone (CORT) plasma concentration (F(2, 33) = 6.787, p<0.01;
of Kiev, Kiev, Ukraine and 2Institute of Physiology named after P. Verhovetsky in Kiev, Ukraine.

The role of the central dopaminergic system and D2-dopaminergic receptor in the pathogenesis of experimental ulcerative colitis was studied. The authors confirmed that the experiments described here conform with the Physiological Society ethical requirements.

Role of central dopaminergic system and D2-dopaminergic receptor in the pathogenesis of experimental ulcerative colitis in rats

K. Nesteruk1, A. Prysiazhniuk1, T. Dovbnychuk1, B. Kopiak2, T. Chervinska1, S. Talanov2 and G. Tolstanova1

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Ulcerative colitis (UC) is a chronic inflammatory disease with poorly understood etiology. Others and we showed disturbance of dopaminergic system in UC pathogenesis. Treatment with D2 receptor (D2R) agonist, which are able to cross blood brain barrier, ameliorated clinical and morphological signs of experimental UC. In present study, we tested the hypothesis on the protective role of central vs peripheral dopaminergic system in UC pathogenesis.

Study was done on male Wistar rats (200-250g, n=35). The unilateral nigrostriatal lesion was caused by intranigral injection of 6-hydroxydopamine (6-OHDA). The area of destroyed central dopaminergic neurons were estimated by apomorphine test: <180 turns/30 min – 44% of destroyed central dopaminergic neurons; >180 turns/30 min – 95% (2). Experimental UC was induced by 0.1 ml of 6% iodoacetamide enema. Level of colonic endothelial permeability by Evans blue extravasation were measured after treatment with central quinpirole D2R agonist (10 mg/kg, per os) and domperidone, peripheral D2R antagonist (20 mg/kg, per os). These procedures were done under Nembutal anesthesia (50 mg/kg, i.p.). Disease activity index was calculated by estimation of the lethargy, diarrhea, and weight loss. The myeloperoxidase activity, proteins level by Western blots were detected in colonic mucosa. Rats were euthanized by CO2 inhalation followed by cervical dislocation.

The disturbance of central dopaminergic neurons by 6-OHDA administration increased disease activity index 1.5-fold (p<0.05); the ratio of colon wet weight/100g body weight 2-fold (p<0.05); and myeloperoxidase activity 2.5-fold (p<0.05) in rats with UC vs sham rats with UC. These effects positively correlated with area of destroyed central dopaminergic neurons. Basal level of colonic endothelial permeability was equal in sham and 6-OHDA rats. Development of iodoacetamide-induced inflammation was associated with 1.4-fold (p<0.05); higher levels of colonic endothelial permeability in 6-OHDA vs sham rats. It was accompanied by upregulation of phosphorylated caveolin-1 (Tyr 14) (1.4-folds) and ICAM-1 (5.5-folds) levels in colonic mucoa vs sham rats. There was no changes in level of VEGF. Quinpirole decreased colonic endothelial permeability in 1.7-fold (p<0.05) in rats with UC. Treatment with domperidone didn’t affect quinpirole-induced changes in colonic endothelial permeability.

We showed for the first time that damage of central dopaminergic neurons aggravated severity of UC in rats. These effects at least partially dependent on increased colonic endothelial permeability and adhesive potential of endothelial cells via dysregulation of central D2R activity.

Tolstanova, G. et al. Role of Dopamine and D2 Dopamine Receptor in the Pathogenesis of Inflammatory Bowel Disease. Digestive Diseases and Sciences. – 2015. - 92(1), p. 9-21

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

The role of central dopaminergic system and D2-dopaminergic receptor in the pathogenesis of experimental ulcerative colitis was studied. The authors confirmed that the experiments described here conform with the Physiological Society ethical requirements.

The Vitamin D receptor regulates skeletal muscle mass in vivo

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Vitamin D (VitD) is proposed to have actions upon skeletal muscle. VitD deficiency is prevalent (~25% of the population) and associated with impaired muscle mass, function, and metabolism. Moreover epidemiological studies have linked VitD deficiency to age-related sarcopenia (1). In contrast, VitD supplementation has been shown to improve muscle function and increase exercise-induced muscle growth (2). VitD transcriptionally regulates and acts through the vitamin D receptor (VDR), with VDR expression recently being confirmed in muscle (3). The VDR has been linked to muscle regeneration (3), with expression increasing acutely in response to resistance exercise (4). However, results from clinical studies on VitD/ VDR are contentious, with poorly defined mechanistic links between VitD status, VDR and muscle mass/metabolism.

We hypothesized the VDR has a functional role in the regulation of skeletal muscle metabolism.

To probe the role of the VDR in the regulation of muscle mass, Tibialis Cranialis (TC) of Wistar rats were electroporated (under 2.5% isoflurane and 50mg/kg carprofen) to constitutively
Melatonin and alendronate synergistically preserved bone matrix and increased trabecular thickness in rats with ovariectomy

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BACKGROUND: Post-menopausal osteoporosis is frequently treated by bisphosphonates (e.g. alendronate) to maintain bone mass. Anti-inflammatory agent melatonin was suggested to have a regulatory role in bone physiology. The aim was to evaluate the possible additive anti-osteoporotic effect of melatonin.

METHODS: Under anaesthesia with ketamine (100 mg/kg, intraperitoneally) and chlorpromazine (0.75mg/kg, intraperitoneally), female Sprague-Dawley rats (n=56) underwent bilateral ovariectomy (OVX), while control group had sham-surgery (n=8). Four weeks after the surgery, OVX rats were treated with saline, alendronate (70 µg/kg/week, subcutaneously), melatonin (25 mg/kg/day, orally), melatonin+alendronate, melatonin+melatonin receptor antagonist (luzindole, 10 µg/kg/day, intraperitoneally) or alendronate+melatonin+luzindole for 8 weeks. Rats were euthanized at the end of 12th week, while an additional saline-treated OVX group (n=8) was euthanized at the end of 4th week. Runx2 expression in bone marrow was determined using real-time polymerase chain reaction. Excised tibiae, fixed in formalin, were treated with decalciﬁer. Parafﬁn sections were stained with TUNEL kit for evaluation of apoptotic cells and with Masson’s trichrome to evaluate bone matrix, mineralization and trabecular thickness. Statistical analysis was performed using Kruskal-Wallis and ANOVA tests.

RESULTS: Runx2 expression was depressed in all OVX groups. Serum oestrogen level in the saline-treated groups was decreased at both the 4th and 12th weeks following OVX (p<0.05), while melatonin abolished this reduction. At the 12th week, saline-treated OVX group presented an extreme decrease in calcified area along with increased un-mineralized area and reduced thickness of the trabecular bone with separation of lamellae, while these alterations were milder at 4th week. In melatonin- or alendronate-treated groups, trabecular bones were mostly calcified, with new bone formation in some regions and less separated lamellae. In alendronate+melatonin-treated group, quite regular, mostly calcified trabecular bones were present, while trabecular thickness was similar to sham-operated group. Moderate decreases in calcified areas and in trabecular thickness, increased decalciﬁed areas with severe separation of lamellae in trabecular bones were observed in melatonin + luzindole-treated group, while these histopathological alterations were milder in melatonin+alendronate-treated group. Quantitative TUNEL analysis also revealed signiﬁcant decreases in both alendronate-treated and melatonin-treated groups.

CONCLUSION: Similar to alendronate, melatonin has direct anti-apoptotic and bone-mass-preserving effects without any additive action. The stimulatory effect of melatonin on trabecular thickness appears to be receptor-mediated.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Attenuation of aluminium chloride-induced reproductive toxicity by silymarin in animal model

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Over the past years the male gonads have been exposed to various degrees of toxicants (Aluminium). Thus, the impact of aluminium on human health has been increasingly alarming in recent years. This study investigated the "Attenuation of Aluminium Chloride-Induced Reproductive Toxicity by Silymarin in Animal Model. Thirty male Wistar rats (180-200g) were divided into five equal groups (6 rats per group). Group A served as control group (received distilled water), group B served as positive control and received corn oil (2ml/kg B.W), group C received Silymarin (SYL) (50 mg/kg B.W), group D received (100 mg/kg B.W) of aluminium chloride (AlCl3), group E received (50 mg/kg B.W) of SYL + (100 mg/kg B.W) of AlCl3. Drug administration was done via oral gavage. At the end of the experimental period (30 days), the animals were anesthetized using sodium thiopentone (30 mg/kg bw); then sacrificed and blood samples collected through cardiac puncture were allowed to clot and then centrifuged in order to obtain serum for hormonal profile. Hormonal assays (FSH, LH and testosterone) were done by ELISA technique. Semen analysis was carried out by WHO standard. The liver, gonads and the accessory organs were also removed for histopathological examination. Values are means ± S.E.M., compared by ANOVA. The results obtained showed an insignificant difference (p>0.05) in the percentage change in body weight of the experimental groups in comparison with their control counterpart. However, there were significant (p<0.05) reductions in sperm motility 25.00±0.02 (%), sperm count 5.0±0.02(X10^6/ml), gonado-somatic and epididymal indices of group treated with AlCl3 alone compared with the control 80.00±0.02 (%); 42.10±0.05 (X10^6/ml) respectively. Significant reductions (p<0.05) were also recorded in serum FSH:14.07±1.14 (mIU/ml), LH:13.55±6.11 (mIU/ml), and Testosterone (T) level 4.95±0.46 (mIU/ml) of AlCl3 treated rats when compared with the control group FSH:29.89±11.53 (mIU/ml), LH:46.29±0.79 (mIU/ml), and T:10.04±0.09 (mIU/ml). However, with SYL supplementation, there were significant increases in sperm parameters and hormonal profile of SYL+AlCl3 treated group when compared with the AlCl3 alone (100 mg [kg bw]) group. AlCl3 could have induced the reproductive dysfunction via a direct effect on the testis and/or at the level of the hypothalamic-pituitary-gonadal axis. Thus, it can therefore be concluded from the results obtained in this study that silymarin has a potent ameliorating potential on the deleterious effects of AlCl3 on reproductive functions. Thus, silymarin supplementation could be considered in the management of male infertility.


We appreciate the Ethical committee of Afe Babalola University

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

The effect of maternal and post-weaning high-fat diet on markers of skeletal muscle insulin-mediated glucose uptake and growth in adult mouse offspring


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Adult obesity is linked to skeletal muscle structure, metabolism and contraction. In the adult female mouse soleus muscle (postural muscle; 37% type I, 63% type II myofibres), the reduction in adult skeletal muscle isometric contraction force by post-weaning (POST) high-fat (HF) diet is minimized by a prior exposure of their mothers to a pregnancy and lactation (PRE) HF diet (1). The POST HF diet was also associated with reduced soleus glucose GLUT-4 and insulin receptor (InsR) mRNA levels (2), which may mediate in part the glucose intolerance previously observed in this model (2) and be linked to muscle contractile capacity. However, muscles are heterogeneous in fibre structure and function, for example, the extensor digitorum longus (EDL) muscle is 96% type II myofibres and is involved in dorsiflexion and extension of the foot and toes. Therefore in this study we determined the expression of GLUT-4 and InsR (markers of insulin-mediated glucose uptake), and Akt-1 (marker of muscle fibre growth and inhibitor of apoptosis) in the EDL muscle.

Female C57BL/6j mice were fed a control (C: 7% kcal fat) or high fat (HF: 45% kcal fat) diet 6 weeks prior to mating and throughout lactation (PRE). Offspring were weaned (POST) onto the same C or HF diet creating 4 different diet groups: C/C, C/HF, HF/C and HF/HF (n=6-8 per group). Female 30 week offspring EDL muscle GLUT-4, InsR, and Akt-1 mRNA levels were measured by RT-qPCR. Data are mean relative gene expression ± SEM and were analysed by 2-way ANOVA. Female offspring EDL muscle GLUT-4 mRNA levels were reduced in POST HF mice regardless of PRE diet (C/HF+HF/HF, 0.95±0.02 vs. C/C+HF/C, 1.14±0.04; p<0.001). There was no significant difference in InsR mRNA levels between groups. Akt-1 mRNA levels were higher in POST HF mice regardless of PRE diet (C/HF+HF/HF, 1.00±0.03 vs. C/C+HF/C, 0.86±0.03; p<0.01).

Our finding of lower GLUT-4 mRNA in POST HF diet group EDL muscle is similar to previous observations in the soleus muscle (2) and may contribute to the glucose intolerance in these animals. Unlike the soleus muscle, InsR mRNA levels in EDL muscle were unaltered by diet, highlighting muscle bed heterogeneity in the insulin-mediated glucose uptake pathway. Increased Akt-1 mRNA levels in POST HF EDL muscle suggests an alteration in muscle growth which could impact on whole body glucose homeostasis and muscle contraction in these animals.

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Silver nanoparticles affect regulation of testicular activity in the young rats

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Silver nanoparticles (nano-Ag) are highly appreciated for their physical, chemical and antibacterial properties (Gromadzka-Ostrowska et al., 2012). But the toxicity possessed of these particles is still not well investigated (Asare et al., 2012). The effects of nano-Ag on the morphophysiology of the testis are not completely understood. That’s why we investigated testicular effects of nano-Ag at different physiological conditions. The experiment was conducted on the male albino rats Rattus norvegicus (1-month-old, 130-150g, n=35). Animals were divided into 7 groups (5 rats each).

The experiment lasted for 10 days. To stimulate or inhibit the reproductive system, we administrated kisspeptin-10 or peptide-234 intracerebroventricularly (I.C.V.) respectively (Qureshi & Abbas, 2013). Silver was injected in the dose of 1 mg/kg, kisspeptin-10 and peptide-234 – 3 µg/kg. Intraperitoneal injections were made on days 1-10, I.C.V. – days 8-10. To perform I.C.V. injections, animals were anesthetized with a ketamine-xylazine solution (80mg/kg + 10 mg/kg i.m.). On the last day, animals were sacrificed; their left testicle was taken and routinely histologically processed for further examination.

We measured the diameter of seminiferous tubules and the cross-sectional area of Leydig cells’ nuclei and Sertoli cells’ nuclei. The means were compared using T-test. The obtained data is summarized in Table 2.

The hypoglycaemic effect of Ocimum Gratissimum (OG) is well documented and morphologic alterations in the intestinal mucosa due to diabetes mellitus is well known. This study investigates the effect of oral administration OG on in vitro intestinal glucose absorption in diabetic rats.

Twenty-five male wistar rats (150–200 g) were randomly grouped into 5 as control, normal + OG, diab-Unreated, diab+OG, and diab+glibenclamide groups. Diabetes was induced by 100 mg/kg of alloxan monohydrate in the diab-Untreated and diab+OG groups followed by treatment with distilled water and 400 mg/kg OG, respectively; whereas control, normal+OG, and diab+glibenclamide groups were treated with distilled water, 400 mg/kg OG, and 5 mg/kg glibenclamide, respectively. Body weight and fasting blood glucose level were monitored weekly. After 28 days of treatments, under anaesthesia by sodium thiopental (50 mg/kg, i.p.), a midline laparotomy was carried out to obtain the small intestine for in vitro intestinal glucose uptake using the everted sac protocol. Histomorphometry of the ileum and jejunum was carried out to measure the villi height, width and crypt depth using standard methods. Data were compared using analysis of variance and Student’s-t test.

There was a progressive reduction in the fasting blood glucose of diabetic animals treated with the extract. While the weight of the diabetic untreated animals consistently decreased, the normal rats treated with OG showed an apparent increase in their weight throughout the experiment and the diabetic animals treated with OG showed an initial reduction in weight.
at the second week but the weight gain increased thereafter. The jejunal and ileal glucose uptake in all the diabetic rats were not significantly different from the control group while normal rats treated with OG showed a significant increase in jejunal intestinal glucose uptake (p<0.05). In spite of the no significant difference observed in the diabetic animals, histomorphometry analysis showed significant increases in the jejunal villi height (p<0.05) of diabetic untreated and diabetic treated with OG. It was concluded that Ocimum gratissimum has no effect on intestinal glucose absorption and intestinal histomorphometry in diabetic rats however; it has growth promoting properties in normal rats as it facilitates glucose absorption and increase in body weight.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**PCA218**

**Metformin treatment in pregnant mice alters expression of genes involved in de novo lipogenesis and β-oxidation in fetal livers**

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Introduction: Prevalence of maternal obesity during pregnancy is on the rise, and may contribute to increased offspring susceptibility to non-alcoholic fatty liver disease (NAFLD) in adulthood. Metformin (MET) is now being prescribed to treat gestational diabetes but may also impact on future NAFLD risk in the offspring. Our previous study in mice shows that maternal MET treatment during pregnancy can either offer protection or further increase offspring susceptibility against NAFLD depending on maternal body condition during pregnancy. However, it is still unknown if maternal MET treatment is already priming the fetal liver to future NAFLD risk.

Aims: To examine the effects of MET treatment in lean and obese pregnant mice on expression of genes involved in de novo lipogenesis, β-oxidation and inflammation in the fetal livers.

Methods: Female C57BL6J mice were fed a control (C, 7% kcal fat) or an obesogenic high fat (HF, 45% kcal fat) diet six weeks prior to conception and during pregnancy, with half of C and HF dams given metformin in drinking water (250mg/kg bodyweight/day) throughout pregnancy. This generated four dam groups: C (n=6), C+MET (n=6), HF (n=6), HF+MET (n=13). Dams were killed on day 16 of pregnancy and fetal livers taken for gene expression analysis. Expression of genes involved in de novo lipogenesis (ACLY, ACC, FASN), β-oxidation (CPT-1) and inflammation (CCL, TLR2) were assessed by real-time RT-PCR. Data was analysed by two-way ANOVA to determine effects of maternal diet and metformin.

Results: In the non-MET groups, HF diet-induced maternal obesity reduced fetal hepatic ACC mRNA levels vs levels in fetuses from lean C-fed dams (0.70-fold p<0.05). Hepatic mRNA levels of ACLY, FASN, CPT-1, CCL and TLR2 were similar in fetuses from both C and HF dams. On the other hand, MET treatment to lean C-fed and obese HF-fed pregnant dams reduced fetal hepatic FASN mRNA levels vs those in fetuses from untreated lean (C+MET vs C, 0.36-fold p<0.001) and obese (HF+MET vs HF, 0.42-fold p<0.001) dams, respectively. Maternal MET treatment also reduced hepatic mRNA levels for ACLY (0.64-fold p<0.05) and CPT-1 (0.36-fold p<0.01) but only in fetuses from C dams. Maternal MET had no effect on hepatic CCL and TLR2 mRNA levels in fetuses from either lean C-fed or obese HF-fed dams.

Conclusion: Maternal obesity did not bring about marked changes on expression of gene involved in de novo lipogenesis, β-oxidation and inflammation in the fetal liver. However, MET treatment to pregnant mothers resulted in significant alteration in expression levels of these genes in the fetal liver. These changes in gene expression during fetal development may contribute to the priming of the offspring liver, and may have long term consequences on disease susceptibility of the offspring in later life.

Supported by Diabetes UK

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**PCA219**

**Different postnatal maturation of AMP-activated protein kinase in skeletal muscle in obesity-prone and obesity-resistant mice**

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AMP-activated protein kinase (AMPK) is a multisubunit protein, which plays a key role in control of skeletal muscle metabolism. Little is known about changes in AMPK subunits expression and AMPK activity in skeletal muscle during the early postnatal development, between birth and weaning. The aims of this study were to (i) characterize the activity and protein level of AMPKα1 and AMPKα2 isoforms, and expression of the genes encoding AMPK catalytic subunits in murine skeletal muscle during postnatal development; and (ii) to assess influence of gender and genetic background of mice on AMPK developmental changes.

Male (M) and female (F) pups of the obesity-prone C57BL/6 (B/6) mice and obesity-resistant A/J mice were born and maintained at 30 °C. Mice were killed by cervical dislocation at the age of 5, 10, 15, 20 and 28 days (D) after birth. Gastrocnemius muscles were collected by freeze-clamping. AMPK activity (n=6 in each age, gender and strain group) was determined using AMARA peptide substrate (1). Protein levels were assessed by Western blot method using antibody against AMPKα1 and AMPKα2 subunits only at 15D and 28D (n=4). Gene expression was assessed by quantitative PCR (n=5). Values are means ± S.E.M. Evaluation of data was performed by ANOVA and considered as significant when p<0.05.

At 10D, the activity of AMPKα1 was significantly higher in comparison with the AMPKα2 activity in all tested groups (A/J F ~1.9-fold; A/J M ~3.3-fold; B/6 F ~2.6-fold; B/6 M ~3.7-fold). Between 10D and 28D, the AMPKα1 activity decreased in mice of both strains except for A/J F (A/J M ~2-fold; B/6 M ~2.6-fold; B/6 F ~3.7-fold). In A/J mice at 28D, activity of AMPKα2 was higher than that of AMPKα1 (A/J F ~1.4-fold; A/J M ~1.6-fold). Total activity of AMPK (α1+α2) in B/6 mice decreased significantly between 10D and 28D (B/6 F ~1.9-fold; B/6 M ~1.5-fold) but it stayed constant in A/J mice. Protein level of AMPKα1 decreased between 15D and 28D, but significantly only in B/6 mice (B/6 F ~2.4-fold). Expression of AMPKα1 gene was constant in both A/J and B/6 mice. Expression of AMPKα2 gene significantly increased between 5D and 28D in
both strains (A/J F ~3.9-fold; A/J M ~4.5-fold; B/6 F ~5.7-fold; B/6 M ~4.8-fold).

Strain-specific changes in AMPK activity in murine skeletal muscle were observed. While in the obesity-resistant A/J mice the activity stayed constant, it declined in the obesity-prone B/6 mice. Developmental changes in total AMPK activity are primarily represent by the activity and protein level of AMPKα1 isoform. However changes in activity and protein level are not corresponding to AMPKα1 gene expression. Changes in AMPK activity in skeletal muscle during early postnatal development may affect propensity to obesity in adulthood, depending on the genetic background of the mice.


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PCA220

Early phase of metformin action in dietary-obese mice: lack of involvement of AMP-activated protein kinase and possible interaction with n-3 fatty acids

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Long-chain n-3 polyunsaturated fatty acids (omega-3) of marine origin exert anti-inflammatory and hypolipidemic effects. We have shown that omega-3 prevent hepatic insulin resistance in obese mice, depending on AMP-activated protein kinase (AMPK) (1). Metformin is the most prescribed antidiabetic drug. However, the precise mechanism of its action remains unknown. Metformin lowers glycemia presumably via mild suppression of mitochondrial complex I activity, leading to AMPK activation and suppression of hepatic glucose production (2). However, AMPK-independent effects of metformin were observed as well (3). We investigated whether pre-treatment of obese mice with omega-3 could enhance acute metformin action and whether AMPK is involved. Adult male C57Bl/6 mice were fed a high fat diet (HFD: 35% lipids wt/wt) for 6 weeks, and then either HFD or HFD with 15% lipids replaced by omega-3 (HFD-F) for 2 weeks. At the end, fasted mice were given a single dose of metformin (400 mg/kg body weight) or saline (placebo) by oral gavage. After 30 min mice underwent oral glucose tolerance test (OGTT; n=6 in each group) or were euthanized by cervical dislocation to collect liver samples for the AMPK activity assay (n=6 in each group) (1). A similar experiment with a lower dose of metformin (60 mg/kg), was performed to study involvement of AMPK in metformin action using mice lacking α2 subunit of AMPK (AMPKα2-KO) (n=6 each group). Data evaluation was performed by ANOVA. During OGTT, glucose levels at 30th minute, as well as an area under the glycaemic curve (AUC; marker of glucose intolerance), were decreased (1.7-fold, 2.0-fold and 2.9-fold, respectively; p<0.05) after single dose of metformin compared to the saline-treated mice. Two-week consumption of HFD-F diet lowered AUC compared to the HFD diet-fed mice (1.5-fold; p<0.05). Omega-3 treatment tended to augment the effect of metformin, but the effect of the interaction between omega-3 and metformin was not significant (3.2-fold vs. the HFD group; 1.1-fold vs. the HFD + metformin group). No changes in AMPK activity in liver in response to metformin were detected. No difference between control and AMPKα2-KO group during OGTT was observed. We demonstrated acute dose-dependent hypoglycaemic effect of metformin during OGTT in obese mice. Omega-3 lowered the response to glucose challenge, showing a trend for additive improvement of glucose tolerance when combined with a low dose of metformin. No difference in the response to metformin of control and AMPKα2-KO mice suggests that AMPK is not essential for the acute hypoglycaemic effect of metformin. Possible AMPK-independent interaction between omega-3 and metformin in their effects on glucose homeostasis is possible and requires further characterization.


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PCA221

Antidepressants-induced histopathological changes on reproductive organs in female and male rats

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The antidepressant drugs are suggested to cause adverse effects on reproductive parameters. There is hardly any reported literature on the effects of paroxetine, bupropion and agomelatine on the histological changes in reproductive organs. The aim of the present study was to elucidate the effects of paroxetine, bupropion and agomelatine on histology of uterus, ovarian and testis of the rats. For the experimental studies, totally 40 female and 32 male Sprague Dawley rats were used. Female and male animals were randomly divided into four groups (control, paroxetine, bupropion and agomelatine) and each female and male groups consisted of 10 and 8 rats, respectively. The experimental groups rats received with 3.6 mg/kg/day, 17 mg/kg/day and 10 mg/kg/day of paroxetine, bupropion and agomelatine orally from post-natal day 21 to 90 day, respectively. The control group received only vehicle. The rats were decapitated under general anesthesia with xylazine (80 mg/kg) plus ketamine (12 mg/kg), and histological slides of uterus, ovarian and testis were prepared and stained by using hematoxylin and eosin, periodic acid-Schiff and Masson’s trichrome techniques. The sections were examined in the olympus BH2 photomicroscope.
On examination, extreme vascular dilatation and congestion, follicular degeneration in the ovary were found in the treated groups. In the uterine samples, antidepressants-induced morphological changes such as vascular dilatation, vacuolization and inflammatory cell condensation in the endometrial stroma and picnosis were seen in some of the endometrial gland. It was shown that all of these antidepressants affected testicular tissue by different ways. When compared to control group, paroxetine treatment caused formation of atrophic seminiferous tubules, and bupropion treatment resulted in interstitial edema formation. Also it was seen that agomelatine caused vacuole formation at seminiferous tubules and cessation at some phases of meiosis. Present study showed that chronic peripheral treatment of paroxetine, bupropion and agomelatine produced several histological changes both female and male reproductive organs, and these changes may interfere with fertility.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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PCA222

Altered Na⁺ delivery to the distal nephron reveals the unimportance of aldosterone in nighttime urinary potassium excretion

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Urinary Na⁺/K⁺ ratio has been important in monitoring the daily intake of Na⁺ and K⁺. It has also been reported to influence blood pressure changes greater than changes in either Na⁺ or K⁺ alone (Kwok et al., 2003; Cook et al., 2009). We have recently reported increased nighttime urinary Na⁺/K⁺ ratio in humans, which was shown to be dependent on a significant dip in nighttime K⁺ excretion by an aldosterone independent mechanism (Asowata et al., 2016). In the current study, we aimed to investigate if increasing the delivery of Na⁺ to the distal tubules will alter the previously established dip in nighttime K⁺ excretion. Ethical approval was obtained for 10 male apparently healthy subjects and we assessed the excretion of aldosterone, Na⁺ and K⁺ as well as the Na⁺/K⁺ ratio in 12 hour-Day (12h-D) and 12 hour-Night (12h-N) urine samples in control and following furosemide administration. Furosemide was administered to increase the delivery of Na⁺ to the distal tubule. Control values for the excretion of aldosterone, Na⁺ and K⁺ in 12h-D (7am-7pm) and 12h-N (7pm-7am) urine samples were collected on the first day. On the following day, 20mg of furosemide was given orally to the subjects twice (7am and 7pm) and 12 hours (Day and Night) urine samples were collected. Na⁺ and K⁺ were analyzed using flame photometry while aldosterone concentration was analyzed using the enzyme immunoassay method (DRG International, Inc., USA). Values are expressed as Mean ± SEM and analyzed using Student’s t-test. In control subjects, consistent with a previous report (Asowata et al., 2016), higher Na⁺/K⁺ ratio was observed in 12h-N compared with 12h-D urine samples (p<0.01) while aldosterone excretion remained unchanged. Day and night changes in Na⁺/K⁺ resulted from a significant difference in urinary K⁺ excretion, which was lower at night compared with the day urine (p<0.05). Correlation analysis revealed a significant direct relationship between Na⁺ and K⁺ in 12h-D (r = 0.82, p<0.01; n = 10) and 12h-N (r = 0.87, p<0.01; n = 10) in controls and after furosemide administration (12h-D: r = 0.97, p<0.001 and 12h-N: r = 0.99, p<0.001; n = 10). However, consistent with our previous finding, no relationships exist between urinary Na⁺ and K⁺ with aldosterone in controls and after furosemide. Following the administration of furosemide, urinary Na⁺/K⁺ ratio remained significantly higher at night than during the day (p<0.001; n = 10) due to a decrease in urinary K⁺ excretion at night compared with daytime (p<0.05; n = 10). Even though the administration of furosemide increased aldosterone activity, nighttime dip in K⁺ excretion was unaffected. Our results support our previous findings that an aldosterone independent mechanism is responsible for the nocturnal fluctuation in urinary potassium excretion.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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PCA223

Effect of predator-induced psychosocial stress on implantation and pregnancy outcome in rats

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Maternal stress is commonly cited as a potential cause for idiopathic pregnancy loss (Knackstedt et al., 2005). However, the mechanisms through which stress affects implantation and pregnancy are yet to be totally deciphered. This study was designed to determine the effect of predator-induced psychosocial stress on implantation and pregnancy in rat. Cycling rats (n=48) at proestrus phase were pair fed overnight with sexually experienced male in ratio 2:1. Following mating confirmation in the morning, rats were registered to be on day one of pregnancy and randomly assigned to either control (n=24) or stress (n=24) group. Stress was induced by the method of Figueiredo et al., 2003; exposing rats to cat for 60 minutes/day for 14 consecutive days. Subsequently, six animals from each group were sacrificed by cervical dislocation on days 6, 8, and 19 and blood was collected through cardiac puncture for hormonal analysis. Remaining six animals in each group were allowed to deliver at term. Number and weight of implantation sites (IS) were determined by intravenous injection of 0.3ml of 0.5% Evans blue dye (Iranloye et al., 2010) and litter size was also determined and recorded for statistical analysis. Results reveal significant (P<0.05) reduction in number of IS on day 6 (5.30±0.56) and 8 (6.40±0.72) compared with control (10.83±0.48 and 10.50±0.56). Mean weight of IS was significantly reduced in stress group on day 6 (0.035±0.002g) compared with control (0.064±0.010g). There is a significant (P<0.05) reduction in the number of fetuses on day 19 (6.00±0.37) and litters at term (6.17±1.01) compared with their corresponding days in control (9.00±0.37 and 9.83±0.54) but no significant difference between stress groups. Hormonal analysis reveal significant (P<0.05) elevation of corticosterone in the stress group (320.80±22.45;
Depletion of c-Cbl impairs mitochondrial function and compromises OXPHOS mitochondrial complex assembly in muscle cells.

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The Cbl family of proteins are multidomain proteins that have a dual function, as a protein adaptors for tyrosine kinase receptors and as E3-ubiquitin conjugating enzymes. As such, they perform a variety of functions in different tissues and in response to various stimuli. In adipocytes and skeletal muscle c-Cbl is rapidly phosphorylated in response to insulin both in vitro and in vivo (Gupta, 2006). In adipocytes, c-Cbl is found associated with CAP (Cbl Associated protein) which helps recruit c-Cbl to the lipid raft microdomains of the plasma membrane. c-Cbl null animals were reported to be more insulin sensitive and be resistant to the deleterious effects of a high fat diet (Molero, 2004). The aim of this study was to understand the function of Cbl and CAP in muscle cells and their contribution to cell metabolism. Cbl or CAP were constitutively knocked down in C2C12 cells using lentiviral transduction of specific shRNAs. Western blot analysis were carried out to determine activation of insulin and nutrient signalling pathways in control and shRNA-expressing cells. Cellular oxygen consumption was determined with a Seahorse XF bioanalyzer and ATP levels and activity of OXPHOS were determined in vitro using spectrophotometric and bioluminescent methods. Native blue gel electrophoresis (1D and 2D) were used to determine the assembly of OXPHOS mitochondrial complexes. We found that Cbl KD cells displayed normal activation of phosphatidylinositol 3-kinase (AKT and ERK pathways in response to insulin. However, ATP levels were reduced compared to control cells. Consistent with this, AMP-regulated kinase phosphorylation levels were elevated in Cbl KD cells. Maximal mitochondrial oxygen consumption was reduced in Cbl KD cells, together with reduced activation of long chain fatty acyl-CoA formation. In vitro, the activity of mitochondrial OXPHOS complexes I, IV and V were reduced compared to that of non-targeting shRNA-expressing cells. Consistent with these findings, we found that the assembly of these proteins into supercomplexes SCI+III+IV and SC III+IV was reduced in Cbl KD cells compared to controls. Overall, our data demonstrates that c-Cbl depletion in muscle cells results in mitochondrial impairment and suggest that this may be due in part to a reduced capacity for OXPHOS complex assembly into supercomplexes. Gupte A and Mora S. Activation of the Cbl insulin signaling pathway in cardiac muscle: dysregulation in obesity and diabetes. 2006. Biochem Biophys Res Comm 342:751-757

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PCA225

Hematopoietic effect of the aqueous calyx extract of Hibiscus sabdariffa in rats

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Cyclophosphamide, a widely-used immunosuppressive agent, may adversely affect the bone marrow. This study investigated the hematopoietic effect of the aqueous calyx extract of Hibiscus sabdariffa (HS) in cyclophosphamide-induced anaemic rats. H. sabdariffa (HS) is used locally as a hematinic and it is reputed to raise blood level. 50g of pulverized HS leaves were boiled in 500ml distilled water and the filtrate air-dried at 40°C. Wistar rats (n = 24) treated as approved by LASUCOM Experimental Animal Ethics Committee, were grouped into 4: Control - water ad libitum; Group I- 200mg/Kg/day HS Extract; GP II 30mg/kg/day Cyclophosphamide for 5 days and Group III – HS 200mg/Kg/day for 30 days + 30mg/kg/day Cyclophosphamide for 5 days. Blood sample was collected directly from the heart chambers of the rats after a light anaesthesia with diethyl ether. In Group I, HS extract caused increase in Hb, PCV, RBC, platelet (PLT) count, MCV, and MCH (p<0.05) compared with Control. In Group II, there was significant reduction in Hb, PCV, RBC, MCV, MCH and PLT count (p<0.05) compared with control (p<0.05) and Group I (p<0.05). In GP III, Hb, PCV, RBC, and platelet count were similar to that of GP I (p > 0.05). The results from this study suggest a hematopoietic potential for the aqueous leaf extract of H. sabdariffa.


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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

### PCA226

**Upregulation of mitofusin-2 - friend or foe to the diabetic heart?**

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Background: Mitochondria are the powerhouses of the cell, supplying a continuous supply of energy in the form of ATP. Maintenance of mitochondrial structure and function is governed by two opposing, dynamic processes known as fusion and fission. These protect the integrity of the mitochondrial genome as well as removing unhealthy mitochondria via PINK1-Parkin-mediated mitophagy. However, mitochondrial dysfunction is closely associated with diabetic cardiomyopathy. Interestingly, the fusion protein mitofusin-2 (Mfn2) has been implicated in the pathogenesis of diabetes. Nonetheless, the role of Mfn2 in the heart is poorly characterized. Therefore, the aim of this study was to investigate changes to cardiac mitochondrial structure and function in a diabetic model, with a particular focus upon fusion/fission.

Methods: Diabetes was induced in male Wistar rats via a single intraperitoneal injection of streptozotocin (55 mg/kg). Mitochondrial OXPHOS function was investigated using isolated and changes to protein expression assessed using Quantitative MS identified 1437 protein and analysis using Ingenuity Pathway Analysis® software highlighted an increase in the expression of both glucagon and insulin release. However, because of their low number in the islets (5% of total cell number), the properties of the δ-cells have not been extensively investigated and little is known about the regulation of SST secretion. Here we have generated and characterized a mouse model in which the genetically encoded Ca²⁺ sensor, GCaMP3, is expressed under the somatostatin promoter (SST-CRE-GCaMP3).

Results: STZ-treated rats developed significant hyperglycaemia 16 weeks post-injection (p<0.0001, n=6). Western blot and quantitative mass spectrometry (MS). Mitochondrial OXPHOS function was investigated using enzyme activity assays. Data was expressed as mean±SEM and analysed by t-test or ANOVA. P values <0.05 were deemed significant.

Results: STZ-treated rats developed significant hyperglycaemia 16 weeks post-injection (p<0.0001, n=6). Western blot and quantitative mass spectrometry (MS). Mitochondrial OXPHOS function was investigated using enzyme activity assays. Data was expressed as mean±SEM and analysed by t-test or ANOVA. P values <0.05 were deemed significant.

### PCA227

**SST-CRE-GCaMP3: a new mouse model to study δ-cell physiology**

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Aim: Pancreatic islets consist of glucagon-producing α-cells, insulin-secreting β-cells and somatostatin-releasing δ-cells. Increased plasma glucose levels stimulate insulin and inhibit glucagon release. Conversely, a decrease in plasma glucose levels suppresses insulin and stimulates glucagon secretion. Somatostatin (SST), which is released in a glucose-dependent manner, is an important hormone as it is a powerful inhibitor of both glucagon and insulin release. However, because of their low number in the islets (5% of total cell number), the properties of the δ-cells have not been extensively investigated and little is known about the regulation of SST secretion. Here we have generated and characterized a mouse model in which the genetically encoded Ca²⁺ sensor, GCaMP3, is expressed under the somatostatin promoter (SST-CRE-GCaMP3).

Methods: Islet hormone release was measured in static incubations of groups of 15-20 mouse islets. Confocal microscopy was used to monitor [Ca²⁺]i in islets from SST-CRE-GCaMP3 mice. Glucose tolerance and insulin tolerance tests were used to measure glucose clearance and insulin sensitivity in wild-type and in SST-CRE-GCaMP3 mice.

Results: We ascertained by immunostaining and by mRNA levels in pure δ-cells fractions obtained by FACS that the expression of GCaMP3 is confined to the δ-cells (90±3% and 98±0.2%, respectively). Glucose and insulin tolerance tests showed no significant differences between SST-CRE-GCaMP3 and control wild type mice. An increase in [Ca²⁺], correlated with stimulation of SST secretion. Glucose (20 mM) stimulated SST and insulin secretion 6- and 40-fold, respectively (p<0.001 and p<0.001 respectively), over basal (1mM), whereas glucagon secretion was inhibited by 60% (p<0.05). The K₅₆ ATP channel opener, diazoxide, abolished glucose-induced SST secretion (p<0.001). FACS-sorted δ-cells express L-, N-, P/Q-, R-, T-type voltage gated Ca²⁺-channels (VGCCs). Glucose-induced increases in cytosolic [Ca²⁺] were suppressed by the L-type blocker isradipine (p<0.005) and the R-type inhibitor SNX-482 (p<0.05). Application of 50 μM yyanadine (an inhibitor of Ca²⁺-induced Ca²⁺ release CICR) inhibited SST secretion by 30-40% (p<0.005).

Conclusion: SST-GCaMP3 mice are physiologically comparable to control littermates. Our data suggest that glucose-induced SST release depends on K₅₆ ATP channel-dependent initiation of CICR. Therefore, we conclude that the established
SST-CRE-GCaMP3 mouse model is a valuable tool to study δ-cell physiology and pathophysiology.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**PCA228**

**An association analysis between psychophysical characteristics and genome-wide gene expression changes in human adaptation to the extreme climate at the Antarctic Dome Argus**

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Genome-wide gene expression measurements have enabled comprehensive studies that integrate the changes of gene expression and phenotypic information to uncover their novel associations. Here we reported the association analysis between psychophysical phenotypes and genome-wide gene expression changes in human adaptation to one of the most extreme climates on Earth, the Antarctic Dome Argus. Dome A is the highest ice feature in Antarctica, and may be the coldest, driest and windiest location on earth. It is considered unapproachable due to its hostile environment. In 2007, a Chinese team of 17 male explorers made the expedition to Dome A for scientific investigation. Overall, 133 psychophysical phenotypes were recorded, and genome-wide gene expression profiles from the blood samples of the explorers were measured before their departure and upon their arrival at Dome A. We found that mood disturbances, including tension (anxiety), depression, anger and fatigue, had a strong, positive, linear relationship with the level of a male sex hormone, testosterone, using the Pearson correlation coefficient (PCC) analysis. We also demonstrated that significantly lowest-level Gene Ontology groups in changes of gene expression in blood cells with erythrocyte removal were consistent with the adaptation of the psychophysical characteristics. Interestingly, we discovered a list of genes that were strongly related to significant phenotypes using phenotype and gene expression PCC analysis. Importantly, among the 70 genes that were identified, most were significantly related to mood disturbances, where 42 genes have been reported in the literature mining, suggesting that the other 28 genes were likely novel genes involved in the mood disturbance mechanism. Taken together, our association analysis provides a reliable method to uncover novel genes and mechanisms related to phenotypes, although further studies are needed.

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**PCA229**

**Heat shock protein 72 regulates mitochondrial integrity and function in the prevention of hepatic insulin resistance**

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Nonalcoholic Fatty Liver Disease (NAFLD) is characterized by an excessive accumulation of lipids in the liver which can lead to inflammation, hepatic insulin resistance, and type 2 diabetes. Mitochondria are critical to liver function and decreased mitochondrial function can contribute to NAFLD and metabolic disease. Induction of the chaperone Heat Shock Protein 72 (HSP72) through heat treatment, exercise, or transgenic overexpression improves glucose tolerance, insulin sensitivity and mitochondrial function in skeletal muscle. The role of HSP72 in liver metabolism is largely unknown. The purpose of this study was to determine the ability of HSP72 to protect against hepatic mitochondrial dysfunction and lipid accumulation in the presence of a high fat diet (HFD). Male Wistar rats (~160g, n=9 per group) were fed a HFD for 15 weeks and were anesthetized with pentobarbital (5mg/100g bw) and given weekly heat treatments (41°C for 20 min) or sham-treatments (37°C for 20 min) for the last 7 weeks. In addition, hepatocytes (n=6 per group) were isolated from 2-month old male C57BL/6 mice and treated with HSP72 siRNA (10nM) for 24 hours and analyzed for mitochondrial oxygen consumption rate using Seahorse Bioscience XF24 Analyzer (substrates used: 2 μM oligomycin, 0.5 μM FCCP, 1μM rotenone/antimycin A). Hepatocytes were also stained with Mitotracker green (100nM) and TMRE (600nM) following siRNA treatment in order to evaluate mitochondrial integrity. Values are means ± S.E.M. compared by ANOVA. Weekly in-vivo heat treatments resulted in upregulation of HSP72 protein content (p<0.01), decreased liver triglyceride storage (p<0.05), and modulation of protein content of the autophagy markers microtubule-associated protein light chain 3 (LC3-II) (p<0.01) and p62 (p<0.05). Loss of HSP72 in primary hepatocytes resulted in decreased basal, maximal, and ATP-coupled oxygen consumption (p<0.05). The siRNA treatment also reduced phosphorylation of AMP-activated protein kinase (AMPK) (p<0.05) and Akt (p<0.05), increased Parkin protein content (p<0.05), and there was a trend toward decreased LC3-II protein content (p=0.07). Decreased expression of HSP72 also reduced mitochondrial quality which was reflected through the decreased TMRE/Mitotracker ratio in siRNA-treated primary hepatocytes (p<0.05). This data suggests that HSP72 regulates mitochondrial function by maintaining mitochondrial integrity through regulation of mitophagy. Future therapies could target HSP72
Insulin is known to increase brain nitric oxide (NO) level but the significance of the increase is not well understood. There is paucity of information on the mechanism by which the increase affects lipid peroxidation and oxidative stress in the brain. The aim of this study was to determine the level of NO and oxidative stress status in the brain of normal non-diabetic mice following sub-acute systemic administration of insulin. Eighteen mice of both sexes, weighing 20.26 ± 1.13 g, were used for the study. They were divided into three groups (n = 6) and treated subcutaneously (s.c.) daily with 0.2 ml deionized water (control), 10 I.U/kg insulin, and 10 I.U/kg insulin (s.c.) + 50 mg/kg L-NAME (N\textsuperscript{\textomega}-nitro-L-arginine methyl ester hydrochloride) intraperitoneally (i.p.) for seven days. Animals were sacrificed following adequate anaesthesia using ketamine + xylazine 65/4 mg/kg, i.p. (Buitrago et al., 2008). Forebrain tissue was immediately harvested from which homogenates were prepared over ice blocks (Shen et al., 2011). Nitric oxide (Biovision, Catalog #262-200) and malondialdehyde (MDA) (Oxford Biomedicals Research, UK, Product number: FR40) levels, as well as glutathione peroxidase (GPx) (Biovision, Catalog #K762-100) activity were determined using commercially available kits according to manufacturers’ instructions. Values were presented as mean ± S.E.M, and compared using ANOVA. Nitric oxide values (nmol/μL) were higher in the insulin group (1.96 ± 0.1) (P < 0.05) but not in the insulin+L-NAME (P > 0.05) group (1.66 ± 0.1) when compared with the control (1.52 ± 0.1). Values of MDA (μM) were higher (P < 0.05) but not in the insulin+L-NAME (P > 0.05) in the insulin (4.06 ± 0.1) and insulin+L-NAME (3.12 ± 0.1) groups, respectively, compared to those of controls (3.06 ± 0.1). The activity of GPx (nM/min/mg protein) in the insulin group (6.44 ± 0.1) was lower (P < 0.05), but that of the insulin+L-NAME (6.84 ± 0.1) was the same (P > 0.05) when compared with the value obtained in the control group (6.92 ± 0.1). Insulin increased the levels of NO (Kamal et al., 2012) and oxidative stress (Monnier et al., 2011) as indicated by increased MDA, and decreased GPx activity (Agrawal et al., 2009) in the administered mice. This effect was reversed by L-NAME (a non-specific NO inhibitor). These data suggest that insulin increased lipid peroxidation and oxidative stress in the brain through elevation of NO (a free radical) level.


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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

**PCA230**

**Sub-acute insulin administration increases nitric oxide level and oxidative stress in brain of non-diabetic mice**

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**PCA231**

**Skeletal muscle and adipose mechanisms contributing to metabolic dysfunction in chronic alcohol administered simian immunodeficiency virus-infected male macaques**

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Alcohol use disorders (AUD) occur frequently in people living with HIV/AIDS (PLWHA). The risk of comorbid conditions such as myopathy, insulin resistance, and lipodystrophy arising from both chronic alcohol consumption and HIV infection has risen significantly with the increased survival due to antiretroviral therapy (ART). Our studies have identified loss of skeletal muscle mass as a critical determinant of time to end-stage disease in chronic binge alcohol (CBA) administered non-ART simian immunodeficiency virus (SIV)-infected (CBA/SIV) male rhesus macaques. Moreover, we have shown decreased whole body insulin sensitivity in asymptomatic CBA/SIV macaques irrespective of ART status. We examined the underlying skeletal muscle and adipose tissue alterations in functional phenotype that could contribute to progression of metabolic dysfunction in CBA/SIV macaques. Skeletal muscle isolated at 11 months post-SIV showed decreased anabolic signaling, enhanced pro-inflammatory cytokine expression and collagen deposition, increased expression of ubiquitin ligases and myostatin expression that was associated with decreased myogenic potential of muscle stem cells in CBA/SIV vs. sucrose (SUC/SIV) macaques. Mesenteric adipose tissue showed decreased adipocyte size, increased inflammatory cell infiltration, and increased collagen deposition, which we predict leads to decreased differentiation of adipose derived-stem cells isolated from CBA/SIV macaques. These findings suggest that CBA exacerbates dysregulation of skeletal muscle and adipose tissue phenotype contributing to metabolic dysfunction in SIV infection. Future studies are aimed to determine the metabolic interrelationship and cross-talk of signals between skeletal muscle and adipose tissue contributing to metabolic dysregulation in SIV infection.

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Sinapic acid ameliorates gut inflammation induced by trinitrobenzene sulfonic acid in rats

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Intense immune response, associated with recruitment and activation of lymphocytes and macrophages, and subsequent release of cytokines and inflammatory mediators all cause tissue damage and contribute to clinical features of colonic inflammation. Sinapic acid (SA), a phenolic acid and cinnamic acid derivative, which is widely distributed in the plant kingdom and is obtained from various sources such fruits and vegetables. Anti-inflammatory effects of SA have been shown in several in vitro experimental models; however it has not been investigated the effects in the gastrointestinal system. The present study was undertaken to examine the anti-inflammatory effects of SA on trinitrobenzene sulfonic acid (TNBS) induced colitis in rat. The colonic inflammation was induced in Wistar-Albino rats (250-300 g; n=9-10/group) by intrarectal administration of 0.8 ml of 30 mg/ml TNBS in 40% ethanol under light isoflurane anesthesia. The control rats received the same volume of isotonic saline by the same route. In the treatment groups, the rats were treated with either SA (20 mg/kg suspended in olive oil per oral) or the vehicle (olive oil; 1 ml/kg/day po). Medications were given 10 min after induction of colitis and continued for 3 consecutive days. On the 4th day, rats were decapitated and distal 8 cm of the colon were removed for the macroscopic and microscopic damage scoring, determination of tissue wet weight index (WI), malondialdehyde (MDA) levels, an end product of lipid peroxidation; glutathione (GSH) levels, a key antioxidant; and myeloperoxidase (MPO) activity, as an indirect index of neutrophil infiltration. Trunk blood was collected for the assessment of serum tumor necrosis factor (TNF-α) and interleukin (IL)-1β levels. Values are mean ± S.E.M., compared by ANOVA. The macroscopic score and WI of colitis group (8.4±0.37 and 0.75±0.02, respectively) is significantly higher compared with control group (0.2±0.13; p<0.001 and 0.18±0.01; p<0.001, respectively) and SA treatment (6.3±0.45; p<0.01 and 0.62±0.02; p<0.01, respectively) reduced these parameters. Incresad microscopic score (8.6±1.1) was reduced by SA treatment (4.7±1.0; p<0.05). Increased colonic lipid oxidation (27.36±4.26 nmol/g) and MPO activity (39.2±4.8 U/g) in rats with colitis were attenuated by SA (14.24±1.19 nmol/g; p<0.05 and 13.3±0.9 rlu/mg; p<0.01, respectively). Increased serum TNF-α (26.8±1.8 pg/ml) and IL-1β (76.8±10.7 pg/ml) levels in colitis group were improved by SA treatment. (15.4±0.9 pg/ml; p<0.001 and 49.2±2.1 pg/ml; p<0.05, respectively). In addition, the GSH depletion in colitis group (0.19±0.02) is prevented by SA treatment (0.64±0.19; p<0.05). Results demonstrate that SA exerts anti-inflammatory effects by inhibiting tissue neutrophil infiltration, suppressing the activation of proinflammatory cytokines and release of oxygen free radicals.

Dietary nitrate modulates gut microbiome profile and prevents the activation of mucosal inflammatory pathways induced by broad-spectrum antibiotics

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Dietary nitrate, mainly found in green-leafy vegetables, is sequentially reduced to nitrite in the oral cavity and to nitric oxide in the stomach. Here, nitric oxide increases mucosal blood flow, mucus thickness and prevents microbial infections. Gut microbiota is now recognized as a pleiotropic organism essential to maintain gastrointestinal (GI) and systemic welfare; indeed, dysbiosis has been associated with increased epithelial permeability and with the activation of inflammatory pathways. Such physiological disturbances are likely to elicit GI symptoms, such as heartburn, dyspepsia and diarrhea during antibiotherapy. Herein, we investigated the impact of nitrate on gut microbiome profile and ensued mucosal effects during dysbiosis.

All animal experiments were performed according to the ARRIVE guidelines and the European Community Council Directive for the Care and Use of Laboratory Animals (86/609/ EEC). Male Wistar rats (n=32) were used in this study. Animals were randomly distributed in 4 groups (n=8, per group) and the drinking water was supplemented for 7 days as follows: 1) antibiotic cocktail (neomycin 5 mg/mL, bacitracin 5 mg/mL, imipenem 1.25 µg/mL), 2) antibiotic cocktail + sodium nitrate (10 mM), 3) sodium nitrate and 4) control (no supplementation). Animals were weighted daily and feces were collected before and after the treatment. Animals were anesthetized (halothane) and euthanized by cervical dislocation. Cecae were collected and weighted. The stomach and ascending colon were isolated and occludin, claudin-5, -15 as well as myeloperoxidase (MPO) and iNOS were analyzed. Bacterial DNA was analyzed in fecal samples by DGGE. Values are mean±SEM.

Nitrate prevented antibiotic-induced body weight loss (1.9±1.8 vs 8.7±1.8, % of increase in respect to day 0, p<0.05) and cecamegalia (7.1±0.5 vs 5.6±0.4, % of total body weight, p<0.05), likely through a more efficient harvesting of nutrients and preserved motility. Gastric expression of occludin and claudin-5 was decreased during dysbiosis, but both protein levels were recovered by nitrate (p<0.05). Similarly, nitrate inhibited MPO and iNOS overexpression under dysbiosis (p<0.05) in the rat stomach. In the large intestine, nitrate increased claudin-5 expression under dysbiosis (p<0.01) but had the opposite effect on occludin (p<0.001). Microbial richness was highly decreased by the antibiotic cocktail but the group also exposed to nitrate showed similarities in specific bacterial groups in comparison to control animals.

This data supports that dietary nitrate may be envisaged as a functional food with a beneficial impact on gastric mucosal integrity and microbial profile during dysbiosis and therefore its consumption may be useful throughout antibiotherapy. BSR acknowledges FCT fellowship SFRH/BPD/84438/2012.
Early oxytocin inhibition of salt intake after furosemide treatment in rats?

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Extracellular fluid depletion and body sodium loss produce neural and behavioral responses to restore body fluid homeostasis, and these responses are influenced by sex hormones. Furosemide, a natriuretic-diuretic, increases urine flow and urinary sodium loss within an hour after treatment, but an 18-24 hour delay typically transpires before rats consume sodium solutions. In other methods of producing hypovolemia and hyponatremia, sodium ingestion is delayed in association with increased oxytocin (OT), suggesting an inhibitory role for OT in sodium intake. We hypothesized that acute furosemide-induced sodium loss and/or the associated volume loss produces central activation in centrally-projecting OT neurons that provides an initial inhibition of sodium intake. Accordingly, we examined early activation in OT neurons after furosemide using fos immunolabeling, a marker for neural activation, and assessed whether activation depends upon the presence of estrogen. Adult female Sprague Dawley rats were OVX under pentobarbital anesthesia (Pbt; 50mg/kg bw, i.p.), treated with Meloxicam (1.5mg/kg bw) for postoperative pain management, and allowed 7 days to recover; then given estradiol benzoate (EB; 10 μg/0.1 ml sesame oil, s.c.) or sesame oil vehicle (OIL; 0.1 ml, s.c.). Rats were given two s.c. injections 1-hour apart of 0.15 M NaCl (ISO; 1.0 ml/kg bw; ns = 4 OIL, 5 EB) or furosemide (5 mg/kg bw; ns = 8 OIL, 8 EB); injections were separated by 1 hour. One hour after the 2nd injection, rats were anesthetized with Pbt and perfused with paraformaldehyde. Brains were removed, cut in 40-µm sections prior to immunolabeling for fos (Santa Cruz; 1:30,000) and OT (Chemicon; 1:25,000). After furosemide, fos immunoreactivity (fos-IR) was prominent in the hypothalamic supraoptic nucleus (SON) but was not different between EB: (42.4 ± 9.8) and OIL: (53.1 ± 15.2) treated rats. Fos-IR also was present in parvocellular cells of the paraventricular nucleus (PVN) after furosemide, and again, was not different between EB: (40.3 ± 8.6) and OIL: (57.6 ± 13.5) treated rats. Virtually no fos-IR neurons in the paraventricular groups of PVN were also immunolabeled for OT. Thus, acute sodium/volume loss produces selective neural activation that is not influenced by estrogen, and the early inhibition of sodium intake after furosemide does not appear to require activation of centrally-projecting OT neurons. All procedures were approved by the Oklahoma State University Center for Health Sciences Animal Care and Use Committee. Supported by OCAST HR12-196.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Poster Communications

PCA235

The role of IGF2 in regulating maternal-fetal resource allocation in mouse pregnancy

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During pregnancy, adequate nutrients must be supplied to the fetus for growth with sufficient resources also partitioned to the mother to maintain her health. Failure to appropriately allocate resources can lead to pregnancy complications and abnormal fetal development with long-term consequences for maternal and offspring health. The placenta is central to this “tug-of-war” over resources as it is responsible for maternal-fetal nutrient transport and secretes hormones with suspected effects on maternal physiology. However compared to placental transport function, less is known about the regulation of placental endocrine function and its significance for fetal nutrient allocation and growth. Insulin-like growth factor-2 (IGF2) is a growth-promoting paternally-expressed imprinted gene abundantly expressed in fetal-placental tissues. Previous work shows that altering conceptus IGF2 modifies both the maternal metabolic profile, and placental nutrient transfer in a manner consistent with the maternal ability to support the fetal genetic drive for growth1–5. However the precise role of IGF2 in placental endocrine function and materno-fetal resource allocation is unknown.

Aim: To determine the effect of selectively manipulating IGF2 expression in the mouse placental endocrine zone, on maternal metabolism, nutrient allocation and fetal growth.

Methods: Transgenic mice were crossed to produce entire litters with complete deletion or over-expression of IGF2 in the placental endocrine zone using the TpbpaCre line (leaving the placental transport zone, fetus and mother un-manipulated, IGF2/Tpbpa and H19DMR/Tpbpa respectively). On day 16 of pregnancy (term ~20 days), dams were anaesthetised with fentanyl-fluanisone:midazolam in sterile water (i.p. at ratio of 1:1:2) and blood was collected before schedule 1 killing. Maternal organs and conceptuses were weighed and placental structure analysed. Procedures were performed abiding to the UK Home Office Animals (Scientific Procedures) Act 1986.

Results: Deletion of IGF2 in the placental endocrine zone reduced maternal kidneys and liver weights and circulating lipid concentrations (Figure). However, over-expression of IGF2 in the placental endocrine zone increased maternal heart weight and circulating glucose, but reduced non-esterified fatty acid (NEFA) and triglyceride concentrations. In both models, placental weights were heavier and related to an increase in the endocrine zone or the transport zone when IGF2 was over-expressed or IGF2 deleted, respectively. In addition, deletion of placental endocrine zone IGF2 marginally reduced fetal weight.

Conclusion: IGF2 expression in placental endocrine zone is important in determining maternal metabolic profile in pregnancy, which affects resource allocation and potentially fetal growth. Work is underway to further assess changes on day 19 of pregnancy.
Leptin pharmacokinetics in the digestive tract of mice

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Leptin is a pleiotropic hormone known for its effects in regulating appetite. Recently data have shown that leptin in circulation enters the lumen of the digestive tract in a form with signaling potential (1). To explore this further an experiment was designed to examine the fate of leptin in the digestive tract. Leptin was labeled with $^{125}$I using the iodogen method. Random bred male Swiss mice aged 8 weeks were lightly anaesthetised using ether to prevent damage to the esophagus before receiving 12 ng $^{125}$I-leptin by intragastric gavage. Animals were returned to individual cages until being euthanized over a time course of up to 120 min (n = 4 per time) before tissues were collected to determine $^{125}$I-leptin distribution. Samples were collected and subjected to trichloroacetic acid (TCA) precipitation to confirm intactness of the tracer. All procedures were performed in accordance with the NHMRC Code for the Care and Use of Animals for Scientific Purposes. Data are presented as mean ± SE. $^{125}$I-Leptin was detected in all samples examined. Recovery from the lumen of the stomach declined to 23.5 ± 2.2 % of the dose 120 min after administration. In the lumen of the small intestine a wave of approximately 3 – 8 % of the dose was recorded in increasingly distal portions of the small intestine over the duration of the experiment. In the hindgut 0.7 ± 0.4 % of the administered dose was recovered from the lumen 30 min after administration, increasing to 3.9 ± 1.7 % 120 min after administration. At all times sampled $^{125}$I-Leptin was recovered from the blood, with calculated recovery for the entire circulation ranging between 3 ± 1.2 % 90 min after administration and 4.1 ± 0.5 % 45 min after administration. $^{125}$I-leptin in the circulation was found to be 73 ± 6.2 % (n = 3) intact after TCA precipitation, indicating signaling potential. The experiment described is the first to examine leptin pharmacokinetics in the digestive tract. The data indicate that leptin in the digestive tract is capable of entering the circulation in a form with potential signaling capacity. Leptin introduced into the stomach progressed distally over the course of the experiment with less than 4 % of the administered dose detected in the hindgut, while at all times sampled approximately 3.7 % of the administered dose was calculated to be in the circulation. These data suggest that leptin in the digestive tract is potentially being absorbed from the small intestine. When considered with a recent report (1), it appears that leptin may cycle between the circulation and the digestive tract. The potential reason for this is unclear, however knockout of Lep-Rb (the principal leptin receptor) from mouse small intestine has conferred a resistance to diet induced obesity (2), suggesting leptin may play a role in regulating nutrient absorption from the digestive tract.


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PCA237

Bioavailability, transport and absorption characteristics of Aspalathin: a bioactive polyphenol shown to elicit a positive effect on glucose tolerance

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Improving the quality of life for individuals with diabetes through optimal glycemic control is a major challenge. Polyphenols show great potential as adjuncts to current therapies in ameliorating metabolic disturbances. The C-glucosyl dihydrcalconaline aspalathin, a polyphenol unique to rooibos (Aspalathus linearis) is poorly absorbed, with metabolites detectable in urine but not in plasma of mice (unpublished data), yet it displays antidiabetic (1,2), cardioprotective (3) and antimutagenic effects (4). In this study we demonstrated hypoglycaemic properties of an aspalathin-enriched green rooibos extract (GRE) in vivo, as well as propose the mechanism for intestinal transport, bioavailability and metabolism of aspalathin in vitro. Diet-induced pre-diabetic (PD) (n=8) vervet monkeys (Chlorocebus aethiops), together with normal controls (n=4) were treated with GRE (90mg/kg body weight) 3 x daily, with meals for 28 days. Intravenous glucose tolerance tests (IVGTT) were performed at base line (pre-treatment), during treatment (14 and 28 days) and after 28-day washout. The extract treatment significantly increased glucose elimination during treatment (14 and 28 days) and after 28-day washout. The rate of transport significantly increased glucose tolerance as measured by decreased area under the curve in PD monkeys, after 14 (791.3±183.0 vs 524.8±103.1, p<0.01) and 28 (588.5±107.7, p<0.05) days returning nearly to normal (626.9±132.5) after washout. As expected, treatment showed no effect on normal controls. Following this positive effect on glucose tolerance, passage of aspalathin was monitored across CaCo2 cell monolayers in the presence and absence of inhibitors to expose mechanisms of transport and thus provide evidence for dose optimisation. Samples were analysed by HPLC-DAD and LC-MS, where aspalathin concentration was used to calculate Papp values (S) representing rate of transport. Aspalathin was transported at a rate typical of poorly absorbed compounds (1.59±1.08 x 10^-6 cm/s2). Major glucose transporters, SGLT-1 and GLUT-2, were shown not to be primary transporters, nor was aspalathin effluxed into the gut lumen (1.9±0.85 x 10^-6 cm/s^2, efflux ratio: 1.2). The rate of transport was not affected by other polyphenols present in aspalathin-enriched extracts (1.6±0.75 x 10^-6 cm/s^2), but was affected by glucose concentration (2.9±0.76 x 10^-7 cm/s2, p<0.05 at 20.5 mM glucose). Statistical significance was calculated using a student t-test, data is shown as mean ± SD. No metabolites were detectable in basolateral samples. Results confirm bioavailability of aspalathin, yet glucose lowering ability of extract in PD monkeys are significant. Mechanistically we showed that aspalathin was not actively transported by glucose transporters across Caco2 monolayers, but presumably passes paracellularly. To exploit bioactivity potential of aspalathin, further enquiry into delivery systems to increase aspalathin bioavailability are needed.


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PCA238

Glycogen availability alters oxidation of β-hydroxybutyrate in the perfused rat heart

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Introduction: B-hydroxybutyrate (BHB) is an oxidative fuel substrate, primarily produced to sustain cerebral metabolism when carbohydrate availability is low (1). BHB is usually only elevated with concomitant depletion of muscle glycogen, and the permissive role of glycogen content on BHB oxidation in the heart is therefore poorly understood. However, it is possible to elevate BHB using exogenous ketones (2), so we sought to determine whether low, control or high cardiac muscle glycogen content could influence ketone body oxidation in the isolated perfused rodent heart.

Methods: Following euthanasia by sodium pentobarbitone injection in accordance with UK legislation, hearts from 150-200g male Wistar rats were perfused with modified Krebs-Henseleit buffer (KH) in a sealed apparatus using the Langendorff mode. Heart glycogen was manipulated to determine whether low, control or high cardiac muscle glycogen content could influence ketone body oxidation in the isolated perfused rodent heart.


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groups (L=33.9±3.6, C=41.4±6.4, H=64.4±10.7), indicating glycogen re-synthesis. Increased BHB oxidation correlated to increased glycogen availability (L=0.84±0.14 µmol/min/heart, C=0.93±0.15 µmol/min/heart, H=1.41±0.25 µmol/min/heart) (R²=0.35). In the H condition, glycolytic intermediates were higher in L and C, apart from phosphoenolpyruvate, which was significantly lower. TCA cycle intermediates were higher with H glycogen vs. L and C.

Conclusions: Our results demonstrate a permissive role of cardiac glycogen in the combustion of increasing quantities of BHB. These findings suggest an obligate requirement for glucose in the oxidation of BHB, possibly because BHB is not inherently anaplerotic, with ensuing cataplerosis limiting TCA capacity (3). Further work on the interactions between carb hydrate and BHB metabolism in working muscle is underway.


Enterococcus faecalis requirements. Described here conform with the Physiological Society ethical requirements.

Role of an Enterococcus faecalis strain on hypertension. Effects on urine nitric oxide and 8-isoprostane levels.

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Hypertension is considered the most serious risk factor for cardiovascular disease. Usually, it is associated with other metabolic disorders, as obesity and glucose intolerance. In addition to drug therapies, different functional foods have been used for its treatment, indicating an important role of gut microbiota in the control of blood pressure (BP) (Ettinger et al., 2014). Moreover, other components of foods have been widely related to the increase of blood pressure, mainly the amount and quality of dietary fat, being the intake of saturated fatty acids and cholesterol an important risk factor in the development of hypertension. However, the relationship between dietary fat, gut microbiota and hypertension has been scarcely studied. Previous results of our laboratory have demonstrated a link between changes in gut microbiota, BP and high fat diets. Mice fed with a diet enriched with butter (20%) increased its BP levels and visceral adiposity compared with control; and different fats had different effects on gut microbial composition (Hidalgo et al., 2014). The levels of Enterococcus faecalis in fecal samples of these animals, showed a linear positive correlation with BP at the end of the experiment. When mice fed with a standard cow diet were inoculated with E. faecalis 12M3-5 during six weeks, the levels of BP were higher than controls, but they did not develop obesity (Prieto et al, 2015).

In the present work, we analyze the relationship between the increase in the BP of mice inoculated with E. faecalis 12M3-5 and the changes in urine nitric oxide and 8-isoprostane. Male Swiss Webster mice were divided in two groups: One Group were inoculated daily with a sample of E. faecalis 12M3-5 (Enterococcus group), and the other received a placebo (Control group). After six weeks of experiment, BP data were measured. Animals were placed into individual metabolic cages in order to determine water intake and diuresis. Urine samples were taken to measure Nitric Oxide and 8-isoprostane levels. Blood pressure was 19% higher in Enterococcus than in control group (211±10.3 vs 177±7.8 mmHg; p<0.5). Water intake (3.3±0.43 vs 7.0±1.30 mL/day; p<0.05) and diuresis (1.2±0.16 vs 2.4±0.71 mL/day) were lower in Enterococcus animals, although no significant differences were reached in urine excretion. No differences were also found in urine Nitric Oxide (70.2±8.48 vs 51.8±15.98 µM/L), but a significant high level of 8-isoprostanes was measured in the urine of Enterococcus group (595.3±23.18 vs 476.3±40.76 µg/mL; p<0.05).

These results indicate that the hypertensive effect of E. faecalis 12M3-5 does not seem to be due to natriuresis or enhanced renal nitric oxide synthesis. However, the increased levels of 8-isoprostane in urine will be related to an increased lipid peroxidation in animals inoculated with Enterococcus.


Iron status influences cognitive development in Santal children of Purulia District, West Bengal, India

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The impairment of children’s cognitive development has been found to be closely associated with the iron deficiency. Recently, poor cognitive development, poor nutritional status and a positive association between these two have been evaluated in Santal children. Deficiency of iron in their diet was also reported in recent past. The association between cognitive development and iron status has not been identified in Santal tribal population. It is pertinent to investigate this association as it may provide a valuable contribution in the field of cognitive research. Therefore, the purpose of the present study was to characterize a relationship between iron status and cognitive development in 5 to 12 years aged Santal children of Purulia district of India. The present study was cross-sectional and conducted on 170 (90 boys and 80 girls) school children aged 5-12 years from Balarampur and Bagmundi areas of the Purulia district. Some biochemical parameters such as hemoglobin concentration, serum iron, serum ferritin, total iron binding capacity (TIBC), serum transferrin, and transferrin saturation were measured to assess the iron status of each subject. Stages of iron depletion have also been measured in surveyed children. Raven’s Coloured Progressive Matrices (RCPM) was applied for measuring the general intelligence. The protocol and procedures employed were in accordance.
with the human ethical guidelines of the Institutional Human Ethical Committee, Department of Physiology, University of Calcutta (Ref. No. IHEC/TKG/P07/11 dated 13.07.2011). The Hb concentration (p<0.05), serum iron (p<0.01), serum ferritin (p<0.05), transferrin saturation (p<0.05) levels of both boys and girls having RCPM scores below 5th percentile value were significantly lower than that of RCPM scores above 5th percentile (maximum 25th percentile) value of standard. The Hb concentration, serum iron, serum ferritin and transferrin saturation levels of ‘intellectually deficit’ group (for both boys and girls) and ‘below average’ group (only in girls) were significantly lower (p<0.05) than that of ‘above average’ group of IQ level. According to IQ classes based on RCPM scores, about 9.53% of ‘below average’ class were found in stage III whereas about and 19.04% of boys of ‘below average’ class were found in stage II of iron depletion. RCPM scores of children (boys, girls and total children) belonging to iron depletion stage II and III were significantly lower (p<0.05) than that of normal children. Pearson’s correlation study indicated that all the iron status parameters were found to be significantly correlated (p<0.05) to RCPM scores. The present study suggests that the iron status of surveyed children is linked with their cognitive development. Vulnerable iron status may be one of the causes of the impairment of the cognitive functions in Santal children. Cognitive development was found to be affected more in girls than boys as iron status is poor in girls than boys.

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PCA241

Effects of kisspeptin on firing rate of habenular neurons and locomotor activity in adult male mice following intra-habenular infusion

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Kisspeptin plays an important role in hypothalamic regulation of gonadotropin releasing hormone secretion. Specific receptor of this neuropeptide (GPR54) is expressed in brain areas other than the hypothalamus. Lateral Habenula (LHb) is a brain area where direct and indirect effects on dopaminergic and serotonergic systems are processed. In the present study, effects of kisspeptin through GPR54 receptors in the LHb on motor activity and anxiety behavior in adult male mice were investigated. Cell-attached recordings were made from adult male Balb-C mice LHb in coronal brain-slice preparation. Following decapitation and quick removal of the brain, 300-μm thick brain slices were prepared with a vibratome and placed in the recording bath filled with artificial cerebrospinal fluid containing (in mM) 119 NaCl, 2.5 KCl, 2 CaCl2, 1.25 MgCl2, 11 D-glucose 25 NaHCO3 and NaH2PO4. Patch pipettes had 3–5 M Ohm resistances. Signals were acquired using a Multi-clamp 700B amplifier connected to a Digidata 1440A. After baseline recording, kisspeptin (100 nM) was applied in the bath. Adult male Balb-C mice were divided into three groups (n=3/group) as control, kisspeptin and kisspeptin antagonist (P234). The mice were placed in the stereotactic frame under isofluranaesthesia (2%) and intra-habenular infusions were performed. The animals were observed to return normal feeding-drinking behavior within 30 min after anesthesia. Following the infusions, elevated plus maze and open field tests were performed to evaluate anxiety and locomotor activity respectively. At the end of the experiments, all animals were decapitated. Brains were dissected out and stored at -80°C for confirming LHb infusion region and future analysis. These preliminary findings suggest that 100-nM kisspeptin strongly activates neuronal firing in the LHb. It was determined that stereotypic, ambulatory and horizontal locomotor movements were significantly reduced by P234 compared to the kisspeptin group (p<0.01). In addition, distance parameter was significantly increased by kisspeptin compared to the P234 group, and resting time in this antagonist group was significantly higher than the kisspeptin group (p<0.01). No significant change was observed in the elevated plus maze findings among the groups. Electrophysiological recordings from brain slices showed that kisspeptin increased firing rate of neurons in the LHb. Locomotor activity was increased by kisspeptin and decreased by kisspeptin antagonist following intra-habenular infusion. It’s thought that motor activity inhibition processed by the dopaminergic system in the LHb is inhibited by kisspeptin, and this inhibition is further increased by P234. Since habenular neurons project to the substantia nigra, kisspeptin-GPR54 system in this brain area may be of importance for neurodegenerative diseases affecting locomotion.

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PCA242

High frequency pelvic nerve stimulation blocks reflex urinary voiding in urethane-anaesthetised rats

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Urgent urinary incontinence (UUI) is a widespread clinical problem characterised by involuntary leakage of urine accompanied or preceded by “a sudden compelling desire to pass urine which is difficult to defer” (Abrams et al, 2002). One approach to mitigate this condition would be to inhibit bladder emptying via short term block of efferent activity in the pelvic nerve at the first sensation of urgency, thus ‘buying time’ to reach a toilet. To test the concept we investigated whether high frequency electrical stimulation of the pelvic nerve could inhibit reflex voiding during continuous cystometry in anaesthetised rats.

Female urethane anaesthetised Wistar rats (n=15) (1.4g Kg-1 i.p.) were instrumented to record blood pressure, heart rate, tracheal air flow and for intravenous infusion of fluids. Rectal temperature was maintained at 37°C. The left preganglionic pelvic nerve bundle was exposed via a laparotomy and fitted with a bipolar cuff electrode for stimulation. Catheter was inserted via the bladder dome to allow infusion of saline and simultaneous recording of bladder pressure. A balloon catheter was used to monitor pressure within the uterus (n= 8) or rectum (n=4).

Continuous infusion of saline into the bladder (6ml h-1) evoked repetitive cycles of filling and voiding (0.32-1.96min-1). Unilateral pelvic nerve stimulation (1-3kHz sinusoidal waveform, 1-5mA for 60s) initiated within 1s of the onset of the sharp rise in bladder pressure signalling an imminent void aborted the void and no urine was expelled. Urinary continence could be maintained for the entire 60s stimulation period but voiding
resumed within 60s of stimulus offset. High (10kHz) or low (500Hz) stimulation did not block micturition (See figure). Cardiovascular/respiratory effects were minimal and there was no significant change in intra-uterine or rectal pressure. Stimulation applied during filling i.e. in between voids produced only small transient ‘on’ and ‘off’ fluctuations in bladder pressure.

We conclude that unilateral high frequency electrical stimulation of the pelvic nerve is sufficient to suppress an imminent void and prevent urine release, without causing significant ‘off-target’ effects. This technique may hold therapeutic potential for management of Urge Urinary Incontinence.

Fig 1A-C. Frequency dependent effect of 1min pelvic nerve stimulation (grey panels) on urinary voiding evoked by continuous infusion of saline into the bladder. Top traces show change in bladder pressure, lower trace shows timing of drops of urine expelled from the urethra. A. Low frequency stimulation (500Hz) partially supressed voiding; B. stimulation at 3KHz supressed voiding completely for the duration of the stimulation period. Voiding resumed within 30s of terminating the stimulus. C. Stimulation at 10kHz had no effect on voiding. All traces from the same animal.


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PCA243

Reefnition of an anaesthesia protocol in laboratory mice to reduce acute stress related to intraperitoneal injections

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Acute stress has been previously reported as a source of unexplained variation in in vivo studies, thus resulting in an increase in a number of animals used for experiments. The intraperitoneal (i.p.) injections, most commonly used in laboratory mice for general anaesthesia (GA) may act as acute stressors, both due to immobilization of the animals and, in particular due to the pain related to a needle insertion into the peritoneum. Therefore, this study evaluated the effects of two GA protocols with i.p. injection of anaesthetics, in which one protocol was preceded with inhalation anaesthetic allowing for animals’ insensitisation to acute stressors related to immobilization/i.p. injection. The effect of the two GA protocols was assessed based on the volume of urine present in the urinary bladder, as it was previously shown that urination is one of physiological responses to acute stress in rodents (Antoniadis and McDonald, 2001). This study was based on a “sharing scheme” and the samples were obtained from animals included in other experiments that were destined to be sampled under GA without recovery; only animals that fulfilled pre-set inclusion/exclusion criteria were selected. A total of 56 mice of three strains (C57BL/6, n=32, 39.5+2.5g; scid, n=18, 20.5+1.03g; CPU2VEGF, n=6, 36.2+4.1g) were included. In 24 mice (IP group) GA was carried out with a dissociative anaesthetic (ketamine) and α2-agonist (xylazine) in a 10:1 mixture administered with a single i.p. injection (1.2ml/kg live body mass, LBM). For the remaining 32 animals (ISOIP group), anaesthesia was induced with volatile anaesthetic gas (5% isoflurane) in a drop-closed anesthetic chamber; when a loss of righting reflex was observed these animals were dosed with a single i.p. injection of a ketamine and xylazine mixture, as described above. Under GA, an abdominal incision was made through the linea alba for needle aspiration of total urine from the urinary bladder. Out of a total of 56, the bladder was empty in 17 of the animals. However, this included a significantly higher (p<0.0001, the Pearson chi-square test) number of mice from IP group (14) vs ISOIP group (3). After exclusion of animals with empty bladder, the effect of anaesthesia protocol on collected urine volume normalized for LBM [µL/1 g LBW] was evaluated with two-way ANOVA with strain and anaesthesia protocol as independent variables. The effect of strain of anaesthesia protocol on collected urine volume was significant (0.95 + 0.32 vs. 5.03 + 0.29 µL/1 g LBW, for IP and IOSIP group respectively, p < 0.001), while the effect of the strain was not significant (p = 0.861). The results of this study suggest that an induction with inhalable anaesthetics can reduce acute stress related to i.p. injections in laboratory mice thus offering a refinement to many experimental procedures carried out on those animals.


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PCA244

Representation of the whisker pad macrovibrissae within the rat trigeminal mesencephalic nucleus

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Among the open questions regarding the rodent whisker-dependent behaviors, an important aspect is undoubtedly represented by the way the rat brain processes signals generated by the active movements of the whiskers to extract a real image of the surroundings. Contact between whiskers and objects produces time-varying stresses at the base of the macrovibrissae that are transduced into action potentials by mechanoreceptors of the follicles and relayed to the CNS by the trigeminal nerve. However, it has been recently demonstrated that besides the trigeminal ganglia even the trigeminal mesencephalic nucleus is involved in detecting kinetic information from macrovibrissae receptors. Spontaneous
and artificial movements of whisker pad macrovibrissae elicit in fact evoked responses in the trigeminal mesencephalic nucleus (Me5). Besides that, neuroanatomical procedures showed that the Me5-macrovibrissae neurons are localized in the medial-caudal part of the nucleus and have their peripheral terminals coiled around the macrovibrissae shaft [Mameli et al. 2010, 2014].

The present study was aimed at understanding whether or not within the Me5-nucleus a neuroanatomical representation of the whisker pad macrovibrissae exists.

The experiments were performed on different groups of anaesthetized Wistar rats, (diazepam 30 mg/kg, ketamine hydrochloride 45 mg/kg), which were submitted to a tracer injection in selected points of the whisker pad. In animals of group 1 only a single macrovibrissae was injected, in group 2 five macrovibrissae along a single row, in group 3 the straddler whiskers (i.e. the vibrissae α, β, γ, δ). Finally, in group 4 the whole whisker pad was uniformly labeled. Fluorescent histological analysis was performed by Confocal Microscopy to detect the Me5 neurons labeled by Dil.

Results showed that the whisker pad macrovibrissae are represented at the level of the nucleusmedial-caudal part, and that the Me5 labeled neurons were globular, oval or triangular in shape. They had variable sizes (30-55 μm) and were provided with two or multiple processes that could be followed for some distance. Moreover, it has been demonstrated that they are clustered in arrays of cellular aggregates, which although did not correlate with the geometrical architecture of the macrovibrissae onto the whisker pad, did resemble the special arrangement of other trigeminal neurons within their relay stations. Finally, since the number of the labelled neurons matched that of the macrovibrissae, this data raises the question as to whether such correlation is related to a specific functional role of single Me5 macrovibrissae-neurons. Specific electrophysiological experiments will be therefore needed to address this issue.


Mameli et al. (2014) Brain Res Bull 102, 37-45.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**Protease-mediated effects of commensal bacteria on nociceptive dorsal root ganglia neurons**

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The mucosal barrier that separates the microbiota from the nervous system is disrupted during gastrointestinal (GI) inflammation. It is presently unknown whether translocation of bacteria during GI inflammation modulates visceral sensation and thereby contributes to pain. The primary aim of this study was to determine if a defined community of 33 commensal GI microbes (MET-1) alters the excitability of mouse dorsal root ganglion (DRG) neurons. To determine whether supernatant containing the secretory products of MET-1 altered the excitability of DRG neurons, and identify the intracellular signaling pathways involved. In addition, we sought to identify candidate bacterial mediators of this effect. DRG neurons were dissociated from C57Bl6 male mice and incubated overnight in sterile MET-1 supernatant (1:10 – 1:10,000 dilution in sterile media) or sterile control media, in the presence or absence of various inhibitors. Current and voltage clamp experiments were performed after 24 hours. MET-1 decreased the excitability of DRG neurons in a concentration-dependent manner by significantly increasing rheobase. 1:100 MET-1 was used in subsequent experiments. The resting membrane potential of DRG neurons was hyperpolarized by MET-1, -68 mV (n=7; MET-1) vs. -58 mV (n=30; controls), p < 0.05; Mann-Whitney test. In addition, MET-1 increased voltage-gated K+ current (p<0.001; 2 way ANOVA). Addition of a nuclear factor kappa B (NFkB) inhibitor (SC-514, 20 mM) or an ERK1/2 inhibitor (PD 98059, 30 mM) blocked the effects of MET-1. A bacterial protease inhibitor cocktail (1:10,000) abrogated MET-1 effects on DRG neurons. The serine protease inhibitor (FUT-17S, 50 mM), but not inhibitors of cysteine proteases, acid proteases, metalloproteases, or aminopeptidase, abolished the effects of MET-1. The serine protease, cathepsin G (100 nM) recapitulated the effects of MET-1 on the excitability of DRG neurons. Blocking protease activated receptor (PAR) 2 (GB83, 10mM) or PAR4 (P4pal10, 10mM) did not block the effects of MET-1 on the excitability of DRG neurons. Serine proteases secreted by MET-1 can directly impact the function of DRG neurons, through NFXB and ERK1/2-dependent pathways. On the basis of these observations, pain signaling may be modulated by microbiota-neuronal interactions.

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Hyphothalamic c-Fos/CRF activations during acute stress and expression of β-endorphin fibers in an animal model of perimenopause

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Perimenopausal changes are characterized by neuroendocrine changes that may be involved in how women react to stressful situations. Using an animal model of perimenopause i.e. rats treated with 4-vinylcyclohexene diepoxide (VCD), this study aimed to explore the association of the main central nuclei of stress system (Paraventricular nucleus-PVN and Locus Coeruleus-LC) with acute stress. Progesterone increases the amount of β-endorphin fibers in the LC which inhibit its neurons. As we demonstrated in this animal model that progesterone levels are lower while the response of LC to stress is higher than control rats, we aimed to correlate the neuronal stress response of LC with the amount of β-endorphin fibers. Also, PVN response to stress was analyzed by c-FOS expression. Female rats from 28 days old were injected (SC) daily with VCD (160 mg/kg; VCD group) or Control oil (2.5 µl/g BW, Control group) for 15 days. At 85-95 days of age at diestrus morning, rats were exposed to 30 min restraint stress followed by 60 min of recovery. Rats were then anaesthetized with a solution of ketamine hydrochloride (55mg/kg BW, IP) associated with xylazine (10 mg/kg BW, IP) and then perfused. Brains were processed immunohistochemically for c-FOS/CRF in PVN and density of β-endorphin fibers in the LC. The increase in number of c-Fos/CRF positive neurons induced by stress in PVN was higher (P < 0.05) while density of β-endorphin fibers in the LC was lower (P < 0.05) in the VCD treated compared to Control rats. These results show that neurons of the stress systems are overactive and suggest that during perimenopause the over reaction of the LC neurons may be due to the lower progesterone levels that induce a decrease of β-endorphin inhibition of LC neurons.

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incomplete bladder emptying and dysuria (Fukuoka et al., 1991). Both drugs have been shown to cause bladder hyperactivity in experimental models suggesting changes in sensory activity may be involved in the bladder dysfunction. The aim of this project was to investigate the hypothesis that the cytotoxic drugs CPO and IFO affect the activity of high and low threshold bladder sensory nerves. Male mice (12wks) were administered either CPO (100mg/kg) or IFO (200mg/kg) by i.p. injection and sacrificed for experimentation after 24hrs. Intravesical pressure and bladder afferent nerve activity were then measured during bladder filling and emptying in vitro. Intravesical pressure changes in response to electrical field stimulation of the isolated bladder were also measured. Values are represented as means ± S.E.M. and analysed using ANOVA. As volume in the bladder increased both intravesical pressure and bladder sensory nerve activity increased. Nerve activity after treatment with CPO or IFO was enhanced throughout bladder filling. At maximum bladder distension the total nerve activity was increased significantly from 182 ± 13 pulses per second (pps) in control animals, to 230 ± 14 pps in CPO treated mice (p<0.05) and 226 ± 17 pps in IFO treated mice (p<0.05) (n=6). Single nerve fibres were identified from each recording and the individual responses of each fibre determined. The number of single fibres located in each treatment group was similar while the firing rate per fibre was enhanced after CPO or IFO treatment compared to control (p<0.05). Each fibre was also characterised as either low threshold (activation at pressures <15mmHg) or high threshold (activation at pressures >15mmHg). The activity of high threshold nerves was unchanged after treatment with CPO or IFO (Fig 1B), but treatment did cause enhanced activity in the low threshold nerves (p<0.05) (Fig 1A). Bladder compliance (pressure-volume relationship) was not affected by systemic CPO or IFO pre-treatment. Similarly, increases in bladder pressure to electrical field stimulation (5s train, 20Hz) were not changed after treatment with CPO or IFO. Increased afferent sensitivity and firing may explain the pain, urgency and feelings of incomplete bladder emptying experienced by patients after treatment with CPO and IFO and provides a target for treating these adverse effects.

Fig 1: The effect of cyclophosphamide (CPO) (100mg/kg) or ifosfamide (IFO) (200mg/kg) treatment on low threshold (A) and high threshold (B) sensory nerve activity (pps per second [pps]) in the mouse bladder.


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The influence of dopamine antagonism on circulating ghrelin levels

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The intestinal motility is regulated by several neurotransmitters and neuropeptides including dopamine and ghrelin. Although ghrelin is known to bear a prokinetic role in the intestinal motility through the recruitment of the cholinergic pathways (Swartz et al., 2014), its relation with dopamine has remained to be investigated. To reveal the influence of type 2 dopamine receptor antagonist on circulating ghrelin levels, enteral and parenteral dopamine antagonists (20 mg/kg, p.o. domperidone and 20 mg/kg, i.p. metoclopramide, respectively) as well as a cholinergic agonist (6 mg/kg, p.o. erythromycin; positive control) were administered to adult male Balb/c mice in a single dose (n=8 for each). The enteral control group (n=8) received distilled water whereas physiologic saline was injected to parenteral controls (n=8). The animals were anesthetized with 2% isoflurane and cardiac blood was obtained. Serum ghrelin levels were estimated using a commercial ELISA kit (Elabscience biotech., China). The experimental protocol was approved by the local ethics and animal use committee. Domperidone and erythromycin led to an increase in serum ghrelin concentration as compared to the enteral controls (one-way ANOVA, F(2,23)=3.542, p=0.047; post hoc LSD test, p=0.032 and p=0.032, respectively). With regard to the change of ghrelin, there was no statistical significance between domperidone and erythromycin administered animals, and enteral and parenteral controls (p>0.05). The metoclopramide treatment resulted in an increase of ghrelin compared to the parenteral controls (Student’s t; t=2.539, df=14, p=0.024). No statistical significance was found in the multiple comparison of the treatments with metoclopramide, domperidone, and erythromycin (p>0.05). Type 1 dopamine receptors induce the secretion of ghrelin (Iwakura et al., 2011), and type 1 and 2 dopamine receptors possess antithetic features in the central nervous system (Eyny and Horvitz, 2003). Thus the change in the levels of ghrelin may be a consequence of the increment of type 1 dopamine receptor activity, although the opposing characters of these two types of dopamine receptors are not obvious yet in the enteric nervous system. On the other side, since type 2 dopamine receptors inhibit the acetylcholine release (Benaroch, 2007), the enhancement of the cholinergic transmission via the antagonism of type 2 dopamine receptors may be responsible for the increase of ghrelin. Conclusively, this study has revealed a stimulating effect of dopamine antagonism on circulating ghrelin levels, and it can be presumed that this influence on ghrelin may contribute to the prokinetic effects of dopamine antagonists.


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PCA251

Cardiovascular autonomic function in hyperthyroid patients

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Background: Autonomic nervous system has a pivotal role in the regulation of cardiovascular system. Thyroid hormones affects sympathetic activity by modulating cardiac adrenergic receptor functioning.

Objective: To study the cardiovascular autonomic function in hyperthyroid patients.

Materials and Methods: The study was conducted on consenting newly diagnosed hyperthyroid patients (n=31) and age and sex matched controls (n=30). For assessment of cardiovascular autonomic function activity, we used heart rate variability (HRV) and for reactivity, autonomic function tests (AFT). Statistics used were independent t-test for parametric and Mann-Whitney U test for non-parametric variables.

Results: Resting systolic BP [(122.52±4.2 vs. 117.6±3.3) mmHg, p=0.004], diastolic BP [(83.17±5.24 vs. 78.87±5.65) mmHg, p=0.003] and pulse rate [(72.07±2.81 vs. 69.24±3.08) bpm, p=0.000] were more in hyperthyroid patients as compare to controls. The BMI [(22.31±2.25 vs. 24.73±3.1) kg/m², p=0.000] was less in hyperthyroid patients. In HRV, parasympathetic activity markers: SDNN [35.2(30.4-45.2) vs. 43.75(34.8-58.825) ms, p=0.011], RMSSD [49.95(36.1-75.75) vs. 34.85(20.05-50.95) ms, p=0.019], NNN50 [8(1-26) vs. 47.5(7-104.5), p=0.007], pNN50 [2.9(0.3-8.7) vs. 13.7(1.675-35.15)%, p=0.007], HF power [182(82-279) vs. 606.5(170.25-1026.25) ms², p=0.006] and HF percent [15.6(8.4-25) vs. 28.75(14.2-41.4) percent, p=0.014] were less in hyperthyroid patients. The sympathetic activity markers: LF nu and LF/HF ratio between groups were comparable. The Poincare plot, SD1 [16(11.2-20.9) vs. 24.7(14.18-36.13) ms, p=0.019] was less in hyperthyroid patients. The parasympathetic reactivity: Valsalva ratio [(1.36±0.08 vs. 1.48±0.11) p=0.003] and heat rate variation in lying-to-standing [(10.04±1.41 vs. 13.3±2.25) bpm, p=0.000] were significantly less in hyperthyroid patients. The sympathetic reactivity: diastolic BP rise in handgrip test [(11.23±2.66 vs. 16.27±3.27) mmHg, p=0.000] was less and systolic BP drop in lying-to-standing [(11.3±5.48 vs. 1.94±2.32) mmHg, p=0.000] was significantly more in hyperthyroid patients as compared to controls.

Conclusion: The newly diagnosed hyperthyroid patients have reduced parasympathetic activity and reactivity, and sympathetic reactivity indicative of reduced stress coping ability.

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Interaction of transient receptor potential vanilloid 1 and anoctamin 1 in primary sensory neurons

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Capsaicin receptor, transient receptor potential vanilloid 1 (TRPV1) is activated by various noxious stimuli, and the stimuli are converted to electrical signals in primary sensory neurons. It is believed that cation influx through TRPV1 causes depolarization, leading to the activation of voltage-gated sodium channels, followed by action potential generation (1). Anoctamin 1 (ANO1), a calcium-activated chloride channel, is also expressed in the TRPV1-positive DRG neurons (2). Therefore, we hypothesized that calcium entering the cells through TRPV1 activation induces ANO1 activation followed by further depolarization in DRG neurons because the intracellular chloride concentrations are maintained at a high level due to low expression of potassium-chloride co-transporter type 2 (3). We suggest the pain enhancement mechanism through the interaction between TRPV1 and ANO1 in DRG neurons. Capsaicin-activated currents were significantly larger in HEK293T cells expressing both TRPV1 and ANO1 than in cells expressing TRPV1 or ANO1 alone. Furthermore, direct interaction between TRPV1 and ANO1 was suggested by immunoprecipitation in both HEK293T cells and DRG, which could effectively drive the TRPV1-ANO1 functional interaction through the increase in intracellular calcium. In addition, in mouse DRG neurons, capsaicin-activated inward currents were significantly inhibited by a specific ANO1 antagonist, T16Ainh-A01 (A01) in the presence of a high concentration of EGTA, but not BAPTA. And the concomitant administration of A01 inhibited capsaicin-evoked action potential generation in DRG neurons probably through blocking the interaction because A01 did not affect the action potential generations by current injection and the currents of voltage-gated sodium, potassium and calcium channels. Furthermore, capsaicin-evoked pain-related behaviors were inhibited by A01.

TRPV1 and ANO1 work as receptors activated by noxious stimuli in sensory nerve endings. It is believed that activation of the two channels causes cation influx and anion efflux, respectively, both of which lead to depolarization. We show that ANO1 is activated by calcium ions entering neurons through TRPV1 activation based on their physical binding on the cell membrane. Indeed, both capsaicin-activated inward currents in sensory neurons and capsaicin-induced pain-related behaviors in mice were significantly inhibited by ANO1 blockade. Thus, the interaction between TRPV1 and ANO1 functions is a pain-enhancing mechanism.


Anxiolytic and antidepressant potentials of chebulinic acid in Swiss mice
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Introduction: Chebulinic acid, an ellagitannin, present in the fruits of Terminalia chebula has shown various positive neuro-logical properties. However, there has not been a report of its antidepressant and anxiolytic potentials. This study was conducted to investigate the antidepressant-like and anxiolytic-like potentials of chebulinic acid, and possible mode of activity in the laboratory Swiss mice.

Methods: Chebulinic acid was evaluated for its antidepressant ( Forced swimming test and Tail suspension test) and anxiolytic (Light-dark box test, Hole-board test and Elevated plus maze) potentials in mice. Moreover, the possible involvement of the serotonergic (metergoline, 4mg/kg, i.p.); cholinergic (atropine, 1mg/kg, i.p.); dopaminergic (sulpiride, 50mg/kg, i.p.), and adrenergic (prazosin, 62.5μg/kg, i.p.) systems in depression were explored. Also, the histology of the hippocampus, and neuronal cell proliferation through immuno-histochemistry were explored. Results were expressed as mean ± SEM. Variance was analyzed using ANOVA, followed by Newman-Keuls multiple comparisons test. P<0.05 was considered to be statistically significant.

Results: Our findings showed antidepressant-like activity of chebulinic acid (10, 20 and 40 mg/kg) with significant reduction in the immobility time (59.2±4.5, 52.7±1.6, 53.5±5.6 sec), respectively in forced swimming test (p<0.001) and (145±2.6, 136.0±9.0, 140.2±3.1 sec) respectively at p<0.05 in tail suspension test when compared with the controls (160.0±4.5 and 164.0±8.9) respectively. However, doses 20mg/kg and 40mg/kg showed significant anxiolytic potentials in elevated plus maze, light-dark box test and hole-board paradigms when compared with the control (P<0.05). Also, mobility was reversed in animals pre-treated with sulpiride, prazosin and metergoline. Histological slides of the hippocampus in chronic pre-treatment with chebulinic acid after 7, 14 and 28 days showed expression of ki-67, which corresponds to the presence of neurogenesis. However, vehicle-treated group showed no expression of ki-67.

Conclusion: Chebulinic acid may possess antidepressant-like and anxiolytic-like potentials which may correspond to its folkloric use in the treatment of neuropsychological disorders. It may exhibit its antidepressant-like potential through cholinergic, adrenergic and serotonergic.

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Poster Communications

Effects of intraventricular ghrelin on synaptic plasticity and long-term potentiation in area CA1 following streptozotocin-induced diabetes
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Hippocampal dysfunction may contribute to hyperglycemia related cognitive impairment, such as that which manifests with diabetes mellitus. The activation of ghrelin receptors in the brain including the hippocampus facilitates high frequency stimulation (HFS)-induced long-term potentiation (LTP) and also improves learning and memory. Here, it is reported that intraventricular ghrelin (200 ng/rat for 7 days) before and after the induction of diabetes by streptozotocin (STZ) prevented impairment of LTP and led to restitution of long-lasting potentiation of excitatory postsynaptic potentials (EPSPs) and population spikes (PSs) in CA1 area of anesthetized rats (male Wistar, 220-300 g, n=18). Anesthesia was induced by intraperitoneal injection of a mixture of ketamine 10% (100 mg kg-1) and xylazine 2% (10 mg kg-1). Animals were divided into three experimental groups: intact (n=6), sham operated (n=6) and STZ-treated (n=6) rats. STZ-treated group were intraperitoneally administered STZ (60 mg kg-1) then received daily ghrelin injections into the brain ventricle for a week. To assess cognitive performance, open-field and passive avoidance tests were performed. Intrahippocampal field potential recordings were done, brains were removed and immunohistochemistry to Bcl-2 and Bax was examined to observe the expression of these proteins in CA1 neurons (2 replicates). Values are means ± S.E.M., compared by one-way ANOVA followed by Newman-Keuls multiple comparison test. The results demonstrated that ghrelin enhanced memory by significantly reducing step-through latencies in diabetic rats and also increased the EPSP slope and PS amplitude [F (2, 17) = 22.59; p<0.001], suggesting the involvement of ghrelin in postsynaptic mechanisms of hippocampal LTP. Additionally, it seems that the Bcl-2/Bax ratio is enhanced and the expression of Bcl-2 was sufficient to prevent apoptosis of hippocampal neurons. It was revealed that neuroprotective effects of chronic ghrelin not only can enhance but also can restore LTP in CA1 area of STZ-induced diabetic rats through inhibition of mitochondrial apoptosis. Therefore, it is suggested that exogenous ghrelin could have therapeutic value in cognitive deficits in diabetes.

Keywords: Diabetes mellitus, Ghrelin, Streptozotocin, CA1 region of hippocampus, Long-term potentiation

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Protease-activated receptor-1 expression is not responsible for high reactivity of platelets and leukocytes in ischemic stroke survivors

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Ischemic stroke is associated with abnormal platelet and leukocyte reactivity [1]. This determines the induction of pro-inflammatory cascade and further brain injury [2]. As it was shown previously cerebral injury progression and complications development depends on expression of one of the thrombin receptors – protease-activated receptor-1 (PAR1), encoded by F2R [3]. The aim of this study was to estimate whether PAR-1 is involved in platelets and leukocytes abnormal reactivity among patients with ischemic stroke.

Methods. Platelets and leukocytes were assessed among 10 patients (males, 54±2.3 years old) with ischemic stroke confirmed by cranial computer tomography. The National Institutes of Health Stroke Scale (NIHSS) scores obtained within 24 hours of acute ischemic stroke symptom onset from patients enrolled in this study was 6-10. In addition to blood cells count, we assessed platelets aggregation induced by thrombin (1,5 NIH) and platelet-leukocyte aggregates (PLA) number in peripheral blood at the time of hospital admission, and after 3, 7 and 14 days after treatment. The PAR-1 mRNA expression was detected by RT-PCR method in isolated platelets and leukocytes. 10 healthy volunteers were taken as a control group.

Results. Ischemic stroke was associated with leukocytosis due to increase of segmented neutrophils percentage rates (p=0,026) and PLA formation in peripheral blood. Their number was significantly higher at the moment of hospital admission (p=0,001) and significantly decreased after 7 days after treatment (p=0,001). Thrombin induced prominent platelet aggregation and PLA formation (p<0,01). However, assessment of PAR-1 expression showed alternative results. Expression of PAR-1 in leukocytes was not detectable both among stroke and control groups. In addition in platelets the PAR-1 expression was low in the most of observed patients (p<0,001).

Recovery of patients was accompanied with increase of the level of PAR-1 mRNA expression by 14 day (p<0,05), however it didn’t reach the control values (p<0,05).

Conclusion. High reactivity of platelets to thrombin and increased PLA formation among patients with ischemic stroke were associated with low PAR-1 expression. It could be related with changes either in PAR-4 expression or in signalling cascades, involved in proinflammatory activation of platelets.

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The rat suprachiasmatic nucleus: the master clock ticks at 30Hz

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The suprachiasmatic nucleus (SCN) of the hypothalamus has an essential role in orchestrating circadian rhythms of behaviour and physiology. In the present study, we recorded from single SCN neurons in urethane-anaesthetised rats (male, 250-450g) using the ventral surgical approach, categorized them by statistical features of their electrical activity and by their responses to light, and examined how activity in the light phase differs from activity in the dark phase. We classified cells as light-on cells or light-off cells according to how their firing rate was acute response to light, or as non-responsive cells. In both sets of light-responsive neurons, responses to light were stronger at subjective night than in subjective day (mean difference of light on cells or light off cells evoked by light; 4.1±0.6 and 1.8±0.4 spikes/s or -1.7±0.4 and +0.2±0.4 spikes/s, each). Neuronal firing patterns were analysed by constructing hazard functions from interspike interval data. For most light-responsive cells, the hazard functions showed a multimodal distribution, with a harmonic sequence of modes, indicating that their spike activity was driven by an oscillatory input with a fundamental frequency of close to 30Hz; this harmonic pattern was very rarely seen in non-responsive SCN cells. The frequency of the rhythm was the same in light-on cells as in light-off cells, the same in subjective day as at subjective night, and was unaffected by exposure to light. Paired recordings indicated that the discharge of adjacent light-responsive neurons was very tightly synchronized, consistent with electrical coupling. Whether the activity of light responsive cells in the SCN is globally synchronised, giving rise to a coherent 30Hz output the amplitude of which is modulated by light intensity, remains to be determined; it is possible that there is only a localised synchronisation with multiple subpopulations discharging out of phase with each other. As both light-on cells and light-off cells discharge in rhythms locked to a 30Hz cycle, an interesting possible is that interactions between these populations mean that their discharge is out of phase (1).


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Post-ictal behavior: Effects of quercetin and rutin

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Since epilepsies are bi-directionally linked to behavioral disturbances (1) and almost one fourth of epileptic patients do not benefit from anti-seizure drugs (2), in this study, we investigated the effects of chronic quercetin and rutin treatments on seizures and post-ictal behavioral alterations. Forty adult male Balb/c mice were equally assigned to four groups which intraperitoneally received either vehicle (Con and PXT), quercetin (50 mg/kg/day; QuePTX), or rutin (50 mg/kg/day; RutPTX) for 21 days. Thereafter, seizure was provoked by 3 mg/kg, i.p. picrotoxin in PTX, QuePTX, and RutPTX groups and graded as defined by Lüttjohann et al. (3). Afterwards, behavioral tests, including Morris’ water maze (MWM), novel object recognition (NOR), open field (OFF), forced swim (FST), and hot plate tests (HPT), were conducted. The Kruskal-Wallis and Dunn’s tests were used for non-parametric data while one-way ANOVA and post hoc Tukey’s tests were used for parametric data. To the results, quercetin and rutin significantly decreased the seizure stage (p<0.01 for both) and delayed onset of seizures (p<0.01 and p=0.04, respectively). The animals in PTX and RutPTX groups exhibited increased locomotion in the OFF which was estimated by the total distance moved (p=0.02 and p<0.01) and velocity (p<0.01 for both). There was no significance for anxiety-related parameters (i.e. time spent in the center zone, grooming, defecation)(p>0.05). In the probe trial of the MWM, the platform latency, time elapsed in the target quadrant, and crossings over the platform area were similar in all groups (p>0.05). Similarly, short-term (1.5 h) and long-term (24 h) memory was not significantly affected by either seizure or the treatments in the NOR (p>0.05). In the FST, the total immobile time was lower (p=0.02 and p=0.03) whereas the climbing duration was higher in QuePTX and RutPTX (p=0.01 and p<0.01) than PTX, although there was no difference between Con and PTX groups (p>0.05). Furthermore, neither treatments were generated a significance in the HPT (p>0.05). In brief, chronic quercetin and rutin treatments seem to attenuate the stage and onset of picrotoxin-induced seizures. Neither a single seizure nor the treatments altered spatial and recognition memory, anxiety-like behavior, and nociception. The seizure did not result in a depressive behavior even though quercetin and rutin generated an antidepressant-like effect. Therefore, quercetin and rutin may be considered as complementary anti-epileptic agents; however, their effects on the post-ictal behavior should be investigated in chronic epilepsy or status epilepticus models to unveil any further potential benefits since a single seizure did not induce behavioral disturbances.


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Kisspeptin in the medial amygdala and sexual behaviour in male rats

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Kisspeptin (Kiss1) plays an important modulatory role in sexual function. Mice that have had the kisspeptin receptor (Kiss1r) knocked out display no sexual behaviour. Recently Kiss1 and Kiss1r have been discovered in the posterodorsal subnucleus of the medial amygdala (MePD) [1], however the role of kisspeptin in this region remains unclear. Given that the medial amygdala is a vital brain region involved in sexual behavior, we hypothesized that Kiss1 in the MePD may have an influence on male sexual behaviour. Adult male Sprague Dawley rats (200-225g) were anaesthetized with ketamine hydrochloride USP (100 mg/kg) and Rompun (10 mg/kg) and positioned in a stereotaxic frame for chronic bilateral cannulation of the MePD [2]. After 3 days of recovery, ex-copula behavioral tests were performed. Ex-copula erections were scored by the emergence of the glans penis from the penile sheath and intensive penile grooming [3] during a 30 min period following infusion of kisspeptin (Kp)-10, kiss1r antagonist (Kp-234) or artificial cerebrospinal fluid (aCSF) into the MePD or lateral cerebroventricle. Correct cannula placement in the MePD was confirmed by microscopic inspection of 30 µm brain sections at the end of the study. Only data from animals with correct cannula placement were analyzed. Values are expressed as mean±S.E.M., and compared by ANOVA. Intra-MePD infusion of 1 nmol Kp-10 caused a significant increase in the number of ex-copula erections compared with aCSF in the 30 min post-treatment period (1.17±0.12, n=12 vs 0.17±0.07, n=5 respectively, P<0.05). These erections were attenuated by Kiss1r antagonist (Kp-234; 5 nmol). (Kp-10; 1.17±0.12, n=12, Kp-234; 0.25±0.06, n=3, P<0.05). There were no observed erections following intra-cerebroventricular injection of Kp-10 (1 or 5 nmol). These results indicate that Kiss1 may play a facilitatory role on sexual arousal by acting as an erentogenic stimulus in the MePD. We conclude that Kiss1 has a role in male sexual behaviour, which is specific to the MePD.


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PCA259

PCA260

Physical exercise training restores the cardiovascular autonomic dysfunction in ageing spontaneously hypertensive rats

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The ageing process is related to a number of disorders that lead to the onset of several pathologies, among them, autonomic dysfunction and hypertension. A sedentary lifestyle impairs cognitive development and cardiovascular performance. By contrast, maintenance of physical activity confers long-term, neuroprotective and cardiovascular benefits. The mechanisms by which physical exercise improve cardiovascular fitness with age are poorly understood. Here, we hypothesized that exercise training is able to improve autonomic dysfunction of hypertensive ageing animals. This study was approved by the Ethical Committee for Animal Research of the University of Sao Paulo (#115/11/03). The hemodynamic effects of aerobic training and physical inactivity was evaluated in ageing (14-months-old) spontaneously hypertensive rats (SHR) compared to normotensive Wistar Kyoto (WKY) rats. Animals from each strain were divided into two cohorts: trained (T) and sedentary (S). The exercise training lasted 8 weeks (1h/day, 5 days/week), with adjustments of speed to 50-60% of the maximum test. Animals were anaesthetised with a mixture of ketamine (100 mg kg$^{-1}$) and xylazine (20 mg kg$^{-1}$, i.p.) for catheterization of femoral artery and blood pressure (BP) and heart rate (HR) monitoring. The baroreflex gain (BRG) was tested with bolus injections of phenylephrine and nitroprussiate (i.v.), and indices of autonomic function from the BP and HR variabilities by spectral analysis of cardiovascular at week zero (S0/T0), in the second (T2) and eighth (T8) weeks of exercise training. Finally, we performed an immunoperoxidase staining for ChAT at the dorsal motor vagus (DMV). The performance on the treadmill of trained WKY animals was higher both in the T2 (1.01±0.05 km/h, n=18) and in the T8 (1.04 ± 0.06 km/h, n=18) when compared to their respective controls S (0.55 ± 0.05, n=27 and 0.35 ± 0.03 km/h, n=27). For SHR the performance on the treadmill of T SHR, were also higher both in the T2 (1.52±0.1 km/h, n=18) and in the T8 (1.27 ± 0.04 km/h, n=18) when compared with their S controls (1.1 ± 0.14, n=27 and 0.87 ± 0.18 km/h, n=27). Mean arterial pressure of T SHR was lower (147±6 mmHg, n=9) than in control group S8 (166±7 mmHg, n=8). The baroreflex sensitivity was improved in T8 (2.9±0.6 bpm/mmHg, n=10) SHR when compared to the S8 group (1.5±0.2 bpm/mmHg, n=10). The HF component of the pulse interval was higher in T8 SHR (22.4±5.9 A.U., n=5) than in the control S8 group (6.3±2.4 A.U., n=5). Finally, ChAT immunoperoxidase reaction was higher in the DMV of T8 SHR compared to S8. Collectively, our data show that exercise training of 8 weeks was effective.
to improve the cardiovascular autonomic dysfunction caused by hypertension in ageing rats.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**PCA261**

Assessment of magnetofection using nanoparticles on sodium and potassium currents in primary cultured cortical neurons

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Magnetofection is a gene targeting technology that uses magnetic iron oxide nanoparticles (MNPs) to enhance vector delivery to target cells through application of a magnetic field. It is suitable for viral and non-viral gene delivery. We have recently established a cell culture of primary cortical neurons from mice that have been genetically engineered using DNA-tagged MNPs. This technique could prove to be useful in establishing genetically modified primary neurons that could promote repair following transfer into sites of injury or degeneration in the central nervous system. To assess whether this type of transfection altered neuronal electrical properties, we have carried out an electrophysiological characterisation of the sodium and potassium currents in these neurons, comparing transfected with non-transfected groups. Cortical primary neurons were harvested from embryonic mouse brains (E18, CD1) and cultured for 7 days at 37°C (5% CO2 / 95% O2) in Neurobasal–B27 supplemented medium without serum at a density of 60x10^3/cm2. MNPs (3.5 µl, mean diameter 160 nm, Neuromag) were complexed with 1 µg DNA (pMAX-GFP, green fluorescent protein) and exposed to neurons for 24 hours on day 7. During this time a magnetic field was applied (4 Hz, 30 min) to promote MNP uptake. When examined under blue excitation light, GFP+ cells exhibited strong green fluorescence in both cell soma and processes. Neurons were whole cell patch clamped at 20°C in neurobasal medium. The intracellular (pipette) solution contained (mM): KCl 140, MgCl2 3.5, Na2ATP 2.5, EGTA 1, HEPES 10, pH 7.4. We found voltage-dependent sodium currents and potassium currents in nearly all neurons studied (two neurons had no clear sodium currents and were excluded from the analysis). A 50 ms voltage clamp step from the holding potential (-60 or -70 mV) to voltages between -40 mV and 0 mV induced a rapidly activating inward sodium current followed by a more slowly activating outward potassium current. We compared sodium and potassium current amplitudes at -30 mV and 0 mV respectively in both groups. Sodium currents were -233 ± 58 pA and -313 ± 96 pA in GFP+ (n = 9) and GFP- (n = 7) groups respectively. Potassium currents were 755 ± 198 pA (GFP+, n = 9) and 787 ± 215 pA (GFP-, n = 7) (mean ± SEM). These means were not significantly different (T-test, p > 0.05). The sodium currents were completely blocked by short (0.7 s) focal applications of 25 µM tetrodotoxin. In some of these cells we examined spiking behaviour in current clamp, and found small action potentials (20-30 mV) in both groups in response to depolarising current steps from -50 mV. We conclude that transfection with GFP via MNP uptake does not significantly alter the sodium and potassium currents in these neurons.
Antinociceptive effect of agomelatine in mice model of acute pain

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Antidepressant drugs are also used in clinical pain treatment in addition to standard analgesics owing to development of tolerance and serious side effects. In this study, the efficiency of new antidepressant agomelatine, as melatonin receptor agonist with high affinity and serotonin receptor antagonist with low sensitivity was scrutinized in acute pain sensitivity in mice.

Adult male Balb/C mice, weighing 30–35g, were obtained from Firat University Medical School Experimental Research Unit (Elazig, Turkey). Animals were allowed to acclimate to the hot plate (50±0.1 °C) for a period of 1 week prior to the experiments. The latency to the first sign of Paw withdrawal latency (PWL) or jump response to noxious heat (pain threshold) of mice was determined using hot-plate analgesia meter. All procedures were in accordance with “Ethical guidelines for investigations of experimental pain in conscious animals”. To prevent tissue damage, a cut-off time of 60 sec was used and after the allocated cut-off time had elapsed, mice were removed from the plate. Physiological saline was administered to the control group (n=7). Pain threshold values were determined and analyzed by Kruskal–Wallis one-way analysis of variance followed by a pairwise comparison using a Dunnett’s t-test. The analgesic effectiveness of different doses of agomelatine was measured. Agomelatine (0.1 mg/kg, 1 mg/kg and 5 mg/kg) didn’t change the PWL, compared to same period of control group (n=7 each group). The highest dose of agomelatine (10 mg/kg) significantly increased the pain threshold in 30th, 60th and 120th minute compared to control (n=7, P<0.05).

Data from this study demonstrated, for the first time, that agomelatine has analgesic action in healthy mice. This result may provide additional insight for a novel target in the development of analgesic drugs.

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Cerebrospinal fluid (CSF) is mostly secreted by choroid plexus (CP) and has a unique composition that is different from plasma. The CSF is in direct contact with the brain interstitial fluid, and the composition of the CSF can reflect the biological processes of the brain (1). Identifying CSF biomarkers in neurodegenerative diseases could monitor the functioning of the brain and aid in the diagnosis. However, the CSF protein changes with advancing age in the absence of disease are not fully elucidated. This study aimed to characterize age-related protein changes in ovine CSF by a proteomic approach. The advantage of using sheep in this study is that adequate CSF samples of all ages can be obtained with easier control over gender selection and environmental factors. Unlike humans, sheep do not develop neurodegenerative diseases in ageing (2). Clun Forest strain adult female sheep aged between 1 and 10 year old were anaesthetized with i.v. thiopentone sodium (20 mg/kg−1) and the CSF samples were collected from the cisterna magna (3). All procedures were within the Home Office Scientific procedures Act, 1986 (HMSO, London, UK). Equal volume of CSF samples from seven of each young (1-2 year old), middle aged (3-6 year old), old (7-10 year old) group were pooled. Up to 90 μg of protein from each group were labelled with iTRAQ reagents and were combined. Proteins were fractionated by 2-dimensional high-performance, liquid chromatography. Tryptic peptides in each spot were both identified (using MS/MS fragmentation ions for sequencing) and quantified from iTRAQ reporter ion intensities at m/z 114, 115, 116 and 117. Biomarker validation were performed with immunoassays, such as ELISA and Western immunoblotting. There were 230 peptides analysed by the MS/MS, among which 224 peptides were identified and belonged to 152 proteins. Seven peptides (6 proteins), neuropeptide Y, neuroendocrine protein 7B2, fibrous sheath interacting protein 1, haptoglobin, haemoglobin, IgM were gradually increased, while histone deacetylase was gradually decreased more than one fold following increased age. Glutathione S-transferase was decreased in middle aged CSF for more than one fold compared to both young or old CSF. Serum paraoxonase/arylesterase 1 (PON1) was increased, while nuclear factor of activated T cells was decreased in old CSF more than one fold compared to both young and middle aged CSF. Some of these biomarkers were validated with commercially available ELISA kits and with Western blotting, such as Neuropeptide Y, Neuroendocrine protein 7B2, IgM, haptoglobin, haemoglobin. In conclusion, this study has identified a number of age-related proteins in ovine CSF, which may help us to elucidate the causes for some neurodegenerative diseases that occur at the old age.

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PCA265

Agomelatine activates intracellular calcium signals in rat primary nociceptive dorsal root ganglion neurons in culture

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Agomelatine is a novel antidepressant, which is a melatonergic agonist for MT1 and MT2 receptors and an antagonist at 5-hydroxytryptamine 2C receptors. Antidepressants have been used a long time at clinic as adjunctive drugs for relief of chronic pain and particular neuropathic pain. However, effects of agomelatine on pain and nociceptive transmission are unknown. The aim of this study was to investigate the effects of agomelatine on intracellular calcium ([Ca2+]i) signaling in cultured rat dorsal root ganglion (DRG) neurons. The effects of agomelatine on [Ca2+]i in DRG neurons were investigated by using an in vitro calcium imaging system. DRG neurons were cultured on glass coverslips following enzymatic dissociation and loaded with the calcium sensitive dye fura-2 AM (1 µM). Intracellular calcium responses in individual DRG neurons were quantified using standard fura-2 based ratiometric calcium imaging technique. Here, we found that agomelatine activated [Ca2+]i transients, selectively in medium and small cultured rat DRG neurons. Agomelatine (10–100mM) activated the Ca2+ signals in a dose-dependent manner in small- and medium diameter DRG neurons (P>0.05). But agomelatine slightly activated the Ca2+ signals in large DRG neurons. We first demonstrated that agomelatine significantly activates calcium signaling in DRG neurons, but further studies are needed for bringing to light the other action mechanism(s) by which agomelatine exerts its nociceptive effect in rat.

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PCA266

Functional rescue of nNOS deficient mouse colon following in vivo enteric nervous system stem cell transplantation

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Enteric neural stem cells (ENSC) have been identified as a possible treatment for enteric neuropathies following successful colonization of recipient gut after transplantation. However, the ability of ENSC to rescue pathophysiological conditions remains unclear. Interestingly, loss of neuronal subtypes, including neuronal nitric oxide synthase (nNOS), has been implicated in enteric neuropathies. nNOS−/− mice display slow colonic transit providing a model to test ENSC rescue in a pathological setting.

Our aim was to assess the functional integration of transplanted ENSC within recipient nNOS−/− colon. Donor ENSC were obtained from Wnt1-cre;YFP transgenic mice for fluorescent labeling and fate mapping of cells. Integration and functionality were assessed using immunolabeling and in vivo and organ bath physiology utilizing electrical field stimulation (EFS).

After 1 month, transplanted cell networks were identified in recipient nNOS−/− colon with spread of 5.19±0.5 mm2; n=8. YFP+/nNOS+ neurons were identified and transcriptional analysis showed specific expression of nNOS in recipient nNOS−/− colon. In vivo analysis showed significant recovery in total gastrointestinal (GI) transit time in transplanted nNOS+ (114.8±3.6 mins, n=5), similar to C57BL/6J controls (117.4±2.6 mins, n=5), compared to non-transplanted nNOS−/− (177±8.15 mins, n=5; P=0.0001). In NANC (non-adrenergic non-cholinergic) conditions, organ bath physiology revealed significant increases in EFS-induced relaxation (Area under curve; AUC) in transplanted nNOS+/− (11.4±0.16g.s,n=5) compared with non-transplanted nNOS−/− (0.30±0.08g.s, n=5; P=0.0016). In transplanted colon segments, addition of the nitric oxide synthase blocker L-NAME resulted in significant reductions in the observed EFS-induced relaxation (0.74±0.17g.s vs -0.12±0.16g.s, n=4; P=0.0389) demonstrating restoration of nitricergic responses after ENSC transplantation.

In addition to partial restoration of nitricergic responses significant increases in basal contractile amplitude were observed in transplanted nNOS+/− colonic segments (0.30±0.06g.s, n=5) compared with both C57BL/6J (0.10±0.01g.s, n=5; P=0.0093) and non-transplanted nNOS−/− mice (0.05±0.08g.s, n=5; P=0.0025). Interestingly these high amplitude contractions were unaffected by application of tetrodotoxin, suggesting that transplantation of ENSC can also lead to potential changes in underlying myogenic motility patterns.

Our experiments show, for the first time, that transplanted ENSC not only integrate but effect restoration of function, at the organ level, in a pathological GI disease model. This recovery of function appears to be associated with both ENSC-specific and non-ENSC-specific processes. Ongoing work is targeting the non-specific processes associated with ENSC transplantation including possible modification of the transplanted cellular microenvironment.
Unravelling mechanotransduction in the locust ear

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Rhodopsin, the light-transducing protein that underpins vision, was discovered 65 years ago. Olfactory and gustatory transduction channels have now also been identified and the function of the sensory neurons in which they operate elaborated. In contrast, the identification of mechanosensory ion channels that underpin the senses of touch, hearing and proprioception has proved more problematic. Mechanosensory neurons bear multiple transduction ion channels, whose expression is scarce, and function depends on many other proteins. Stretch-sensitive neurons of insects (so-called chordotonal organs) have emerged as a useful tool to identify candidate mechanotransduction channels and understand cellular mechanotransduction in general (Kernan, 2007). Despite such progress, it is not known how mechanotransduction operates in insect chordotonal organs, and the identity of the mechanotransduction channels is still unclear. At their apical end, insect stretch-sensitive neurons have a mechanosensory cilium, atop a dendrite. At their basal end an axon carries action potentials to synaptic terminals in the thoracic ganglia.

We have developed whole-cell patch-clamp recordings in the Müller’s organ of the locust ear to study the mechanotransduction current and its encoding into action potentials. We dissected out the oval-shaped tympanal ear and placed it within a hole of a divided petri dish. The inside of the tympanum was perfused in saline and the outside was stimulated using a loudspeaker. Mechanosensory neurons were visualised for patch-clamp recordings with a water-immersion objective using differential interference contrast optics.

We acoustically stimulated the ear and, in conjunction with voltage- and current-clamp protocols and pharmacology, recorded the transduction current and two distinct spike types which, due to their inferred position of generation, are denoted as apical spikes and basal spikes (Hill, 1983). To confirm the sodium-selectivity of both apical and basal spikes we changed from perfusion with normal sodium (213 mM Na+) to low sodium solution (7 mM Na+) which significantly decreased the amplitude of the basal spikes from 3.7 ± 2.2 to 0.9 ± 0.5 nA (mean ± standard deviation, n=10) and apical spikes from 1.3 ± 0.5 to 0.6 ± 0.4 nA (n=9) before abolishing spike generation altogether (spikes recovered after washout: p<0.05, ANOVA) (Figure 1). We blocked both spike types with the sodium channel blocker tetrodotoxin, as found previously by Hill (1983). Current injection into the soma revealed that both spike types were voltage-dependent (Figure 2).

This work provides the basis for understanding how mechanotransduction operates in the sensory neurons of chordotonal organs. We are currently using antagonists and agonists of candidate mechanotransduction ion channels to identify their involvement in passing the transduction current.
the central amygdala (CeA) and bed nucleus stria terminals (BNST), regions involved in the consolidation of fear memory. However, mRNA expressions of the classical RAS pathway (AT$_1$R and ACE) were unaltered in these brain regions. Thus we examined the effect of AT$_2$R on long-term fear memory. Mice received intraperialetal (IP) or intracerebral (ICV) injections of either the novel non-peptide AT$_2$-agonist, Compound 21 (C21), or AT$_2$R antagonist PD123,319 (PD) during classical Pavlovian fear conditioning. Twenty-four hours following fear conditioning both C21 (10 mg/kg IP or 2 μg/kg ICV) and PD (15 mg/kg IP or 200 μg/kg ICV) had no effect on extinction of fear memory compared to the vehicle. However, when fear memory retention was tested, the ICV C21 treated mice exhibited a trend for decreased freezing (C21 - 38%) (t(15) = 1.7; p = 0.1) versus saline (55%) and PD (64%) injected animals. Conclusion: These data suggest that brain AT$_2$Rs and ACE 2 may play a role in fear memory formation and retrieval. Future studies are required to understand the differential regulation of brain angiotensin receptor signaling and the implications for the treatment of PTSD.

Values are means ± S.E.M., compared by ANOVA
Marvar P.J., Goodman J., Fuchs S., Choi D.C., Banerjee S and Ressler, K.J. Antagonist Type 1 receptor inhibition enhances the extinction of fear memory. Biol Psychiatry. 2014 Jun 1;75(11):864-72

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PCA269
Inhibition of endocannabinoid degradation improves anxiety-like behavior and promotes recovery of cognitive deficits following mild traumatic brain injury in rats
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Mild traumatic brain injury (TBI) is a common public health burden affecting otherwise young and healthy individuals. In spite of this, TBI is underreported due to the transient nature of clinical symptoms. Although the overt symptoms like unconsciousness dissipate quickly, less obvious symptoms at the cellular level (including inflammation and excitotoxicity) persist weeks post-TBI. These cellular changes are associated with behavioral alterations including anxiety, depression, and excessive alcohol consumption. There are currently no therapeutic interventions for mild TBI, however, the endocannabinoid system is a promising therapeutic target due to its well-known anti-inflammatory role. Previously we showed that inhibition of monoacylglycerol lipase (MAGL), the enzyme involved in 2-acylglycerol (2-AG) degradation, with JZL184 following mild TBI attenuated neuroinflammation and synaptic hyperexcitability up to two weeks post-TBI. The aim of this study was to examine if common behavioral pathologies resulting from mTBI, including anxiety, pain sensitivity, cognitive deficits, and alcohol drinking, could be reversed during the acute recovery period up to two weeks post-TBI. Adult male Wistar rats underwent a 5-mm left lateral craniotomy, and TBI was induced three days later by lateral fluid percussion. Thirty minutes post-TBI, rats received intraperitoneal injections of vehicle (alcohol, emulphor, and saline; 1:1:18) or JZL184 (16 mg/kg). Resulting experimental groups were sham surgery (n=10), TBI-VEH (n=10), and TBI-JZL (n=10). Anxiety-like behavior (open field test), cognitive deficits (Y-maze), pain sensitivity (von Frey test), and motivated alcohol drinking (progressive ratio operant self-administration) were assessed up to two weeks post-TBI. JZL184 administered-TBI animals had significantly improved cognitive performance (p<0.01 compared to cognitively impaired rats; one sample T-test) and reduction in mechanical sensitivity to pain (p<0.2; two-way ANOVA) compared to vehicle-injected TBI animals. JZL184 administration led to a significant reduction in anxiety-like behavior (p<0.05; one way ANOVA) and a reduction in motivated alcohol drinking (p<0.2; one-way ANOVA) compared to TBI-Vehicle animals. Together with our previous data, these results show that EC degradation inhibition 30 minutes post-TBI has potential therapeutic benefits that persist throughout the acute recovery period up to 14 days post-injury. Current studies are investigating the mechanisms of EC degradation inhibition-mediated improvements from TBI.

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PCA270
Neuroprotective effect of oxytocin during in vivo neonatal hypoxia/hypercapnia in rats
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Accumulating evidence suggests that the neuropeptide oxytocin plays an important neuroprotective role during development. We previously found that oxytocin was neuroprotective on immature hippocampal cultures subjected to oxygen–glucose deprivation. Here we explored in neonatal Wistar rats the neuroprotective potential of nasal administered oxytocin (20 IU/kg BW) 30 min before a 90 min hypoxia/hypercapnia exposure. We found that during the first 2 hours postexposure the loss of resting reflex (LRR) was midway between sham-treated and normoxic rats. Consistently, in oxytocin treated rats the hippocampal damage at 24 hours was attenuated, as assessed by S100β, IL1b and TNFa. At 2 months, there was no EEG evidence of seizures or rhythmic slowing in either oxytocin or sham-treated groups. During deep isoflurane anesthesia, the resulting BS patterns
at burst-suppression ratios (BSR) of 40-80% showed bursts of normal duration, and normal intra-burst EEG. Nevertheless, photic stimulation for 60 seconds caused a transient reduction in BSR. In oxtocin-treatment group this reactivity was also midway between the reactivity of sham-treated and normoxic rats. Our data suggest that oxtocin has a acute neuroprotective effect during peri-natal hypoxic/hypercapnic injury with long-term beneficial effects. Furthermore, measurements of B5 reactivity could emerge as a novel approach to derive functional markers of ischemic injury.

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**PCA271**

Absence of dopamine D2-receptor modulation of NMDA responses in neonatal rat substantia nigra dopaminergic neurones

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Dopamine receptor signalling is essential for normal basal ganglia function. In Parkinson’s Disease (PD) there is a loss of dopaminergic (DAergic) neurons of the substantia nigra pars compact (SNc) with consequent loss of dopamine signalling. SNc DAergic neurons express D2 autoreceptors and somatodendritically released dopamine mediates a negative feedback via D2 receptors on DAergic neuron activity. D2 receptors mediate inhibition of NMDA responses in both hippocampus (Kotecha et al., 2002) and striatum (Higley & Sabatini, 2010). Here we tested whether D2 receptor activation modulates DAergic neuron NMDA responses in SNc using ropinirole, a D2 receptor agonist currently used in PD therapy. Whole-cell patch clamp recordings were made from DAergic neurones in the SNc of acute midbrain slices from neonatal (P7) rats. Brain slices were made in accordance with the guidelines of the UK Animals (Scientific Procedure) Act 1986 and following local ethical approval. DAergic neurones were identified by the presence of a prominent hyperpolarisation-activated inward current (amplitude, 193.4 ± 17.4 pA; activation time constant, 937 ± 73 ms; mean ± S.E.M) in response to a voltage step from -60 to -120 mV. In each cell two successive responses to 20 µM NMDA with 10 µM glycine were recorded. Control experiments gave responses of 919 ± 89 pA (1st response) and 881 ± 113 pA, n = 11 (2nd response). Following application of 20 µM ropinirole, the NMDA response was not significantly different (control NMDA response, 946 ± 88.8 pA; in presence of ropinirole, 895 ± 75 pA, n = 24, paired t-test, P = 0.514).

In order to block G-protein activation, intracellular 0.5 mM GDP-β-S was used (in absence of any added GTP) and allowed to equilibrate for 5 minutes prior to ropinirole application. GDP-β-S did not significantly change the NMDA response (control, 1208 ± 63 pA, n = 6, one-way ANOVA, P > 0.05) and there was no significant effect of ropinirole (NMDA response: 813 ± 163 pA, n = 6, paired t-test, P = 0.05).

As dopamine receptor desensitization could obscure an effect of D2 receptor activation, a novel G-protein receptor kinase (GRK2/3) inhibitor, Cmpd101 (Lowe et al., 2015), was applied (10 µM) intracellularly. Surprisingly, compared to experiments in the absence of Cmpd101 the NMDA response was smaller (one-way ANOVA, P = 0.0004) in the presence of Cmpd101 (control, 417 ± 101 pA, n = 10). However, there was no significant effect of ropinirole (570 ± 77 pA, n = 10, paired t-test, P = 0.289). These results suggest that D2-R activation does not modulate NMDA receptor responses in neonatal rat substantia nigra neurones.


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**PCA272**

Activation of spinal dorsal horn inhibitory networks by the C low-threshold Mechano Receptors derived chemokine tafa4

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Pain elaboration results from the integration within dorsal spinal cord networks of sensory and nociceptive information conveyed by primary afferents. Among these, C low-threshold Mechano Receptors (CLTMRs), expressing the chemokine TAF4A4, were recently identified as modulators of pain. Although TAF4A4 was previously demonstrated to modulate pain transmission, the functional repercussions of this regulation on sensory-nociceptive integration remains poorly understood. We investigated the effects of TAF4A4 on synaptoc transmission in Rexed lamina IIIi in dorsal horn. Using in vitrod patch clamp recording on acute spinal cord slices (mice 3-4 weeks), we demonstrate that in WT mice, application of TAF4A4 (20nM) induces a reversible decrease in frequency of spontaneous excitatory post synaptic currents (EPSC) (means ± S.E.M, paired t-test n=12 control 10.2 ±0.9 vs.TAF4A4 treatment 4.8±0.3 p<0.05). This decrease in excitatory activity is mirrored by an increase in frequency of spontaneous inhibitory synaptic events (IPSC) (n=7 control 1.2±0.2 vs. TAF4A4 treatment 2.7±0.7 p<0.05), indicating a shift of the inhibition/ excitation balance toward increased inhibition. This modulation of synaptic activity was preserved with tetrodotoxine (1uM) (mEPSC n=14 control 3.5±0.9 vs.TAF4A4 treatment 2.5±0.6 p<0.05 mIPSC n=8 control 0.86±0.14 vs. TAF4A4 treatment 2.3±0.4 p<0.05), indicating that TAF4A4 alters synaptic transmission through presynaptic mechanisms. Moreover, by blocking inhibitory activity we demonstrate that the decrease of EPSC frequency is a consequence of the increase
of IPSC frequency (n=9 control 19.4±6.3 vs. TAFA4 treatment 18.7±2.3 p<0.05), showing that TAFA4 releasing fibers mainly interact with inhibitory neurons. Using Electron Microscopy, we demonstrate the presence of direct synaptic contacts between CLTMR and GABAergic terminals within lamina III. In inflammatory condition (sub cutaneous injection of Complete Freund Adjuvant 1mg/ml in the right rear paw) there is a great tendency to relief of the CFA induced mechanic hypersensitivity by TAFA4. We also prove that the effect of TAFA4 on EPSC is preserved (T-test, control n=19 5.8±2.2 vs. TAFA4 treatment n=25 2.0±0.4 p<0.05). To go further in the study of modulation of sensory inputs integration within the spinal cord by TAFA4, we recorded the neuronal discharge of neurons responding to a mechanical stimulus. Using in vivo electrophysiology, we found a decrease of the neuronal discharge after a local injection of TAFA4 (200µg/ml) within the spinal cord indicating a decrease of nociceptive inputs transmission (ANOVA, N = 5 ACSF 135.4±11.5 vs. TAFA4 treatment 62.7±11.2 p<0.05). We propose that CLTMR directly contact inhibitory interneurons in dorsal horn, and, through the liberation of TAFA4, reinforce inhibitory synaptic activity which may in turn promote their anti-nociceptive activity.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA273

The role of renal V2-receptors in regulation of sodium excretion

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Vasopressin (AVP) plays an important role in concentration of urine and water balance maintenance. It enhances solute-free water reabsorption stimulating V2-receptors in collecting tubule and sodium excretion via V1a-receptors in distal nephron. V2-receptors were found in distal nephron. The aim of the study was to investigate the role of renal V2-receptors in regulation of sodium excretion.

The experiments were carried out using Wistar rats. Treatment of the animals was performed in accordance with Russian and EU guidelines on the use of animals in research. Protocols were approved by the Ethical Committee of the Institute. We applied antagonist of V2-receptors (Bachem, Bubendorf, Switzerland) 15 or 50 nmol/kg and sodium load consisting in intraperitoneal administration of 18 ml/kg of 2.5% NaCl intraperitoneally. AVP concentrations were quantified using a commercially available enzyme immunoassay kit (Enzo Life Sciences, Inc., Farmingdale, NY, USA). Diuresis was recorded as spontaneous urination. Osmolality (Micro-Osmometer 3300, Advanced Instruments, USA) and sodium concentration in urine (Sherwood-420 flame photometer, Sherwood Scientific, Cambridge) was measured in each sample. Results were normalized per 100 g bw and presented as M±SEM. All measurements between groups were compared by t-test with Bonferroni correction, p<0.05 was considered significant.

Compared to vehicle the injection of V2-antagonist increased renal solute excretion (246±8 vs. 454±31 µOsm/2 h, p<0.05), renal sodium excretion (7±2 vs. 37±9 µmol/2 h, p<0.05), solute-free water clearance (C H2O) (-0.40±0.05 vs. 1.76±0.26 ml per kg per 2 h) and 17-fold increase of renal AVP excretion was observed. The blockade of V2-receptors led to severe water loss what caused AVP secretion and natriuresis consequently. After sodium load the urine solute and sodium excretion rised to 849±42 µOsm/2 h and to 236±15 µmol/2 h (p<0.05), but C H2O decreased to -2.4±0.14 ml/2 h (p<0.05). The injection of 50 nmol/kg of V2-antagonist following sodium load enhanced solute-free water clearance, but enhanced urinary sodium reabsorption that contributes to increased osmotic gradient in renal medulla necessary for maximal water conservation.

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PCA274

Aggressive behaviour: Systems biology of anger physiology

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Introduction: Anger plays a very vital role in the society where hate crimes and intolerance are rampant. Studies have demonstrated the role of molecular interactions in the development of un-controlled anger. However, the holistic molecular picture that underpins the aggression is still vague.

Objective: To develop the composite molecular network of aggression

Methodology: Using approaches of systems biology, we constructed the composite network of molecular events which underscore the anger physiology. Briefly, molecular network of aggression associated genes were explored by text mining, co-expression, co-localization, physical and/or transcriptional interactions using STRING v9.1. Gradient sieving of the confidence filters starting with 0.9, followed by 0.7 and 0.4 were used to link these networks. In addition to these, lacunae in the molecular network were filled using Reactome Pathway Database and KEGG Pathways.

Result and Discussion: NGF and BDNF proteins showed maximum number of intermediate binding partners, which through their intermediate partner, GIPC1, link with DRD2 receptor. DRD2 in turn associated with DRD3, DRD4 and SLC6A3. These interactions congregate them with the physiological dopamine and other proteins involved in cAMP regulation like HTR1B, AKAP5, OXT, AVP and MAOA. Of these SLC6A3, HTR1B and MAOA form a molecular bridge between dopamine and serotonin physiology. The later involve additional proteins like SLC6A4, TPH1 and TPH2. An auxiliary association was also noted between SLC6A3 with AR which in turn result in the transcriptional regulation via CREB, reflecting the role of testosterone with the anger physiology. Finally, the CREB is known to regulate the expression of BDNF making the entire molecular network link together.

Conclusion: The present data provide preliminary composite picture of molecular events that forms the basis of anger and/or aggression. Such resolved molecular systems network will
be helpful in designing therapeutic intervention against ailments associated or involving aggression.

Key words: Anger, Systems Biology, Molecular Network, Dopamine, Serotonin

Franceschini et al., 2013. Nucl. Acid Research.
Croft et al., 2012. Nucl. Acid Research.

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PCA275
Investigation of Possible Alpha Adrenergic Modification of Acute Pain Sensitivity in Absence Epileptic WAG/Rij Rats
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Aim: The aim of this study was to investigate possible modulatory role of noradrenergic system in absence epileptic WAG/Rij Rat model, which determined to have changed (increased) pain sensitivity.

Methods: Experimental pain was induced in adult WAG/Rij epileptic rats by thermal and mechanical stimulation and effects of intraperitoneally administered yohimbine, an alpha-2 receptor antagonist, treatment on acute interictal (confirmed by simultaneous electroencephalography through intracranial implanted electrodes) pain threshold was assessed at 15, 30 and 60 minutes in vivo nociceptive behavioral “plantar (heat-induced)” and “electronic von Frey (mechanical stimulus) tests by comparing their prospective basal pain threshold values. Statistical analysis of normalised pain threshold values were performed by use of Shapiro-Wilk test followed by repeated measure of paired sampled t test for normally distributed data. P<0.05 value was accepted statistically significant. The protocols of this study was approved by local Ethic Committee.

Results: Administration of yohimbine caused dose-dependent increase, although not statistically significant, in pain sensitivity in von Frey test: mechanical pain threshold values were 1.0±0.0 before and 1.50±0.9, 1.56±0.8 and 1.8±0.9 after 15, 30 and 60 minutes after 1 mg/kg yohimbine (n=6, p>0.05 for all). 1 mg/kg yohimbine did not cause any significant difference in thermal stimulated pain latencies either; pain threshold values were 1.0±0.0 before and 1.1±0.3, 1.2±0.1 and 1.1±0.2 after 15, 30 and 60 minutes after 1 mg/kg yohimbine (n=6, p>0.05 for all).

Mechanical pain threshold values were 1.0±0.0 before and 1.5±0.6, 1.1±0.6 and 1.3±0.5 after 15, 30 and 60 minutes after 3 mg/kg yohimbine (n=7, p>0.05 for all). 3 mg/kg yohimbine significantly reduced thermal stimulated pain latencies; pain threshold values were 1.0±0.0 before and 0.9±0.2 (p<0.05), 0.7±0.2 (p<0.005) and 0.7±0.2 (p<0.005) after 15, 30 and 60 minutes after 1 mg/kg yohimbine (n=7 for all).

Conclusion: Results from this study indicate that, noradrenergic pain modulation is probably (alpha-2 sensitive mechanism being only tested) involved in pain modulation in rat model of absence epileptic WAG/Rij and detailed explanation of this modulation, although other endogenous pain modulating system else needs to be considered, may contribute our current understanding of increased/chronic pain development.

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PCA276
Carotid chemoreceptors tune breathing via a multipath brain stem network modulated by coordinated groups of segmental field-parafacial neurons
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Carotid chemoreceptors modulate breathing via circuits that remain incompletely understood. Extending prior work [1-3], we tested the hypothesis that peripheral chemoreceptors tune ventilation via a distributed brain stem network. Data were from 12 recordings in 11 adult decerebrate, artificially ventilated, vagotomized cats initially anesthetized with isoflurane mixed with air (induction: 5%; maintenance: 0.5–3.0%). Immediately prior to decerebration, an anesthetic assessment was performed [2] and cats were neuromuscularly blocked (vecuronium bromide; initial bolus 0.1 mg kg−1 followed by 0.2 mg kg−1 hr−1 iv). Spike trains were recorded with electrode arrays in the ventral respiratory column (VRC) and (i) the dorsomedial reticular formation (DMR) extending out from the nucleus of the solitary tract, (ii) the lateral segmental field-dorsal parafacial region (FTL), and (iii) the medullary
rphaphithom. Phrenic nerve activity, arterial blood pressure, end-tidal CO$_2$, and tracheal pressure were recorded; PaO$_2$, PaCO$_2$, and pH were monitored. Carotid chemoreceptors were stimulated (5 trials) by close 30-s injections of 1 mL of CO$_2$-saturated 0.9% saline. Neuronal responses were identified with a bootstrap-based statistical method [2]; p-value threshold was set with a false discovery rate (FDR) of 0.05 [4]. Cross-correlogram features were identified with Monte-Carlo tests using surrogatespike trains [3,5]; FDR < 0.05. Of 638 neurons tested, 368 responded with a change in firing rate (VRC: 179/266; DMM: 81/133, FTG: 67/128, and rphaph: 41/111 cells). Overall, 62% of the chemoresponsive neurons were elements of at least one pair with a correlational signature indicative of a paucisynaptic interaction. We note 3 major observations. (i) Thirty-eight pairs of DMM-VRC chemoresponsive neurons provided evidence of directed functional connectivity between these areas (offset peaks, DMMàVRC: 9; VRCàDMM: 17; offset troughs, DMMàVRC: 2; VRCàDMM: 10). (ii) Forty-four of 65 peri-columnar tonic excitatory (t-E) neurons were chemoresponsive; 13 of these 44 were putative targets of neuronal circuit “chains” composed of 2 to 14 antecedent correlationally-linked elements distributed among all monitored regions. For 9 of 10 responsive VRC t-E pairs, we identified a putative shared input from other sampled domains (DMM and/or FTG). (iii) We detected FTL neurons with functional interactions extending over 12 mm (AP) and with up to 19 targets. Twenty-seven FTL neurons (including 19 pairs with central correlogram peaks) had offset correlogram features with 72 distinct chemoresponsive target cells, including 59 outside the FTL. We conclude that carotid chemoreceptors tune breathing via a web of multiple reciprocally linked brainstem circuits incorporating feed-forward and recurrent loops.


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**PCA277**

**Perturbation of Akt signaling, mitochondrial potential and ADP/ATP ratio in acidosis-challenged rat cortical astrocytes**

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Cells switch to anaerobic glycolysis when there is a lack of oxygen during brain ischemia. Extracellular pH thus drops and such acidosis causes neuronal cell death. The fate of astrocytes, mechanical and functional partners of neurons, in acidosis is less studied. In this in vitro study we investigated the signaling in acidosis-challenged cultured Sprague Dawley neonatal rat cortical astrocytes and whether these signals were related to mitochondrial dysfunction and cell death. Results are presented as means ± S.E.M. and analyzed by ANOVA. Exposure to acidic pH (6.8, 6.0) caused cytosolic Ca$^{2+}$ elevation (1.3 ± 0.2 and 2.2 ± 0.3 fold of baseline ratio, respectively; fura 2 microfluorimetric assay; 21-34 cells from 4 separate experiments; p < 0.05), p38 MAPK activation and Akt inhibition (Western blot; N = 4). Mitochondrial membrane potential, as measured by JC-1 fluorescence assay, was hyperpolarized after astrocytes were exposed to acidic pH as soon as 1 h and lasted for 24 h (R/G ratio raised to 119 ± 3 % and 148 ± 7% of control (pH 7.4) at pH 6.8 and 6.0, respectively; N = 3; p < 0.05). Such mitochondrial hyperpolarization was abolished by SC79 (Akt activator; 30 mM; N = 3; p < 0.05) but not by SB203580 (p38 inhibitor; 10 mM) nor by cytosolic Ca$^{2+}$ chelation by BAPTA (10 mM), suggesting that only the perturbation in Akt signaling was causally related to mitochondrial hyperpolarization. SC79, SB203580 and BAPTA did not alleviate acidic pH-induced cell death (MTT assay; N = 4). Using 2,7’-dichlorofluorescin diacetate fluorescent assay, pH 6.8 and 6.0 were observed to suppress reactive oxygen species (ROS) production by 31 ± 3 and 53 ± 3 %, respectively; N = 3; p < 0.05), thus ruling out the role of ROS in cytotoxicity. Interestingly, pH 6.8 increased ADP/ATP ratio (ADP/ATP luminescence assay kit) from 0.49 ± 0.03 (pH 7.4 control) to 1.30 ± 0.09 (N = 4; p < 0.05) and caused apoptosis (FITC Annexin V/Propidium iodide detection kit; N = 3); pH 6.0 increased ADP/ATP ratio to 1.79 ± 0.02 (N = 4; p < 0.05) and caused necrosis (FITC Annexin V/Propidium iodide detection kit; N = 3). Therefore, astrocyte cell death in acidosis did not result from mitochondrial potential collapse; in case of acidosis at pH 6.0, necrosis might partly result from mitochondrial hyperpolarization and subsequent suppressed ATP production.

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**PCA278**

**Innate aerobic capacity and adult hippocampal neurogenesis**

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New neurons are generated in select regions of the mammalian brain throughout life. Adult hippocampal neurogenesis (AHN) is known to be enhanced in response to aerobic exercise, especially when it is sustained and voluntary (Nokia et al. 2016). However, the effects of innate low or high aerobic capacity on AHN are less studied. In general, high aerobic capacity associates with a diminished risk for various diseases and longevity (Koch et al. 2012). To study whether innate aerobic capacity is also associated with AHN we examined young and adult male rats selectively bred for either High or Low Capacity for Running (HCR vs. LCR rats) (Koch & Britton, 2001). Currently, there is a 10-fold difference in running capacity between the rat lines. All the experimental procedures were implemented in accordance with the directive 2010/63/EU of the European Parliament and approved by the National Animal Experiment Board, Finland. At sacrifice, the young rats (generation 36) were 8 weeks and the adult rats (generation 35) 6 months old. The brains were quickly extracted, fixed
Haloanisoles suppress activation of the cyclic nucleotide-gated channel in the olfactory cilia

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We empirically know that unpleasant smells are masked by pleasant fragrances. In 2009, we have reported that certain types of odorants suppress the transduction current in olfactory receptor cells (ORCs)\(^1\). Since there was a big correlation between the channel suppression and human masking test (R=0.81), it is likely that the channel suppression by odorants is tightly linked with olfactory masking. In 2013, it was found that 2,4,6-trichloroanisole (TCA) which is the group of haloanisole\(^2\) served anomalously strong channels suppression. TCA has been known for the cause of degradation of the quality of foods/beverages. Especially, in many vineyards, existence of TCA at pM (ppt) concentration causes big financially problem. In this study, we investigate the mechanism of channel suppression by haloanisoles and analogues. We monitored the current response using whole-cell recording from the isolated newt (Cynops pyrrhogaster) ORCs. The experiments were performed under the latest ethical guidelines for animal experimentation at Osaka University, based on international experimental animal regulations. After caged cAMP was introduced to ORCs beforehand, UV light stimulation was applied to the cilia exclusively. First, we obtained pure transduction channel current by caged photolysis. Second, chemicals were applied to ORCs during the same UV stimulation. From the current suppression, we obtained the efficiency of the substances as suppression ratio (SR). In this study, EC\(_{50}\) of TCA was identified to be 0.19 \(\mu\)M (in a puffer pipette). Other natural suppressor geraniol and artificial CNG channel blocker L-cis diltiazem were also tested, EC\(_{50}\) were 5.8 \(\mu\)M and 29 \(\mu\)M, respectively. It was surprising to see that the least effective concentration of TCA was 1 \(\mu\)M with the U-tube system (SR=0.12±0.02, n=3)(Mean±S.D.). Furthermore, to elucidate the mechanism of strong channel suppression, we focused on the high LogD of TCA (3.87) and high channel density in the cilia (1750 channels/\(\mu^2\))\(^3\). Extremely high efficiency of TCA may be based on high surface to volume ratio of the cilia and dissolultion into the ciliary membrane. We examined the SR of TCA that we changed the membrane lipid components. SRs of TCA were decreased by cholesterol incubation for 2h (SR=0). LogD is an index for the hydrophobicity, therefore haloanisole and analogues may dissolve into lipid bilayer. Then, channel suppression could be caused by conformation change by remaining haloanisoles in the ciliary membrane. These results may suggest that CNG channels are suppressed through a partitioning of those substances into the lipid bilayer. The findings not only reveal a mechanism of flavor loss of foods/beverages, but also suggest certain molecular structures as possible olfactory masking agents and powerful channel blockers.


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PCA279

PCA280

The differential recruitment of parasternal intercostal motor units during inspiration is not preserved for a voluntary postural task

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The human parasternal intercostal muscles are obligatory inspiratory muscles, but there is differential recruitment of parasternal intercostal motor units in the first-to-fifth interspaces (Gandevia et al., 2006). The parasternal intercostals are also active in ipsilateral rotation of the chest wall and we showed previously that the same motor units are active in quiet breathing and voluntary rotations (Hudson et al., 2010). However, it is not known if the differential recruitment of motor units across interspaces, as observed during inspiration, is preserved in a non-respiratory task of chest wall rotation. Healthy volunteer subjects (n=5; all male) were seated and breathed through a pneumotachometer. Intramuscular recordings were made from the parasternal intercostal muscles in the 2\(^{nd}\) and 4\(^{th}\) interspaces while subjects performed quiet breathing and ramped ‘isometric’ ipsilateral rotations of...
the chest wall during apnoea. The recruitment behaviour of single motor units was compared during quiet breathing and rotations. For breathing, the onset of each motor unit (TO) was determined and expressed relative to total inspiratory time (TI) to represent the proportion of TI that the unit was active (i.e. [TI-TO/TI]%). For rotations, the torque at which the motor unit began to discharge was determined as a percentage of maximal rotational torque for each subject. Data were compared between the 2nd and 4th interspaces using t-tests and median [IQR] values are shown. Single motor units active in both quiet breathing and ipsilateral rotations were discriminated from the 2nd (n=44) and 4th (n=56) interspaces. Respiratory parameters during these recordings were matched for inspiratory time and mean flow, but tidal volume was higher during recordings from the 2nd interspace. The inspiratory onset time of motor units in the 2nd interspace was significantly earlier than the 4th interspace. However, for the same motor units, the rotation force at which motor units were recruited was similar in the 2nd and 4th interspaces, for rotations of similar peak and rate of torque. With voluntary drive for the rotation task, there was divergence from the differential recruitment observed during inspiration. This suggests that parasternal intercostal motoneurone output at different spinal levels can change depending on task and supports a spinal mechanism that integrates and distributes descending drive to different human inspiratory muscles.

### Table 1. Respiratory and rotation parameters and onset recruitment behaviour of single motor units (SMU) in the 2nd and 4th parasternal intercostal (PSIC) muscles during quiet breathing or rotations. * significantly different from the 4th interspace.

|       | PSIC | Insp. Time(s) | Mean flow(l/s) | Tidal volume(l) | Peak rotation torque (% max) | Rotation rate (% max/s) | SMU breathing onset [TI]
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<td>2nd</td>
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<td>11.94</td>
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<td>4th</td>
<td>1.97</td>
<td>0.40</td>
<td>0.74</td>
<td>10.94</td>
<td>3.20</td>
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### PCA282

**Macrophage-derived high mobility group box 1 enhances neuritogenesis via NMDA receptors in neuron-like NG108-15 cells**

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**<Introduction>** High mobility group box 1 (HMGB1), a nuclear protein, is passively released by dead or dying cells, and actively secreted by certain alive cells such as activated macrophages (Mφ), contributing to various biological events such as inflammation, proliferation, and differentiation. Extracellular HMGB1 targets multiple molecules including Toll-like receptor 4 (TLR4), the receptor for advanced glycation end products (RAGE), CXC chemokine receptor 4 (CXCRI4), and NMDA receptors [1]. It has been reported that HMGB1 contributes to neuropathic pain after peripheral nerve injury [2] and to axonal regeneration after spinal cord injury [3]. Given the activation of resident and infiltrating Mφ around injured peripheral neurons, we hypothesized that HMGB1 secreted by Mφ plays a role in the repair of injured neurons. We thus investigated the impact of exogenous and endogenous HMGB1 on the neuritogenesis of neuron-like NG108-15 cells in the presence and absence of Mφ-like RAW264.7 cells. **<Methods>** Extent of neuritogenesis in NG108-15 cells is shown as the proportion (%) of cells with longer neurites than the cell body diameter. In a co-culture assay, using 24-well plates with transwell inserts, NG108-15 and RAW264.7 cells were seeded in the plate wells and transwell inserts, respectively. Protein expression of TLR4 and secreted HMGB1 were determined by Western blotting. Data are shown as mean ± SEM. Statistical significance was evaluated by Student's t-test or ANOVA followed by Tukey's test. **<Results>** In NG108-15 cells, stimulation with HMGB1 at 0.5 mg/ml for 24 hours increased neurito-
MK-801 at 10 µM (vehicle (V)+V 24.4 ± 2.3% (p<0.01 vs. V+V), n=4). Lipopolysaccharide (LPS) at 1 µg/ml, a TLR4 agonist, did not cause neurogenesis (V 14.5 ± 1.8%, V+LPS 31.6 ± 3.9% (p<0.05 vs. V+V), control IgY+LPS 33.9 ± 2.5% (n.s. vs. V+LPS), HMGB1-Ab+LPS 16.3 ± 3.5% (p<0.01 vs. V+LPS); V+V 20.3 ± 2.3%, V+LPS 33.5 ± 2.1% (p<0.05 vs. V+V), MK-801+LPS 19.2 ± 1.2% (p<0.01 vs. V+LPS), n=4-6). <Conclusions> Our data thus suggest that Mot-1 derived HMGB1 promotes neurogenesis via NMDA receptors in the neuron-like NG108-15 cells.


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PCA284

Developmental role of sigma-1 receptor in the regulation of hippocampal-CA1 area activity and spatial learning

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Long term potentiation (LTP) has been suggested as a cellular mechanism of learning and memory. NMDA receptors (NMDAR) and non-NMDA receptors (nNMDAR) dependent mechanism has proposed as molecular bases of LTP. Among non-NMDAR dependent mechanisms, sigma 1 receptor has a potential to be the molecular bases of gonadal steroids effect on spatial memory and LTP during adolescence. Field potential recording (in vitro) from CA1 area of hippocampus is used to evaluate the effect of castration and BD107 (20 µM), a sigma-1 receptor antagonist, on synaptic plasticity and water maze is used to study spatial memory. Castration or hippocampal bilateral cannulations were done under 25 mg/kg Xylazine and 75 mg/kg Ketamine anesthesia. All procedures were done according international animal care and use program and supervised by Kermanshah University of Medical Sciences animal welfare and use committee. Three-way, two-way and one way ANOVA were used for statistical analysis. P-value was corrected using Holm–Bonferroni method to avoid Familwise error rate. Data are presented as mean ± SEM.

Castration caused a reduction in the magnitude of both field excitatory postsynaptic potential-long term potentiation (fEPSP-LTP) (167.25 ± 8.85% vs. 124.46 ± 6.44%; Both n=6; P < 0.05) and population spike (PS)-LTP (307.26 ± 25.2% vs. 172.58 ± 14.74%; both n=6; P < 0.01) at 35d. BD-1047 reduced PS-LTP in sham-castrated rats (307.26 ± 25.2%; n=6 vs. 182.14 ± 27.61%; n=5; P < 0.05), whereas BD-1047 reversed the effect of castration on PS-LTP (167.25 ± 8.85%; n=6 vs. 172.63 ± 9.70; n=5; P < 0.5) at 35d. Castration had no effect on fEPSP-LTP (137.13 ± 8.85% vs 0.09 vs. V+V), n=4-6). Lipopolysaccharide (LPS) at 1 µg/ml, a TLR4 agonist, did not cause neurogenesis (V 14.5 ± 1.8%, V+LPS 31.6 ± 3.9% (p<0.05 vs. V+V), control IgY+LPS 33.9 ± 2.5% (n.s. vs. V+LPS), HMGB1-Ab+LPS 16.3 ± 3.5% (p<0.01 vs. V+LPS); V+V 20.3 ± 2.3%, V+LPS 33.5 ± 2.1% (p<0.05 vs. V+V), MK-801+LPS 19.2 ± 1.2% (p<0.01 vs. V+LPS), n=4-6). <Conclusions> Our data thus suggest that Mot-1 derived HMGB1 promotes neurogenesis via NMDA receptors in the neuron-like NG108-15 cells.


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and CA1 area plasticity might be modulated by gonads. However, the role of sigma-1 receptor on spatial learning was age-dependent, whereas its role in spatial learning ability is age-dependent. Activity-dependent CA1-LTP is locality- and age-dependent, whereas its role in spatial learning ability is age-dependent. The regulatory role of sigma-1 receptors (spatial learning ability) plays a role in the regulation of both CA1 synaptic efficacy and neural plasticity dependent rat behavior (spatial learning ability). The regulatory role of sigma-1 receptors in activity-dependent CA1-LTP is locality- and age-dependent, whereas its role in spatial learning ability is age-dependent only. However, the role of sigma-1 receptor on spatial learning and CA1 area plasticity might be modulated by gonads.


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**PCA285**

**Tactile history influences perceived location of touch stimuli**

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Why at times we can feel touch on our skin but not know its location is perplexing. Weak electro-cutaneous stimuli applied to the forearm are erroneously mislocalised towards the forearm’s middle [1]. We found that this phenomenon also holds true for mechanical touch stimuli. Therefore, it is does not simply arise from the stimulus modality. This is likely common to other senses when stimuli are presented under conditions of uncertainty. The responsible mechanism is important, as perceiving the ‘what’ and ‘when’ of things in our environment does not afford an action without knowing the ‘where’. For example, part of the reason more accidents occur in foggy conditions is underestimation of driving speed [2]. One salient reason for an error of ‘where’ under uncertainty is the perceptual integration of the recent history of stimuli. We tested if the perceived ‘where’ was biased toward the centre of the forearm or towards the centre of the recent stimuli distribution. We touched volunteer participants (n=16) on the forearm with a filament that was barely perceptible. We also used a filament that was manfold stronger. At each of four locations, stimuli were applied 20 times, in random order. On separate days the locations were centred about either the proximal or distal end of the forearm. Using their other arm participants pointed to the locations expressed relative to strong stimuli, this shift was seen as a significant main effect of distribution (proximal vs distal forearm) in a three way repeated measures ANOVA (distribution X location X time; F(1,15) = 9.71, p = 0.007). The reason for this is not clear. As subjects returned their pointing hand to a single reference point between trials it might have been a strategy to reduce energy costs of movement. Perceived locations of weak stimuli were computed relative to strong stimuli. They were biased toward the centre of the distribution of stimuli presented in a given experimental session. Regression analysis showed a significant overall compression of 7.8 ± 1.7%.
(p<0.001). This bias suggests that sensory input in the previous minutes or seconds is integrated with the current input to give the ‘where’ perception of touch. This likely functions as a mechanism to minimise localisation errors when the location of touch is uncertain.


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**PCA286**

**Theobromine crosses the blood brain barrier in vivo resulting in increased phosphorylation of vasodilator-stimulated phosphoprotein in the mouse brain**

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Theobromine, a caffeine derivative, is the primary methylxanthine in *Theobroma cacao*. Theobromine works as a phosphodiesterase inhibitor to increase intracellular cAMP and has been shown to have antitumor effects in vitro, inhibiting the growth of a cell line derived from a human malignant glioma (Sugimoto et al., 2014). As glioma is a cancer of the brain, to be an effective therapeutic, theobromine would need to cross the blood-brain barrier to exert its antitumor effect. In this study, we investigated whether orally administered theobromine could cross the blood-brain barrier in mice to act centrally. Vasodilator-stimulated phosphoprotein (VASP), a critical factor in regulating actin dynamics, is phosphorylated by cAMP-dependent protein kinases; thus, the level phosphorylated VASP (pVASP) was used as an index of theobromine activity.

All animal experiments were performed according to the Guidelines for Animal Experimentation of Shimane University Faculty of Medicine. Mice were divided into two groups (n = 6/group), fed either a normal diet or a diet supplemented with 0.05% theobromine for 30 days. Mice were sacrificed at the end of the experiments, and brain and plasma were harvested from mice and kept at -80°C until processing. The concentration of theobromine in brain and plasma samples was measured by high-performance liquid chromatography in combination with ESI–MS, using a TSQ quantum mass spectrometer (Thermo Fisher Scientific K.K., Tokyo, Japan). Plasma glucose concentrations were determined using a Student's t-test. Vasodilator-stimulated phosphoprotein in the mouse brain

**PCA287**

**Lack of CIN85 in the brain causes impairment of maternal behaviour in mice**

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Cbl-interacting protein of 85 kDa (CIN85) is a multi-adaptor protein implicated in the regulation of receptor endocytosis, cell division and the cellular cytoskeleton (Haglund et al., 2002). To investigate the function of CIN85 in the central nervous system, we generated mice deficient of the two major CIN85 isoforms expressed in the brain (CIN85Δex2 mice). Mice deficient of brain-specific CIN85 expression show hyperactive phenotypes. As a molecular explanation of this phenotype, we concluded that the absence of striatal CIN85 causes insufficient complex formation of endophilins with dopamine receptors in the striatum and ultimately decreased dopamine receptor endocytosis in striatal neurons in response to dopamine stimulation (Shimokawa et al., 2010). Here we show another phenotype of CIN85Δex2 mice; that of maternal neglect of the newborn pups, and demonstrate that CIN85 contributes to expression of maternal behavior in the next generation through dopamine-prolactin signaling. Even though there is no difference in the number of live births from CIN85Δex2 homozygote and wild-type mice, almost all pups born to CIN85Δex2 homozygote mothers have died within two days of birth. This could be explained by the fact that CIN85Δex2 mothers showed significantly decreased arching back nursing, a kind of maternal behavior and pups from CIN85Δex2 mothers were often found scattered within the bedding. Moreover, despite of the fact that no defect in the mammary glands of CIN85Δex2 mother mice was found, milk was not detected in the stomachs of most pups. Importantly, when measuring the plasma levels of prolactin, we detected significantly decreased prolactin levels in CIN85Δex2 mice compared to heterozygote and wild-type mice. Prolactin injection in CIN85Δex2 mice during pregnancy (0.07 µg/g body weight, s.c., twice a day) could however partially rescue the defect in maternal behavior of the next generation. Taken together, the low nursing ability by CIN85Δex2 mothers of their newborn pups may partially be due to the lack of exposure to prolactin during their fetal development.
Period. Our findings indicate an important role of CIN85 in the regulation of the dopamine-prolactin system in the brain and provide new insight into a molecular explanation for maternal behavior.


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Relationship of fluoxetine with growth factors and apoptotic proteins in neuron cell culture differentiated from PC-12 cells

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Fluoxetine is a traditional and widely used antidepressant which is used as a selective noradrenaline reuptake inhibitor. Antidepressant therapy has been associated with broad based mechanisms in the brain which growth factors are part of it (Duman et al, 2001). It’s been known that growth factors levels in the brain changes during neuropsychiatric disorders or we can say as changes in the levels of these factors may underlie the mechanisms of these disorders. BDNF (Brain-derived neurotrophic factor) and NGF (nerve growth factor) are widely used growth factors in the brain. The well-known receptor of BDNF is TrkB (Tyrosine receptor kinase B) and its signaling is involved in neuronal differentiation, plasticity and survival. Nonetheless, BDNF causes the phosphorylation of CREB (cAMP response element-binding protein) that is one of important transcription factors for memory and learning in different species (Pizzorusso et al, 2000). Moreover, some studies show that, BDNF, NGF and activation of TrkB protect cells from apoptosis (Nguyen et al 2010, Davey & Davies, 1998). In this study, relationship of fluoxetine was examined with stated neuronal growths, regulator factors and apoptotic proteins by evaluating these protein levels with Western Blot (WB) method. For this purpose, PC12 cells were cultured in standard culture conditions and converted to neuron cells. After transformation, cells were incubated with 10 µM Fluoxetine during 72 hours period. Following the incubation, cells were harvested and lysed. SDS-PAGE and WB were performed for the evaluation of BDNF, TrkB and NGF protein levels. Protein bands were visualized with NBT/BCIP, image J was used for densitometric analysis of proteins and statistical analysis was performed. The data are representative of three independent experiments. p < 0.05 was considered statistically significant. Fluoxetine treatment neuron cells BDNF, TrkB and NGF and CREB protein levels were found to be higher when compared to cells were not treated with Fluoxetine. And also, Caspase 3, BCL-2, BAX, BAK, protein levels were determined as the markers of apoptotic process. Previously, it’s been reported that BDNF levels of depression patients were reduced. Also many other growth factors underlie the mechanism of various neuropsychiatric disorders. This is the preliminary results of our study. Our main study mainly focus on the mechanisms of fluoxetine on some growth factors, secondary messengers and cytokines in the neuron cell culture differentiated from PC 12 cells.

Nguyen TL et al. (2010). Experimental and Molecular Medicine 42, 583-593.

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PCA292

Involvement of macrophage-derived high mobility group box 1 in paclitaxel-induced neuropathic pain in mice

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<Introduction> High mobility group box 1 (HMGB1), a nuclear protein, is released from various cells including macrophages (Mφs), and aggravates inflammation through multiple targets including the receptor for advanced glycation end products (RAGE) and Toll-like receptor 4 (TLR4). NF-kB participates in HMGB1 secretion and also in downstream signals of RAGE and TLR4. Given our recent evidence for the involvement of peripheral HMGB1 in pain processing [1, 2], we investigated and TLR4. Given our recent evidence for the involvement of peripheral HMGB1 in the neuropathic pain induced by paclitaxel (PTX), an anticancer drug, in mice, and analyzed the effect of PTX on HMGB1 secretion in Mφ-like RAW264.7 cells, particularly in relation to NF-kB signals. <Methods> Male ddY mice (20-25 g) received i.p. administration of PTX at 4 mg kg⁻¹ on day 0, 2, 4 and 6, 4 times in total. Neuropathic hyperalgesia was evaluated by determining mechanical nociceptive threshold in the right hindpaw with von Frey test. After decapitation, the sciatic nerve was excised for immunostaining of F4/80, a Mφ marker. An anti-HMGB1 neutralizing antibody (anti-HMGB1) at 1 mg kg⁻¹ and pyrrolidine dithiocarbamate (PDTC), an NF-kB inhibitor, at 50 mg kg⁻¹ were injected i.p. once a day, 7 times in total, from day 0 of PTX treatment. Liposomal clodronate (Cld), known to deplete Mφs, at 1.05 mg per mouse was injected i.p. on day 6 of PTX treatment. RAW264.7 cells were stimulated with PTX at 1 μM for 24 hours in the absence and presence of PDTC at 100 μM. HMGB1 levels in the supernatant were determined by Western blotting. Data are shown as the mean±S.E.M. Statistical significance was performed by Kruskal-Wallis H-test followed by a least significant difference-type test for behavioral data and by ANOVA followed by Tukey’s test for all other data. <Results> PTX treatment caused delayed decrease in nociceptive threshold 9-10 days after the onset of PTX administration, which was inhibited by repeated administration of anti-HMGB1 [vehicle (V)+V 0.59±0.03 g, V+PTX 0.21±0.07 g (p<0.01 vs. V+V), anti-HMGB1+PTX 0.64±0.05 g (p<0.05 vs. V+PTX), n=5], or PDTC [V+V 0.59±0.08 g, V+PTX 0.12±0.02 g (p<0.01 vs. V+V), PDTC+PTX 0.58±0.07 g (p<0.01 vs. V+PTX), n=5]. Depletion of Mφs by Cld also significantly eliminated the PTX-induced hyperalgesia [V+V 0.51±0.04 g, V+PTX 0.15±0.04 g (p<0.01 vs. V+V), Cld+PTX 0.41±0.06 g (p=0.05 vs. V+PTX), n=5]. The F4/80-positive cells increased in the sciatic nerve after PTX treatment [V 6.5±0.7 cells per field, p=0.001, n=16]. In RAW264.7 cells, stimulation with PTX for 24 hours markedly increased HMGB1 secretion, which was inhibited by PDTC [V+V 0.02±0.002 (arbitrary unit), V+PTX 0.88±0.18 (p<0.01 vs. V), PDTC+PTX 0.35±0.10 (p<0.05 vs. V+PTX), n=5]. <Conclusion> Our data suggest that PTX stimulates Mφs, which in turn secrete HMGB1 in an NF-kB-dependent manner, leading to neuropathic pain in mice. Tanaka J et al (2013). Br J Pharmacol 170, 1233-1241.

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PCA293

Sex differences in impaired hippocampal function and memory on a high-fat diet: Peripheral metabolism, neuronal intrinsic excitability, and insulin-sensitivity

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Excess ingestion of energy-dense fats promote obesity, metabolic syndrome and insulin-resistant type-2 diabetes, a worldwide public health crisis. In rat models, a high-fat diet (HFD) fed from weaning severely impairs hippocampal function, critical for memory consolidation. Sex differences are rarely systematically assessed in such models.

Littermate male and female Long-Evans rats were fed from weaning a control or a HFD (57.6% fat, 26.8% carbohydrate, 15.6% protein) for 12 wk. Spatial memory was assessed in spatial objection recognition (SOR) and spontaneous alternation (SAT) tasks. Rats were then deeply anesthetized with 1% isoflurane, and plasma was collected for ELISAs; estrus was assessed by vaginal cytology; and brain slices rapidly prepared. In vitro recordings were made from CA1 pyramidal neurons to assess post-burst AHPs and accommodation, then plasticity induced by bath applied 12.5 nM insulin.

Both sexes consuming HFD showed significantly impaired spatial memory compared to controls. HFD males developed clinically-relevant symptoms of type-2 diabetes (weight gain, loss of blood glucose control, elevated circulating insulin, loss of insulin-sensitivity of CA1 neurons), but HFD females did not (normal body weight, normal blood glucose control, reduced circulating insulin, enhanced insulin-sensitivity of CA1 neurons, with no changes in estrogen nor in estrus). While both males and females exhibited significant deficits on both memory tasks, cognitive deficits in males—presenting with diabetic symptoms—would be easier assess and treat (with strategies appropriate for insulin-resistant diabetes). Females deficits would be covert (no clinical symptoms) and thus likely remain undiagnosed. Given the profoundly different biological responses shown by females to HFD, their cognitive deficits are unlikely to respond to treatments effective in males. These previously undescribed findings suggest different public health issues for females than for males.

Consistent with other findings linking intrinsic excitability to memory, HFD enhanced post-burst AHPs and accommodation in both sexes, i.e. reduced intrinsic excitability. CA1 insulin-sensitivity was lost sex-dependently, only in males, consistent with dysfunctional peripheral glucose regulation and increased circulating insulin, symptoms of insulin-resistant type-2 diabetes. Females maintained glucose control, while circulating insulin decreased, and remained insulin-sensitive both peripherally and centrally, previously undocumented effects. These findings illustrate that while cognitive impairments in females are clinically asymptomatic, diagnostic and treatment strategies for the effects of HFDs on males and females should not be the same.
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PCA294

Contralateral spinal activation after noxious stimulation in an area of secondary hypersensitivity in arthritic rats

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Pain may be present at secondary sites in arthritis, e.g. the contralateral limb; this is termed secondary hypersensitivity. In inflammatory arthritis, contralateral peripheral neurons fire antidromically, suggestive of contralateral spinal neuronal activation. This contralateral neuronal activation is hypothesised to be a protective mechanism, priming the organism for an immediate inflammatory or pain response should the contralateral limb be damaged.

In this study, we tested the hypothesis that noxious stimulation of the ipsilateral hindpaw in knee joint arthritic rats results in greater activation of contralateral spinal neurons than in naïve rats, using Fos-like immunoreactivity (FLI) to identify activated spinal neurons. Inflammatory arthritis was induced in male Wistar rats (250-300g, n=17) under isofluorane anaesthesia (2% in O2) by intra-articular injection of 100µl Freund's complete adjuvant. Controls were naïve (n=14). After 7 days arthritic and naïve rats were anaesthetised (isofluorane induction, followed by i.v. alphaxalone infusion 25mg/kg/h) and A- or C-nociceptors were selectively stimulated in the hindpaw with a contact heat ramp stimulus. Rats were left for 2 hours for development of Fos, overdosed with alphaxalone and perfused fixed with 4% paraformaldehyde. After cryoprotection, 50-100 spinal cord sections from L3-L5 were processed for Fli. The 10 sections in which FLI was highest were previously identified, data on the ipsilateral FLI-positive neurons has been published. Here, FLI-positive neurons were counted in contralateral dorsal horns from naïve (n=5), naïve + A-nociceptor stimulated (4), naïve + C-nociceptor stimulated (5), arthritic (6), arthritic + A-noci stim (5) and arthritic + C-noci stim rats (6). There was a significantly greater number of FLI-positive neurons only in lamina II of arthritic rats when C-nociceptors were stimulated compared to the same stimulation in naïve rats (18±6 vs 6±1.4, mean±SEM p<0.05 Holm-Sidak). There were no significant changes in any other laminae or when A-nociceptors were stimulated. There was no difference in number of FLI-positive neurons in the contralateral dorsal horn between control (naïve) rats and arthritic rats. Stimulation of one hindpaw significantly increased the number of FLI-positive neurons in the contralateral dorsal horn in naïve (2 way ANOVA, effect of stimulation F(2,55)=12.61, p<0.0001; effect of lamina F(4,55)=8.872, p<0.0001, interaction p=0.13) and arthritic rats (2 way ANOVA, effect of stimulation F(2,70)=7.751, p=0.0009; effect of lamina F(4,70)=5.231, p=0.001, interaction p=0.14).

There is greater contralateral activation of lamina II spinal dorsal horn neurons when C-nociceptors are stimulated at secondary sites in arthritic rats compared to naïve rats.

S Kelly, et al. (2007). Sensory nerves have altered function contralateral to a monoarthritis and may contribute to the symmetrical spread of inflammation. Eur J Neurosci. 26: 935-42

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PCA295

Curcumin reverses ethanol-induced locomotor sensitization

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Locomotor sensitization is the augmented locomotory behavior as a result of repeated exposures to an abusable substance, and has been shown to come about with ethanol consumption (1). In a Porsolt’s cylinder which was filled with warm water (25±1°C), two groups (n = 8 for each) of adult male Swiss albino mice were evaluated for their locomotory activity for two minutes following the administration of either solely ethanol (2 g/kg/day, i.p.) or ethanol with curcumin (2 g/kg/day and 50 mg/kg/day, respectively, i.p.) for 15 days. An additional group of mice (n = 8) was allocated as control to which neither treatment, but only locomotion test, was applied. Locomotor activities of mice were evaluated by using a computerized video tracking system (EthoVision XT 10, Noldus, Wageningen, The Netherlands). The data were analyzed with one-way ANOVA following post hoc Tukey’s tests. A p value of less than 0.05 was considered significant. The one-way ANOVA test revealed a significant difference between the experimental groups (F2, 21) = 13.39, p < 0.001). The post hoc test showed that the mice in solely ethanol group had increased locomotor activity as compared to the controls (p < 0.001). However, the locomotor activity of mice on which curcumin applied alongside ethanol was similar to that of the control group (p = 0.112). According to the present results, curcumin reverses ethanol-induced locomotor sensitization. Dopaminergic pathways are involved in the augmented locomotory activity in ethanol sensitization (2) whereas curcumin has been reported to modulate dopamine type 1 and 2 receptor expressions (3). Thus, we speculate that our findings may originate from the modulatory ability of curcumin on dopaminergic innervation.


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Bladder pain accompanying cyclophosphamide (CPA)-induced cystitis in mice involves the upregulation of cystathionine-γ-lyase (CSE), an H2S-forming enzyme, followed by increased activity of Ca3.2 T-type Ca2+ channels, known as targets for H2S [1], and also extracellular high mobility group box 1 (HMGB1) targeting the receptor for advanced glycation end products (RAGE) [2]. We thus analyzed the relationship between the HMGB1/RAGE and CSE/H2S/Cav3.2 pathways in end products (RAGE) [2]. We thus analyzed the relationship between the HMGB1/RAGE and CSE/H2S/Cav3.2 pathways in bladder pain signaling. Female ddY mice (16-24 g) received i.p. CPA at 400 mg kg-1. As reported previously [1], bladder pain-like behavior (BP) was counted from 3.5 to 4 h after CPA treatment, and thereafter, referred hyperalgesia (RH) in the lower abdomen was evaluated by determining nociceptive scores following stimulation with von Frey hairs. After cervical dislocation, the bladder was isolated for measurement of bladder weight (BW) and Western blot analysis of CSE. Data show the mean ± SEM. Statistical significance was analyzed by Kruskal–Wallis H-test followed by a least significant difference-type test for nociceptive score and by ANOVA followed by Tukey’s test for all other data. The anti-HMGB1 antibody (Ab) at 1 mg kg-1, administered i.p., prevented CPA-induced BP [vehicle (V)+V 2.4±1.2, control IgG (IgG)+CPA 35.4±4.8 (p<0.01 vs. V+V), Ab+CPA 13.3±1.8 (p<0.01 vs. IgG+CPA), n=5-6] and RH [total nociceptive score (TNS) from 10 challenges with 0.7 g hair: V+V 6.2±0.58, IgG+CPA 11.4±0.4 (p<0.001 vs. V+V), Ab+CPA 8.1±0.6 (p<0.05 vs. IgG+CPA), n=5-6], but not BW increase, an indicator of swelling [BW (mg g-1 body weight): V+V 0.8±0.04, IgG+CPA 1.77±0.19 (p<0.01 vs. V+V), Ab+CPA 1.89±0.07 (n.s. vs. IgG+CPA), n=4-6]. The CPA-induced upregulation of bladder CSE was also significantly reduced by Ab [V+V 0.057±0.016, IgG+CPA 0.98±0.033 (p<0.01 vs. V+V), Ab+CPA 0.70±0.12 (p<0.05 vs. IgG+CPA), n=5]. Liposomal clonodine (LClo) (1.05 mg per mouse) that depletes macrophages (Mø) prevented the CPA-induced BP [control liposome (cl)+V 0.83±0.48, cl+CPA 39.33±5.06 (p<0.01 vs. cl+V), LClo+CPA 12.5±3.12 (p<0.01 vs. cl+CPA), n=6], RH [TNS: cl+V 5.3±0.61, cl+CPA 13.5±0.76 (p<0.001 vs. cl+V), LClo+CPA 8.6±0.95 (p<0.05 vs. cl+CPA), n=6] and CSE upregulation [cl+V 0.095±0.045, cl+CPA 0.62±0.036 (p<0.01 vs. cl+V), LClo+CPA 0.36±0.095 (p<0.05 vs. cl+CPA), n=5]. FPS-ZM1 (FPS), a RAGE antagonist, preadministered at 1 mg kg-1, suppressed the CPA-induced BP [V+V 1.0±0.63, V+CPA 37.6±2.9 (p<0.01 vs. V+V), FPS+CPA 12.1±1.3 (p<0.01 vs. V+CPA), n=6], RH [TNS: V 3.8±0.75, V+CPA 12.3±0.42 (p<0.001 vs. V+V), FPS+CPA 8.5±0.62 (p<0.05 vs. V+CPA), n=6] and CSE upregulation [V+V 0.11±0.047, V+CPA 1.1±0.10 (p<0.01 vs. V+V), FPS+CPA 0.74±0.10 (p<0.05 vs. V+CPA), n=6]. Thus, Mø-derived HMGB1 is considered to mediate CSE upregulation via RAGE during cystitis, leading to H2S-dependent bladder pain. Matsunami M et al (2012). Br J Pharmacol 167, 917-928.


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Macrophage (Mø)-derived high mobility group box 1 (HMGB1), a nuclear protein, promotes inflammation via Toll-like receptor 4 (TLR4), receptor for advanced glycation end products (RAGE), CXCR chemokine receptor 4 (CXC4), etc. We have shown that HMGB1 mediates bladder pain accompanying cyclophosphamide-induced cystitis in mice, a model for interstitial cystitis (IC)/bladder pain syndrome (BPS) [1]. To study the pathophysiology of BPS without apparent bladder injury, we examined the role of HMGB1 in the bladder pain induced by intravesical (i.v.) administration of substance P (SP) in mice. Under inhalation anesthesia with isoflurane, female ddY mice (17-30 g) received i.v. SP at 6 nmol per mouse (200 µL of 30 µM solution, for 30 min) twice. To evaluate referred hyperalgesia (RH), 24 hours after i.v. SP, nociceptive responses following stimulation of the skin region between the anus and urethral opening with von Frey hairs were scored, as reported previously [1], and the bladder was isolated, weighed and subjected to hematoxylin/eosin staining after cervical dislocation. Data show the mean ± SEM. Statistical significance was analyzed by Kruskal–Wallis H-test followed by a least significant difference-type test for nociceptive score and by ANOVA followed by Tukey’s test for all other data. SP treatment caused RH [total nociceptive score (TNS) from 10 challenges with 0.4 g hair: vehicle (V) 3.17±1.08 vs. SP 10.17±0.4, p<0.01, n=6], whereas it produced no change in bladder histology and only slight increase in bladder weight, an indicator of swelling [V 0.96±0.05 vs. SP 1.23±0.09 mg g-1 body weight, p<0.05, n=6]. The SP-induced RH was prevented by i.p. administration of an anti-HMGB1-neutralizing antibody (Ab) at 1 mg kg-1 [V+V 6.6±0.72, control IgG (IgG)+SP 10.63±0.46 (p<0.001 vs. V+V), Ab+SP 3.5±0.69 (p<0.01 vs. IgG+SP), n=8-10] and also by i.p. liposomal clonodine (LClo) (1.05 mg per mouse) that depletes Mø or ethyl pyruvate (EP), known to inhibit HMGB1 release from Mø, at 80 mg kg-1 [TNS: control liposome (cl)+V 5.4±0.51, cl+SP 9.6±0.24 (p<0.01 vs. cl+V), LClo+SP 6.2±0.37
AMD3100 (AMD), a CXCR4 antagonist, at 8 mg kg⁻¹, but not running in parallel in S1 and S3 sublayers of IPL, while the BC labeled. A lot of varicosities were seen along the AC processes, endings in outer (OPL) and inner (IPL) plexiform layers were the results showed strong TRH immunoreactivity in frog and above mentioned 5-HT receptors were used. More than ten antibodies directed to the key enzyme in sero-retinas were further processed by the indirect immunofluorescence fluorescence for DsRed and c-Fos. All results are mean ± SEM.

PCA298

Tryptophan hydroxylase and serotonin receptors 5-HT1A, 5-HT2A, 5-HT3A, 5-HT4, 5-HT5A, 5-HT6 and 5-HT7 in frog and turtle retina: An immunofluorescence study

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Serotonin (5-hydroxy-tryptamine, 5-HT) is one of the major monoamines in the brain. It is involved in global functions such learning, mood etc. Recently, its neuroprotective function was also revealed. Serotonin is also found in the retina, which is known to be part of brain. A subgroup of amacrine cells (AC) is considered to be the sole retinal source of S-5HT, although some bipolar cells (BC) accumulate and release it (Wilhelm et al, 1993; Shütte, 1994; Chai et al, 2009). Serotonin receptors are poorly studied and the data obtained are too contradictory. That’s why the aim of present investigation was to study the distribution of serotonin synthesizing neurons and serotonin receptors 5-HT1A, 5-HT2A, 5-HT3A, 5-HT4, 5-HT5A, 5-HT6 and 5-HT7 in the retina of frog (Rana ridibunda) and turtle (Emys orbicularis), two lower vertebrates species with mixed and predominantly cone type of retina resp.

All procedures with a frog and a turtle were performed in accordance with the guidelines of Ethical commission of Medical University Sofia, Bulgaria and the EU legislation. The animals were deeply anesthetized in water containing tricain-methansulphonate (1000 mg/L) and decapitated. The retinas were further processed by the indirect immunofluorescence method, as described earlier (Vitanova et al, 2001). More than ten antibodies directed to the key enzyme in serotonin synthesis - tryptophan hydroxylase (TRH), and to the above mentioned 5-HT receptors were used.

The results showed strong TRH immunoreactivity in frog and turtle retinas. The AC and BC perikarya, as well as their final endings in outer (OPL) and inner (IPL) plexiform layers were labeled. A lot of varicosities were seen along the AC processes, running in parallel in S1 and S2 sublayers of IPL, while the BC axons usually had hemi-ring shaped ramifications in S1, S2 and S3. Single horizontal and ganglion cells were also labeled, as well as bundle of parallel axons in the proximal retina. All 5-HT receptors studied were also very well expressed. In frog, numerous labels were found in both plexiform layers that show synaptic localization of serotonin receptors. In turtle, labels in the nuclear layers prevailed that was interpreted as a sign for extra-synaptic effects of serotonin. The 5-HT6 and 5-HT7 receptors, the data for which (as we know) are the first immunocytochemical data seems to be mainly involved in the distal retina information processing. All the results give good ground to affirm that the serotonin retinal sources are more numerous than was previously thought, which is in agreement with recent studies on the TRH mRNA expression in retina (Cornide-Petronio et al, 2013, 2015). The numerous receptors, distributed widely in retinal pool show that serotonin fulfills multiple functions, serving both as neurotransmitter and neuromodulator.

Where applicable, the authors confirm that the experiments described here confrom with the Physiological Society ethical requirements.

PCA299

Functional connection of prolactin-releasing peptide neuromnes from dorsomedial to paraventricular hypothalamic nucleus

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Prolactin-releasing peptide (PrRP) is expressed in three neuronal populations of the brain; the dorsomedial hypothalamus (DMH), the nucleus tractus solitarii and the ventrolateral medulla. We have shown that the thermogenic action of leptin is dependent upon PrRP neurons in the DMH. Others have shown PrRP fibres are present within the paraventricular nucleus of the hypothalamus (PVN), in apposition with oxytocin- and corticotrophin-releasing hormone (CRH)-expressing neurons. Furthermore, intracerebroventricular PrRP activates PVN oxytocin and CRH neurons of wild-type but not PrRP receptor knock-out mice. It is unknown which PrRP population is responsible for these effects, demonstrating the need to decipher the neuronal circuitry involved in mediating the effects of PrRP.

Tracing studies were performed using PrRP-cre::EYFP reporter mice injected with cre-dependent anterograde tracer AAV-hSynaptophysin-mCherry into the DMH (DMH-PrRPmCherry). During surgery mice were initially anaesthetised with 3% isoflurane, and maintained with 1-2%. Mice were left for two weeks before further anaesthesia and perfusion with 4% paraformaldehyde. Projections were confirmed with retrograde tracer injections into the following areas in PrRP-cre::EYFP mice: medial preoptic (MPO), PVN and raphe pallidus (RPa). PrRP-cre::EYFP mice were also injected intra-DHM with a cre-dependent stimulatory DREADD hM3Dq-mCherry (DMH-PrRPmCherry). Neuronal activity was verified by immunohistochemistry using DsRed and GFP antibodies verified spread of tracers and colocalisation with the EYFP reporter. PVN projections were investigated using DsRed and oxytocin antibodies in DMH-PrRPmCherry mice. Neuronal activation of DMH-PrRPmCherry mice was verified by immunohistochemistry for DsRed and c-Fos. All results are mean ± SEM.

DMH-PrRPmCherry injections (n=3) confirmed projections from DMH PrRP neuromnes to the PVN, but also to the MnPO, MPO, as well as local projections in the DMH. No projections were seen to the RPa. Investigation of PVN projections confirmed proximity of PrRP terminals with oxytocin neurons in the PVN (ipsilateral side); 34±6% anterior PVN (n=4), 26±4% medial PVN (n=3) and 49±9% posterior PVN (n=3). Projections to the
PVN (n=4), MnPO (n=5) and MPO (n=4), but not in the RPa (n=3) were confirmed also by colocalisation of retrograde tracer in PrRP DMH neurones. Furthermore, DMH-PrRPβGal mice injected with CNO showed increased c-Fos in the PVN compared with saline-treated mice; 104±17 cells (n=5) vs 28±5 cells (n=6), respectively (p<0.01, unpaired t-test).

Our results show DMH PrRP neurones project to brain areas involved in thermogenesis. In addition, we have shown a functional connection to neurones within the PVN, an important area for modulation of sympathetic activity.


Funded by the BBSRC.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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PCA300

**Hyperoxia increases neuronal responsiveness to hypercapnic acidosis (HA) in caudal solitary complex neurons in rat medullary tissue slices**

G. Ciarelone and J.B. Dean

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Neurons in the caudal nucleus tractus solitarius and dorsal motor nucleus of vagus (caudal solitary complex, cSC) are one of several sites of CO2-chemoreception in the mammalian brain (1). Our previous electrophysiology studies (in vitro) reveal that cSC neurones, particularly CO2/H+-sensitive neurones, are stimulated by hyperoxia and chemical oxidants (2, 3). These findings suggest that CO2-sensitive neurones employ redox and nitrosative signaling mechanisms. In this study, we have tested the hypothesis that CO2-sensitivity in cSC neurones increases with increasing pO2 based on changes in firing rate (AFR, impulses/second = imp/s). A brain slice/intracellular recording (ICR) station was adapted for use inside a hyperbaric chamber so that pO2 could be increased from 0.4 atmospheres absolute (ATA) to 1.95 ATA. Brain slices (400 µm) containing the cSC were harvested from Sprague-Dawley rats (aged P10-P42, both sexes) using methods approved by the USF Institutional Animal Care & Use Committee. ICRs (90-150 MΩ, 3 M K+ acetate) were initiated in cSC neurones in brain slices submerged in artificial cerebral spinal fluid (aCSF; 35-37°C) aerated with (in ATA) 0.4 O2, 0.05 CO2 & 0.55 N2 overlain by an atmosphere of 100% helium (He) pressurized to 2 ATA. aCSF was delivered at 2.5 ml/min using an HPLC pump. Hyperoxia was tested by switching to aCSF aerated with 0.9-0.95 or 1.9-1.95 O2 while HA was tested using 0.1 CO2. Table 1 reports FR responses measured in neurones at various levels of O2 & CO2 dissolved in aCSF at 2 ATA He. The trend was for CO2-chemosensitivity to increase with increasing pO2 with significance at the highest level of O2 tested. These findings suggest that oxidative stimuli, including hyperoxia and potentially redox stress, increase cellular CO2-chemosensitivity and, presumably, the magnitude of the hypercapnic ventilatory response. Parallel, ongoing studies of cSC neurones using fluorescence imaging in rat brain slices indicate that hyperoxia increases various reactive species, including singlet oxygen (4), superoxide, nitric oxide, peroxynitrite, hydrogen peroxide, hydroxyl radical, nitrogen dioxide and carbonate radicals (5). Which reactive species modulate neuronal CO2-sensitivity in the cSC during hypercapnic hyperoxia has yet to be determined.

Table 1. FR responses (imp/s) of cSC neurones to HA as a function of pO2.

<table>
<thead>
<tr>
<th>O2</th>
<th>CO2</th>
<th>FR, impulses/second</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>0.4</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*P = 0.0069 & **P = 0.008, where FR during HA in 0.4 ATA O2 < FR during HA in 1.9 ATA O2; 1-way ANOVA & Sidak’s multiple comparisons.


Ciarelone, G. and J. B. Dean (2016) Hyperoxia and hypercapnic acidosis increase free radical production and cellular excitability in rat caudal solitary complex brain slices. FASEB J. 30, 772.10

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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PCA301

**Role of autophagy in the human microcirculation**

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Background: Autophagy plays a critical role in cellular homeostasis by recycling damaged proteins and organelles. A contribution to the regulation of redox hemostasis has previously been described in vascular endothelial cells. Pathological stress such as elevated ROS can attenuate endothelial function and shear-induced NO formation, which under physiological conditions suppresses mitochondrial ROS production. We have previously shown that onset of disease (coronary artery disease; CAD) changes the endothelial cell response to flow in isolated human microvessels from production of NO to H2O2. We hypothesize that autophagic flux (AF) is necessary for shear-induced release of NO in isolated human microvessels.
(MV) and loss of autophagy results in excess oxidative stress and greater susceptibility to oxidant-induced injury.

Methods: Human MVs (~200µm; MV) obtained from otherwise discarded surgical tissue (adipose and heart) were used for pressure myograph studies. Collection of human tissue samples was approved by institutional review board. Flow mediated dilation (FMD) and its mechanism was evaluated via video microscopy. Statistical significance (p-value of < 0.05) was determined via 2 way RM ANOVA tukey post hoc test (FMD) or t-test (lysotracker).

Results: In cultured endothelial cells shear and Trichostatin A (TSA; 100nM) increased AF significantly and was blockage in the presence of 3-Methyladenine (3-MA; 5 mM) an inhibitor of autophagosome elongation. Using histochemistry and western blots we determined a significant decrease of the autophagy marker LC3B in tissue from subjects with CAD (Fig.1). Incubation of MVs from non-CAD subjects with an inhibitor of lysosome formation, Bafilomycin A (BFA; 10 nM) decreased NO mediated dilation to compare to vehicle treated vessels (Fig. 2a). Interestingly in MVs from subjects with CAD TSA restored the mechanism of FMD to NO form the normally observed pathological H2O2 (Fig. 2b). To confirm these findings we performed IHC (n=3) in isolated vessels of subjects with and without CAD. Similar to the findings in the LV tissue a marked decrease of autophagy was observed vessels from subjects with CAD (B).

Conclusions: Our data suggest, that AF is a critical regulator of endothelial function. Under disease conditions (CAD) AF is reduced and likely contribute to the decrease NO mediated dilation with compensatory increase in H2O2. Pharmacological activation of AF is sufficient to restore a physiological (NO) dilation to flow in subjects with CAD.

Evaluation of Autophagy: LC3B was used to quantify Autophagy in protein from left ventricle tissue (LV; n=4) of subjects with and without CAD. With the presence of CAD autophagy was significantly decreased compared to age matched controls (A). To confirm these findings we performed IHC (n=3) in isolated vessels of subjects with and without CAD. Similar to the findings in the LV tissue a marked decrease of autophagy was observed in vessels from subjects with CAD (B).

Effects of Autophagy inhibition on human microvessels.

Left: Inhibition of fusion of autophagosome and Lysosome with Bafilomycin A1 (BFA) induces a CAD like phenotype in vessels from non CAD subjects after prolonged (15-20h; D) treatment.

Right: In vessels from subjects with clinical diagnosed CAD promotion of autophagosome formation with Trichostatin A (TSA) restores normal NO mediated FMD after prolonged (15-20h) treatment.

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PCA303

Strategical positioning of chromatin anchoring NET protein complexes in the nuclear invaginations of pulmonary arterial smooth muscle cells

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Biomedical Science, The University of Edinburgh, Edinburgh, UK

Nuclear invaginations (NIs) have been identified in multiple cell types, yet their function remains unclear. Because NIs extend the nuclear envelope (NE) into the nucleoplasm, they were proposed to increase the surface area available to support Ca\(^{2+}\) entry into the nucleus and thus regulate gene expression(1). Consistent with this view we have identified that NIs contain chromatin attachment points formed by nuclear envelope transmembrane (NET) proteins that are targeted to the inner and/or outer nuclear membrane (NM) of NI. Pulmonary arterial smooth muscle cells (PASMCs) were isolated from rats, fixed either immediately or after 7 days of culture. Cells were stained with ER tracker or labelled for lamin A, nesprin-1, SUN2 and emerin using immunocytochemistry methods described previously(2). Confocal images were acquired (Nikon A1R), deconvoluted (Huygens) and reconstructed in 3D (Imaris, Bitplane). All data are quoted as mean±SEM, and compared by t-test. Both the Lamin A labelling (n=7) and ER-tracker staining (n=6) revealed trans-nuclear networks of NIs in acutely isolated PASMCs. 61% of cells exhibited deep NIs and 100% of cells harboured superficial NIs. The number of NIs identified per cell was 4±0.41. Labelling for nesprin-1, a trans-outer NM protein, was widely distributed across the entire surface of the NE including NIs, albeit in a punctate manner, and colocalised (Pearson’s correlation coefficient=0.47) with 60% of the lamin A labelling (n=2). SUN2, which combines with nesprin-1 to form the linker of Nucleoskeleton and Cytoskeleton (LINC) complex, colocalised with 46±2% of nesprin-1 labelling (Pearson’s correlation coefficient=0.26±0.09, n=3), and SUN2 labelling appeared to be absent from the centre of some deep NIs. This indicates that the LINC complex, which serves to connect the cytoplasm to the nucleoplasm, may be strategically positioned at the entry point to NIs(3). By contrast, emerin labelling extended across most of the NE and was present along the entire length of the NIs (n=5). Given that this trans-inner NM protein may bind to nesprins directly or through SUN proteins and may either way anchor chromatin to the vicinity of the NE via its binding partners(4), NIs might confer different loci for chromatin attachment through observed targeting of NET proteins to the peripheral NE and to different regions of NIs. Surprisingly, no NIs were found in cultured, proliferating PASMCs and the NE surface area to volume ratio (\(\mu m^2/\mu m^3\)) dropped from 5±0.8 (acutely isolated, n=4) to 2±0.4 (7-day culture, n=4; p<0.01). Given this finding and the fact that the switch from a contractile to proliferative phenotype is underpinned by changes in gene expression(5), it is likely that the NI provides greater surface area for chromatin attachment via NET proteins and thus enhanced suppression of gene expression.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA304

Diabetes differently affects endothelial function in the aorta and pulmonary artery


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Abstract. Diabetes is associated with a higher prevalence of cardiovascular disease, and recently it was shown as a risk factor in the development of pulmonary arterial hypertension (PAH). Regarding the systemic circulation, it is well known that diabetes is associated with vascular dysfunction and an elevation in the systemic pressure, being the endothelial function in aorta compromised in diabetic rats (1, 2). However, it is not consensual that PAH is caused by endothelial dysfunction (3, 4, 5).

Aim. Herein we have investigated the effect of different stages of diabetes on the contractility machinery and endothelial function in the pulmonary and systemic circulation.

Methods. We used 2 pathological animal models: the high-fat (HF) animal, which is submitted to 3 weeks of 60% lipid-rich diet and that represents a prediabetic stage, and the high-fat/high-sucrose (HFHSu) animal, which is submitted to a combined diet of 60% lipid-rich diet and 35% sucrose in drinking water for 14 weeks and that represents an early type 2 diabetes stage. Pathological animal models were compared with age-matched controls. Rats were anesthetized with pentobarbitone (60 mg/kg, ip.) and the pulmonary and aortic arteries were removed and dissected. Contractility was evaluated by small vessels myography in the rings of pulmonary and aortic arteries in response to increasing doses of prostaglandin F2\(\alpha\) (PGF2\(\alpha\)) and expressed as % of the contractile response against 80mM of external K+ (KPSS). Endothelial function was evaluated by monitoring the relaxation effect of acetylcholine over the contraction induced by PGF2\(\alpha\). NO levels in aortic and pulmonary arterial trees were measured.

Results. PGF2\(\alpha\) produced a dose-dependent increase in arterial contractility in pulmonary and aortic arteries in control, HF and HFHSu animals. Pulmonary artery contractility to PGF2\(\alpha\) was significantly enhanced in HF animals, while it was diminished in the HFHSu animals (PGF 30\(\mu M\), expressed as % of KPSS 80mM: control=21.8±2.8, n=20; HF=32.2±4.4 (p<0.001), n=12; HFHSu=10.6±1.8 (p<0.001), n=7. In the aorta we found that the contractile response to PGF2\(\alpha\) increased in both diabetic models (PGF 10\(\mu M\): control=75.3±10.8, n=10; HF=104.8±19.3, n=6; HFHSu=129.4±13.9, n=9). Endothelial function was unaffected in pulmonary artery in prediabetes and early type 2 diabetes animal models, while it was compromised in the aorta in the HFHSu animals, as the dose-response
relaxation curve to ACh was significantly decreased in relation to control animals (p<0.001; control n= 10; HFD+Su n=8).

Conclusions. In diabetes contractility to PGF2α is altered in both the pulmonary artery and aorta. Diabetes affects endothelial function in the aorta but not in the pulmonary artery. This suggests that the pulmonary artery is more resistant to diabetes-induced endothelial dysfunction.


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PCA305

Effect of ambient temperature and obesity on perivascular adipose tissue

P. Aldiss, G. Perez, L. Albustanji, I. Bloom, N. Dellschaft, H. Budge and M.E. Symonds

The Early Life Research Unit, Division of Child Health, Obstetrics and Gynaecology, School of Medicine, University of Nottingham, Nottingham, UK

Obesity and associated excess adipose tissue around the heart and vasculature are major risk factors for atherosclerotic cardiovascular disease (CVD). Perivascular adipose tissue (PVAT) surrounding the aorta is phenotypically brown and thermogenic in nature as demonstrated by the presence of numerous multilocular adipocytes, dense mitochondria and expression of uncoupling protein (UCP). Most rodent studies on adipose tissue function are typically carried out at 20-22°C which is of thermoneutral nature as demonstrated by the presence of numerous unilocular adipocytes, dense mitochondria and expression of uncoupling protein (UCP). Most rodent studies on adipose tissue function are typically carried out at 20-22°C which is well below their thermoneutral zone of 27-30°C (301, H1425-1437, doi:10.1152/amjphisiol.00029.2014 (2014)).


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.  

Poster Communications

Effect of ambient temperature and obesity on perivascular adipose tissue

P. Aldiss, G. Perez, L. Albustanji, I. Bloom, N. Dellschaft, H. Budge and M.E. Symonds

The Early Life Research Unit, Division of Child Health, Obstetrics and Gynaecology, School of Medicine, University of Nottingham, Nottingham, UK

Obesity and associated excess adipose tissue around the heart and vasculature are major risk factors for atherosclerotic cardiovascular disease (CVD). Perivascular adipose tissue (PVAT) surrounding the aorta is phenotypically brown and thermogenic in nature as demonstrated by the presence of numerous multilocular adipocytes, dense mitochondria and expression of uncoupling protein (UCP). Most rodent studies on adipose tissue function are typically carried out at 20-22°C which is well below their thermoneutral zone of 27-30°C (301, H1425-1437, doi:10.1152/amjphisiol.00029.2014 (2014)).


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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Effect of agomelatine on acetylcholine induced contraction of rat bladder in isolated organ bath

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Agomelatine is a new antidepressant drug and has serotonin 2c antagonistic effect together with melatonergic agonist action. There is no evidence about the effect of agomelatine on bladder contractility. Therefore, the aim of this study was to investigate the possible effect of this agent on rat bladder contraction in isolated organ bath.

Adult Wistar rats were terminally anaesthetised and bladder strips were removed from animals after decapitation. All strips placed in a jacketed tissue bath containing Krebs’ solution, constantly gassed with 95% oxygen-5% carbon dioxide. The bladder strips were initially placed under 1g tension and a 30-min equilibration period was allowed before the start of two experimental protocols. Firstly, agomelatine was cumulatively applied to the organ bath for five minutes intervals at a dose range from 10^{-8} to 10^{-3}M for testing its effect on spontaneous contractions. Acetylcholine at 10^{-5}M concentration was administered to the organ bath to induce bladder contraction and same concentrations of agomelatine were cumulatively applied in the second experimental protocol. Peak amplitude values were determined as Mean±SEM and Friedman’s Two-Way Analysis of Variance was used for statistical analysis.

Agomelatine didn’t affect spontaneous bladder contraction (data not shown). The mean amplitude values of acetylcholine induced contractions were 2.01±0.28g (n=7), 1.96±0.18g (n=7), 1.89±0.17g (n=7), 1.80±0.16g (n=7), 1.68±0.13g (n=7), 1.55±0.11g (n=7) and 1.35±0.10g (n=7) in control and after application of 10^{-8}M, 10^{-7}M, 10^{-6}M, 10^{-5}M, 10^{-4}M and 10^{-3}M agomelatine, respectively. Agomelatine significantly inhibited the contractions in 10^{-4}M and 10^{-3}M concentrations (p<0.01 and p<0.001, respectively).

Results from this preliminary study demonstrate for the first time that agomelatine has inhibitory effect on acetylcholine induced bladder contraction in vitro. This effect occurs in a dose dependent manner. Further investigations are needed to clarify the mechanism(s) of agomelatine action on bladder and smooth muscle contractility.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Simvastatin improves microvascular cerebral blood flow and attenuates angiotensin II-induced microcirculatory changes in a hypertension model

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Oswaldo Cruz Foundation - FIOCRUZ, Rio de Janeiro, Brazil

Motivation: Renin-angiotensin system (RAS) plays a central role on the regulation of blood pressure and cerebral blood flow. It has been described that statins may reduce blood pressure through a direct regulation of RAS. Thus, this study was designed to investigate the acute effects of simvastatin (SIM) on cerebral microcirculation and in spontaneously hypertensive rats (SHR), and the possible role of AT2 receptors on statins microvascular effects. Methods: Male Wistar normotensive rats (WKY) and SHR were divided into 4 groups compared with normotensive controls (SHR-CTL 185 ± 2 vs. WKY-CTL 231 ± 13 AU; p<0.05) and SIM treatment was able to increase mCBF (SHR+SIM 208 ± 9 AU; p<0.05) when compared with non-treated SHR (SHR-CTL). Locally applied, Ang II elicited a reduction in mCBF of hypertensive rats and an increase in normotensive rats (SHR-CTL 13.53±2 % vs. WKY-CTL 13.74±4%; p<0.001), which was attenuated in hypertensive rats treated with SIM (SHR+SIM 6.7±1%; p<0.01 vs. SHR-CTL). Additionally, AT2 receptor expression was reduced in the brain of SHR compared with WKY (SHR-CTL 0.5±0.2 vs. WKY-CTL 1.5±0.15 AT2R/GAPDH (AU), p<0). Treatment of SHR with SIM increased brain expression of AT2 receptor (SHR+SIM 3.1±0.9 AT2R/GAPDH (AU); p<0.05 vs. SHR-CTL). Conclusion: Acute treatment with SIM reversed cerebral microvascular rarefaction and restored microvascular cerebral blood flow of hypertensive rats. Furthermore, the increase in AT2 receptor expression might be associated with the positive pleiotropic effects of statins on cerebral microcirculation.

Values are mean ± SD; a Borderline difference vs. CMS-(P<0.08); b Significant difference vs. lowlanders (P<0.05); 1 Recomended Dietary Allowances; 2 Non Defined.


The present research was supported by the University of South Wales and the Bolivian Institute for High-Altitude Studies (IBBA).

Where applicable, the authors confirm that the experiments described here conformed with the Physiological Society ethical requirements.

Table1: Antioxidants intake

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Age-patiarntes (n = 28)</th>
<th>Hypertensives (n = 36)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OMS (n = 20)</td>
<td>OMS (n = 26)</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid (mg)</td>
<td>101 ± 53</td>
<td>95 ± 33</td>
<td>5 ± 30</td>
</tr>
<tr>
<td>a. Tocopherol (mg)</td>
<td>6 ± 0</td>
<td>5 ± 4</td>
<td>1 ± 2</td>
</tr>
<tr>
<td>1-Carotene (µg)</td>
<td>3252 ± 2509</td>
<td>4520 ± 2084</td>
<td>3964 ± 2649</td>
</tr>
<tr>
<td>Values are mean ± SD; a Borderline difference vs. CMS-(P&lt;0.08); b Significant difference vs. lowlanders (P&lt;0.05); 1 Recomended Dietary Allowances; 2 Non Defined.</td>
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</table>
PCA310

Repeated intravesical gemcitabine increases voiding frequency, compliance and urothelial release of ATP and PGE<sub>2</sub> in the murine bladder

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Intravesical chemotherapy for bladder cancer limits systemic absorption but significant local urological side effects including dysuria and urgency of urination still occur. A newer agent, gemcitabine, has a favourable efficacy and toxicity profile in patients compared to other commonly used chemotherapies (Addeo et al, 2010). Here we investigate the effects of repeated intravesical gemcitabine instillations on bladder function. Female C57BL/6J mice (age 32 weeks, n=6 per group), received two intravesical instillations of 0.9% saline (control) or 40mg/mL gemcitabine, 1 week apart under anaesthesia (1-3% isoflurane gas). Micturition was induced 1 hour post-intravesical instillation to expel drug/saline. Voiding pattern analysis (VPA) was conducted (Sugino et al, 2008) prior to both first intravesical instillation and euthanasia by cervical dislocation, 24 h after second instillation. A modified isolated whole bladder preparation (Tanaka et al, 2011) was used to assess bladder compliance and contractile responses. Luminal contents were also collected following distension for analysis of urothelial mediator release. ATP, ACh and PGE<sub>2</sub> levels were quantified using commercially available kits. Data was analysed using one- or two-way ANOVA with Bonferroni post-hoc test. Repeated intravesical gemcitabine instillations significantly increased the number of voiding events (1.9 fold; p<0.05) with a corresponding increase in the number of small voids (2.4 fold; p<0.05) (Figure 1). Release of ATP and PGE<sub>2</sub> into the lumen of the bladder was increased significantly by 2.8 (p<0.001) and 3.2 (p<0.05) fold respectively, while acetylcholine release was unchanged following gemcitabine treatment. Surprisingly, repeat intravesical gemcitabine significantly increased compliance in murine bladders, resulting in enhanced filling volume. Contractile response to carbachol, KCl and ATP was unchanged following gemcitabine treatment, unlike response to electric field stimulation which was significantly depressed at 20Hz (p<0.05). All parameters were unchanged in saline instilled mice. These results indicate that intravesical gemcitabine causes an overactive bladder phenotype, potentially mediated by enhanced urothelial ATP and PGE<sub>2</sub> acting on C-afferent fibres, and depressed effenter nerve mediated contractions.

Figure 1: Total number of voiding events, number of small voids and total voided area were measured pre- and post-repeat intravesical gemcitabine or isotonic saline instillation. Data represents mean ± SEM (n=5). (p<0.05 vs pre-treatment).


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PCA311

Muscarinic receptor-induced contractions of the detrusor are mediated by activation of TRPC4 channels

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Activation of M3 muscarinic receptors (M3Rs) on detrusor myocytes by acetylcholine (ACh), is the predominant mechanism responsible for contraction of the bladder (Andersson, 1993). Muscarinic receptor-mediated contractions of the detrusor rely on Ca<sup>2+</sup> influx through voltage-gated Ca<sup>2+</sup> channels, but the mechanism that links stimulation of M3Rs to activation of voltage-dependent Ca<sup>2+</sup> channels has not been established. Transient receptor potential channel 4 (TRPC4) are receptor operated cation channels that couple muscarinic receptor activation to depolarisation of intestinal smooth muscle cells, voltage-activated Ca<sup>2+</sup> influx and contraction (Tsvilovsky, 2009). The purpose of this study was to investigate if TRPC4 channels are involved in cholineric-mediated contractions of the detrusor.

Isometric tension recordings were made from strips of murine detrusor and intracellular Ca<sup>2+</sup> measurements were made from isolated detrusor myocytes using confocal microscopy. Transcriptional expression of TRPC and IP<sub>3</sub>R isofoms in intact detrusor strips and isolated detrusor myocytes was assessed

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using RT-PCR. All procedures were carried out in accordance with current EU legislation (EU Directive 2010/63/EU) and with approval from Dundalk Institute of Technology Animal Use and Care Committee.

Cholinergic stimulation of the detrusor, induced by electric field stimulation (EFS) or via exogenous application of carbachol (300 mM), or neostigmine (1 μM), evoked two types of contraction; i) a transient, plus tonic response, that was blocked by the TRPC4 blocker, ML204 (10 μM) and ii) a phasic oscillatory response that was blocked by the IP3,R inhibitor, 2-APB (100 μM). ML204 reduced the overall contraction amplitude (expressed as the area under the contraction) in response to 4 Hz EFS from 704.6 ± 127.7 to 400.6 ± 109.9 mN.s, and 2-APB reduced the residual contraction to 15.3 ± 4.8 mN.s (p<0.05, n=7). Similarly carbachol-evoked contractions were reduced from 6220 ± 724 to 1777 ± 232 mN.s, and 2-APB further inhibited the remaining response to 102.2 ± 40.7 mN.s (p<0.01, n=6). Carbachol induced reproducible Ca2+ responses in isolated murine detrusor myocytes. ML204 inhibited the initial component of the response, whereas 2-APB reduced the oscillatory component. For example, ML204 reduced the initial response from 2.85 ± 0.33 to 0.37 ± 0.16 Δ(F/F0) (p<0.001, n=8). RT-PCR experiments showed that TRPC4, TRPC6 and IP3,R1 were selectively expressed in isolated detrusor myocytes. Control experiments showed that ML204 did not affect L-type Ca2+ or BK current amplitude, caffeine-induced Ca2+ transients or KCl-induced contractions of the detrusor. These data show that muscarinic receptor-mediated contractions of the murine detrusor involve activation of TRPC4β channels.


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PCA312

Acetylcholine is an autocrine signalling molecule released by the endothelium in response to flow

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The endothelium is highly sensitive to acetylcholine (ACh) and responds to the neurotransmitter by releasing vasodilators that govern underlying smooth muscle tone. However, ACh released by neurons is not expected to reach the endothelium through the vessel wall or via blood. Despite a lack of a clear physiological role, the endothelium is so exquisitely sensitive to ACh that the neurotransmitter is the most frequently used assay of endothelial function. Here, by studying calcium (Ca2+) signalling in large areas of the endothelium of intact arteries, we show that the endothelium itself is a source of ACh and that ACh underlies flow-mediated vascular responses. Ca2+ signalling was studied in large fields of endothelia (~150 cells) of common carotid and second-observer mesenteric arteries from male (Sprague-Dawley, 150-250g) rats killed by overdose of CO2 (Schedule 1; Animals (Scientific Procedures) Act 1986). In both carotid and mesenteric arteries, flow evoked repeatable, complex endothelial Ca2+ signalling. Flow-evoked responses persisted in Ca2+-free physiological saline solution and were sensitive to 2-aminoethoxydiphenylborate (100μM), cyclopiazonic acid (100μM), and U71322 (5μM), suggesting the Ca2+ rises derived from phospholipase C-dependent, InsP3,-induced Ca2+ release form internal stores. Importantly, flow-mediated Ca2+ responses could be manipulated by modulating cholinergic signalling pathways: the muscarinic receptor blocker, atropine (100nM), and the ACh hydrolase, acetylcholinesterase (AChE; 4U/ml), each abolished flow-induced Ca2+ activity, whilst the AChE inhibitor, neostigmine (10μM), enhanced Ca2+ activity, and the choline acetyltransferase inhibitor, bromaocetylcholine (50μM), attenuated flow-evoked responses. Ca2+ responses were unaffected by the vesicular ACh transporter inhibitor, vesamicol (10μM) but were inhibited by corticosterone (100μM), and abolished by decaenium-22 (1μM), inhibitors of organic cation transporters (OCTs). These results suggest that non-vesicular release of ACh occurs via OCTs. Ca2+ responses were unaffected by the ATP hydrolase, apyrase (4U/ml), the P2 receptor antagonist, suramin (100μM), or the pannexin-1 blocker, probenecid (250μM), suggesting that ATP release does not contribute to the flow response. Flow-evoked signals were also insensitive to tetrodotoxin (10 μM) suggesting that nerves are an unlikely contributor to the response. All treatments tested in the same preparation at multiple time points, and responses compared using one-way ANOVA with Dunnet’s post hoc test (n=3; p<0.01 considered significant).

These results uncover a new role for the classical neurotransmitter, ACh, in endothelial mechanotransduction, identify the endothelium as a source of non-neuronal ACh release by fluid flow and thus demonstrate a physiological reason for the endothelium’s sensitivity to ACh.

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PCA313

Endoplasmic reticulum/ mitochondrial stress in human airway smooth muscle

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Inflammation is a key to a number of diseases that affect airway smooth muscle (ASM) function. Inflammatory cytokines (e.g., TNFα) enhance cytosolic Ca2+ ([Ca2+]cyt) responses to agonist stimulation triggering ASM hyperreactivity. Inflammation also induces ASM cell proliferation and remodeling. We hypothesized that TNFα induces a vicious cycle including reactive oxidant species (ROS) formation, endoplasmic reticulum (ER) stress, mitochondrial fragmentation via increased dynamin-related protein (Drp1) and reduced mitofusin (Mfn2) expression, and uncoupling of mitochondria and ER resulting in dysregulation of mitochondrial and cytosolic Ca2+ responses to agonist stimulation. Human ASM cells were isolated from lung specimens incidental to patient surgery and exposed to TNFα for 24 h. Expression of ER stress markers was evaluated by Western blot including GRP78, PERK, IRE1, ATF6, PDI, ERO-1, and calnexin. Expression of the spliced isoform of XBP1 was also measured by quantitative RT-PCR. Mitochondrial fission/fusion proteins, Drp1 and Mfn2 were measured by Western blot, and mitochondrial morphology was analyzed in ASM cells loaded with Mitotracker Green.
hASM cell proliferation was evaluated using CyQUANT assay. Isolated ASM cells were loaded with Fluo3 and Rhod2 to measure [Ca^{2+}]_{cyt} and mitochondrial ([Ca^{2+}]_{mito}) responses to 1 μM ACh stimulation using high-speed real time confocal imaging. We found that 24-h exposure to TNFα increased ROS formation (MitoSOX fluorescence) and increased and expression of ER stress protein markers, an effect reversed by the ROS scavenger, tempol. Associated with ER stress, we found that Drp1 expression increased and Mfn2 expression decreased and this was associated with increased mitochondrial fragmentation. Exposure to TNFα also resulted in the uncoupling of [Ca^{2+}]_{mito} and [Ca^{2+}]_{cyt} responses to ACh stimulation with a net increase in [Ca^{2+}]_{mito} and reduced [Ca^{2+}]_{cyt}. Exposure to TNFα increased hASM cell proliferation. Together these results indicate that inflammation induces ROS formation leading to ER stress in human ASM cells with downstream changes in Mfn2 and Drp1 expression, mitochondrial fragmentation, uncoupling of mitochondria and ER, and altered cytosolic and mitochondrial Ca^{2+} regulation – all characteristic of ASM hyperreactivity and remodeling in asthma. Supported by a grant from the USA National Institutes of Health - HL126451 and by the Mayo Foundation.

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PCA314

Computational modeling of electrical activities in Isolated Detrusor smooth muscle cells: Role of purinergic neurotransmitter in shaping action potential

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Motivation:
Detrusor smooth muscle (DSM) cells from several families of species invoke spontaneous contractile activity at different frequency [1,5]. Excitation of the DSM cell is achieved through the parasympathetic activation of both purinergic (ATP) and cholinergic (ACh) neurotransmission [3,4]. Spontaneous purinergic neurotransmission was characterized in terms of spontaneous excitatory junction potential (sEJP) that triggers the opening of L-type Ca^{2+} channels to initiate spontaneous action potentials (sAP). Information on how these sEJPs modulate the shape and time course of sAPs in DSM cell is sparse. Here, we have developed an elementary computational model to advance our understanding of sAP modulation due to purinergic synaptic input.

Methods:
We have considered a cylindrical single cell morphology of 200 μm in length and 6μm in diameter. This model has incorporated voltage gated Ca^{2+} (T-type and L-type) channels, three voltage gated potassium (Kdr, Kdrf and Ka) channels and two calcium dependent potassium (BK and SK) channels. These channel models are borrowed from our previous model [2]. The purinergic synaptic conductance profile is consisting of a sum of two exponentials defined in following equation. $g_{\text{syn}}(t) = g'_{\text{syn}} \left( \exp \left( -(t-t_0)/\tau_{\text{rise}} \right) - \exp \left( -(t-t_0)/\tau_{\text{fall}} \right) \right)$ Where $g_{\text{syn}}(t)$ is synaptic conductance, $g'_{\text{syn}}$ is maximum synaptic conductance, $\tau_{\text{rise}}$ is time constant for rising exponential and $\tau_{\text{fall}}$ is time constant for the decay phase exponential. The purinergic synaptic input is injected when time is 300ms.

Results:
The resting membrane potential (RMP) is set at —50mV. The rising time constant $\tau_{\text{rise}}$ and decaying time constant $\tau_{\text{decay}}$ are 5ms and 60ms respectively. The AP is characterized by rising (depolarizing) phase, falling (repolarizing) phase, and presence of an after-hyperpolarization (AHP) or after-depolarization (ADP) phase. In figure 1, Three APs are simulated after injecting Purinergic synaptic input of different values of conductance $g'_{\text{syn}}$. The APs in black, blue and red have $g'_{\text{syn}}$ values of 0.002 μs, 0.0005 μs and 0.0001 μs, respectively. The AP with lower synaptic conductance (red line) shows —14mV of after hyperpolarization and slow rise in depolarization phase. However, the AP with higher synaptic conductance (blue and black line) show after depolarization and fast rise in depolarization phase.

Conclusion:
To the best of our knowledge, this is the first biophysical model of purinergic neurotransmission in DSM cell. As the $g'_{\text{syn}}$ is directly proportional to amount of purinergic neurotransmission, an important implication of our simulation result is that the shape and time course of sAPs are purely neurogenic. Our future efforts will focus on analyzing the impacts of these parameters syncitium model of DSM cells, in order to the AP propagation.

Figure 1. Three APs are simulated after injecting Purinergic synaptic input of different values of conductance $g'_{\text{syn}}$. The APs in black, blue and red have $g'_{\text{syn}}$ values of 0.002 μs, 0.0005 μs and 0.0001 μs, respectively.


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Salvia fruticosa induces vasorelaxation in rat isolated thoracic aorta via a PI3K/AKT/eNOS/NO/cGMP pathway

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Cardiovascular disease continues to be the leading cause of morbidity and mortality worldwide. Accumulating evidence provides reason d’être for the application of herbal therapy in relation to cardiovascular diseases. Herbs of the genus Salvia form a significant component of ethnomedicine approach practiced in the Levant region. One of these herbs, namely Salvia fruticosa (SF) Mill., is traditionally used for its antihypertensive actions. However, there is dearth of data on its pharmacologic and molecular mechanisms of action. Here, after obtaining appropriate ethical approvals, we determined the effects of an ethanolic extract of SF on rings of isolated thoracic aorta from Sprague-Dawley rats. Rings were pre-constricted with norepinephrine (3 µM) and subjected to increasing doses (cumulative) of SF extract in the absence or presence of different inhibitors. In addition, cyclic guanosine monophosphate (cGMP) levels were quantitated using an immunoassay, and Akt phosphorylation was determined by Western blotting. Our results show that SF extract relaxed endothelium-intact rings in a dose-dependent (0.3 µg/ml-1 mg/ml) manner, and the maximum arterial relaxation (E_max) was significantly reduced with removal of endothelial cells. Pretreatment of endothelium-intact aortic rings with L-NAME (a non-selective inhibitor of nitric oxide synthase, eNOS, 100 µM), or ODQ (an inhibitor of soluble guanylyl cyclase, sGC, 10 µM) significantly diminished SF-mediated vasorelaxation. This was paralleled with increased cGMP levels from the aortic rings treated with increasing doses of SF. Prior exposure to PI3-K inhibitors, Wortmannin (0.1 µM) or LY294002 (10 µM), attenuated the SF-induced vasorelaxation by approximately 50% (E_max). The SF-induced relaxation was not affected by indomethacin, verapamil, glibenclamide, tetraethylammonium, pyrimidine or atropine. Taken together, our results indicate that SF induces endothelium-dependent vasorelaxation in rat aortic rings through the PI3-K/Akt/eNOS/NO/sGC/cGMP pathway. Our data illustrate the health-orientated benefits of consuming SF, which would prevent endothelial dysfunction and may thus act as an antihypertensive agent to reduce the burden of cardiovascular complications.


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Selectivity attenuation of inflammatory eNOS stimulation under physiological O2 levels: a role for Ca2+-sensitive PP2A
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Culture of human endothelial cells is routinely undertaken under atmospheric oxygen conditions (18-20% O2), whereas oxygen levels in vivo are typically ∼3-5% within most vascular beds (Ward, 2008). We have previously used an O2-regulated workstation and plate reader to characterise the phenotype of human endothelial cells under defined O2 concentrations and, using an O2 sensitive nanoparticle probe MitoXpress-INTRA, confirmed an intracellular O2 content of 3.6% in HUVEC cultured long-term under physiological (5%) O2 levels (Chapple et al., 2016).

As nitric oxide (NO) signalling is intimately regulated by O2, we here investigate NO signalling in human umbilical vein endothelial cells (HUVEC) cultured long-term under 18% or physiological (5%) O2 levels and then stimulated with the inflammatory mediator histamine or physiological shear stress. Culture of HUVEC under 5% O2 (5 days) significantly decreased histamine (10 μM, 5 min), but not shear stress (15 dynes/cm2, 10 min), stimulated eNOS phosphorylation (3.1- vs 1.8-fold, n=6, P<0.01 2-way ANOVA) and eNOS activity (2.5±0.3 vs 1.6±0.1, mean ± S.E.M., n=4, P<0.05 2-way ANOVA). Despite this, histamine-stimulated cGMP production was unaffected (6.5±1.3 vs 6.5±1.2 pmol/mg, n=6), and exposure to shear stress elicited a significantly greater cGMP production under 5% O2 (5.8±0.7 vs 13.4±2.6 pmol/mg, n=9, P<0.001). Higher NO bioavailability under physiological O2, investigated using mathematical modeling and in vitro experiments with the NO donor DETA NONOate, could account for the disparity between eNOS activity and cGMP production. Treatment with okadaic acid (100 nM, 30 min), a selective inhibitor of protein phosphatase 2A (PP2A), reversed the affects of adaptation to 5% O2 on histamine-stimulated eNOS phosphorylation and significantly increased cGMP production (4.9±0.8 vs 10.1±3 pmol/mg, n=5, P<0.001 2-way ANOVA).

Increased basal microsomal association of PP2A under 5% O2 (14.2±1.3 vs 28.9±3.4%, n=5, P<0.05 2-way ANOVA), facilitated a more rapid interaction with eNOS, assessed by in situ proximity ligation, and therefore accelerated dephosphorylation. This study provides the first evidence that long-term culture of human endothelial cells under physiologically relevant O2 levels significantly alters their phenotype and responses to select vasoactive stimuli. We conclude that culture of endothelial cells under hyperoxic (18% O2) conditions induces an oxidative, inflammatory phenotype that may prime them towards inflammatory stimulation, possibly via delayed targeting of eNOS by PP2A. These novel insights into endothelial physiology in vitro highlight their sensitivity to O2, and the consequences associated with fluctuations away from physiological normoxia (∼3-5%).


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The effect of phosphodiesterase-5 inhibitor tadalafil on the severity of joint injury in rats with adjuvant arthritis

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Rheumatoid arthritis (RA) is an inflammatory systemic and autoimmune disease, characterized by chronic, symmetric and erosive synovitis, mainly of peripheral joints. Although etiology of RA isn’t clearly described, recent research efforts have been focused on oxidative stress. Tadalafil is a selective and potent inhibitor of phosphodiesterase (PDE)-5 for the cure of sexual dysfunction. PDE-5 inhibitors have been proved to reduce oxidative stress to decrease inflammatory events in various experimental models. This study aimed to investigate whether tadalafil has a protective effect on the severity of joint damage using an experimental RA model. Male Sprague-Dawley rats (300-450 g; n=8 per group) were inoculated intradermally into the plantar surface of right hind paw with 0.1 ml of complete Freund’s adjuvant (CFA) containing 10 mg/ml of heat-killed Mycobacterium tuberculosis. Control group received the vehicle (0.1 ml paraffin oil). After being injected with CFA on day-0, tadalafil (10 mg/kg; per oral) was given between days 5-15. Other groups received the quanuyl cyclase inhibitor ODQ (10 mg/kg; intraperitoneally), the non-selective nitric oxide synthase inhibitor L-NAME (25 mg/kg; subcutaneously) or the non-selective cyclooxygenase inhibitor indomethacin (10 mg/kg; intraperitoneally) on day-15 prior to tadalafil. All rats were decapitated after stunning on day-16 and metatarsophalangeal joints, gastrocnemius muscle and trunk blood were sampled. Joints were stained with hematoxylin & eosin and Masson’s trichrome for histopathological evaluation. Muscle samples and blood were used for biochemical assays. The study protocol was approved by Marmara University, Animal Care and Use Committee. Values are means ± S.E.M., compared by ANOVA and Student t-tests. Arthritis group revealed markedly increased muscle luminol- and lucigenin-enhanced chemiluminescence levels showing oxidant production in the arthritis group (4.00±0.69; 13.17±2.47 rlu/mg and 10.69±1.43 rlu/mg, respectively) were attenuated by tadalafil (1.17±0.31, p<0.01; 4.48±1.12, p<0.01 rlu/mg and 3.04±0.33 rlu/mg, p<0.001, respectively); however, other treatments did not change the effects of tadalafil on these parameters. These results suggest that inhibition of PDE-5 enzyme by tadalafil decreases the extent of the histopathological damage in joints and generation of reactive oxygen metabolites in the muscle in a rat model of experimental RA via mechanisms that do not seem to interfere with guanly cyclase, nitric oxide or cyclooxygenase.

PCA319

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PCA320

Urinary aminopeptidase activities in spontaneously hypertensive rats

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The spontaneously hypertensive rats (SHR) is one of the major experimental models of hypertension. In this model, renal damage is pressure-dependent and consists of arterial hypertrophy that leads to the collapse of some glomeruli, tubular atrophy and compensatory hyperfiltration in another population of glomeruli (Hultström, 2012). In previous works, we have demonstrated that urinary aminopeptidase activities can act as early biomarkers of renal dysfunction in cisplatin-treated rats (Quesada et al., 2012; Montoro-Molina et al., 2014). The aim of this work is to study the potential use of urinary aminopeptidase activities as biomarkers of renal dysfunction in SHR.

10 Wistar-Kyoto (WKY) rats and 10 SHR were housed from 8 to 32 weeks of age. Once a month, 24-h urine collection was made. Body weight, food and water intake, and systolic blood pressure (SBP) were also measured. Urine samples were centrifuged and supernatants were frozen at -80 °C. At the end of the experiment, blood samples were obtained from carotid artery under anaesthesia with pentobarbital (50 mg/kg i.p.), and animals were sacrificed with an overdose of pentobarbital (150 mg/kg, ip.). In urine samples we measured dipeptidyl-peptidase-IV (DPP4), glutamyl (Glu) and alanyl aminopeptidase (AlaAP) activities by fluorometry. Proteinuria, urine and serum creatinine (SCR) were analyzed in a Spin120 autoanalyzer. All experimental procedures were performed accord-
ing to the European Union Guidelines to the Care and Use of Laboratory Animals and approved by the Ethical Committee of the University of Jaen.

Urinary AlaAP, GluAP and DPP4 activities (nmol/min/mg creatinine) were significantly increased in SHR at 8, 12, 16, 20, 24, 28 and 32 weeks. Proteinuria (mg/mg creatinine) was increased at 8, 12, 20, 24 and 32 weeks, decreased at 16 weeks and remained unchanged at 28 weeks. We found significant correlations (p<0.001) between aminopeptidase activities and SBP. Proteinuria did not correlate with SBP. At the end of the experiment, Scr (mg/dl) was decreased in SHR (0.36 ± 0.01) vs WKY (0.48 ± 0.02; p<0.001), and creatinine clearance (ml/min/g kidney) was higher in SHR (0.64 ± 0.05 vs 0.46 ± 0.02; p<0.01).

These findings suggest that urinary aminopeptidase activities can be used to assess pressure-dependent renal damage in SHR. Besides, Scr concentration is decreased in SHR due to glomerular hyperfiltration. Therefore, these enzymes could have a potential application in the evaluation of chronic kidney diseases that could course with glomerular hyperfiltration, especially when Scr cannot be used as a marker of renal dysfunction.


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**PCA321**

**Voltage- and calcium-dependent mechanisms of vascular Maxi-K channel activation by plasmonic gold nanocrystals**

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We have previously reported potent activation of large-conductance Ca2+-activated K+ channels (Maxi-K) in thoracic rat aorta smooth muscle (SM) by plasmonic gold nanocrystals (AuNCs) ~5 nm core size having plasmon resonance of 532 nm. Green laser irradiation facilitated this effect and caused SM relaxation (1, 2). The aim of this study was to uncover the intrinsic mechanisms of Maxi-K regulation by AuNCs. Maxi-K currents in isolated rat aortic and pulmonary artery myocytes were recorded at room temperature in the whole-cell and cell-attached configurations. Maxi-K channels are both Ca2+ and voltage-sensitive, hence we compared the effects of NCs on Maxi-K channel activity under weak (0.3 mM EGTA) or strong (10 mM BAPTA/4.6 mM Ca2+) to “clamp” the intracellular Ca2+ concentration at 100 nM. With weak Ca2+ buffering, Maxi-K current density at 70 mV was increased by AuNCs applied at 10^-4 M from 50±5 pA/pF to 130±6 pA (P<0.001; n=6), and further increased to 205±7.6 pA (n=6; P<0.001) by laser illumination. The potentiating effect of AuNCs alone was due to an increase in maximal conductance (Gmax) by about 50% without any shift of the activation curve of K+ conductance (the potential of half-maximal activation V1/2 was -89.6±2.4 mV and -91.0±1.99 mV in control and in the presence of AuNCs, respectively, n=6). Interestingly, green laser illumination (5 mW, 532 nm) had no significant effect on the maximal K+ conductance (2.09±0.08 and 2.18±0.06 nS/pF before and after laser illumination, n=6), but instead shifted the V1/2 value to -79.6±1.5 mV, n=6; P<0.05). Under conditions of strong intracellular Ca2+ buffering, no effect of AuNCs on current density was observed (54±4 pA/pF in control vs 55±4 in the presence of 10^4M AuNCs; P>0.05, n=6). Gmax and the V1/2 values also remained largely unchanged. At the single channel level, the effects of AuNCs were due to a significant increase in channel open probability, while single channel conductance remained unchanged. Intriguingly, the independent gating of maxi-K channels (e.g. binomial distribution test) was not observed, suggesting that plasmon resonance may not equally affect all channels in the patch, which is different from other common types of drug action. We conclude that AuNCs activate Maxi-KCa channels via both Gmax increase and a negative V1/2 shift. These processes are clearly calcium-dependent, as the potentiating effect of AuNCs could be completely abolished by “clamping” the intracellular Ca2+ concentration at 100 nM. This is an important step towards not only better understanding of the nature of this effect, but also the development of new methods of nano-photon control of voltage-gated ion channels function in living cells that, in turn, may have significant promise for developing next generation of ion channel modulators with high target specificity and low toxicity.


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**PCA322**

**Quercetin-filled phosphatidylcholine liposomes significantly increase Maxi-K channel activity in ileum smooth muscle cells**

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Flavonoids are present in many plants and isoflavone 3, 3’, 4’, 5, 7-penta-hydroxylflavone (quercetin) is one of the widely distributed and well known bioflavonoids, which produce a number of biological effects including apoptosis, protein kinase C and lypoxygenase inhibition, and superoxide dismutase-like activation. Considering that protein kinase C and related reactive oxygen species (ROS) overproduction are involved in a number of vascular abnormalities, it seems very relevant to
address the possibility that quercetin may be therapeutically beneficial under oxidative stress. It was shown in preliminary studies (1, 2) that free quercetin and quercetin-filled phosphatidylcholine liposomes (PCL-Q) effectively normalize endothelium-dependent vascular smooth muscle (SM) relaxation and outward macroscopic currents carried by large conductance Ca\(^{2+}\)-dependent K\(^+\) channels (Maxi-K) in rat thoracic aorta smooth muscle (SM) cells inhibited under ionized irradiation but underlying mechanisms remain unclear. The aim of this study was thus to investigate the effects of PCL-Q on single Maxi-K channel activity in mouse ileac myocytes. Cells were isolated from mouse (male, 2 month old) ileum longitudinal SM by enzymatic digestion (1 mg/ml, collagenase 1A, 1 mg/ml, soybean trypsin inhibitor, 1 mg/ml, bovine serum albumin, 18 min at 36.5°C). Single Maxi-K channel currents were recorded in the cell-attached configuration using hyper-K\(^+\) (130 mM) external solution in the bath to bring the resting membrane potential to about 0 mV and normal physiological salt solution containing 6 mM K\(^+\) in the pipette. Channel activity was expressed as NPo. Bath application of PCL-Q (3 µg/ml by quercetin) increased single Maxi-K channel activity about threefold, from 0.010±0.003 to 0.034±0.004 (p<0.05, n=4), whereas there was only a non-significant increase in single channel conductance from 138 to 146 pS. In the presence of PCL-Q multiple simultaneous channel openings were observed, with up to 8 active channels in a patch. Surprisingly, testing the PCL-Q we have also found that "empty" phosphatidylcholine liposomes (PCL, 100 µg/ml) also produced some channel activation, although it was less potent compared to PCL-Q. Thus, PCL increased NPo from 0.010±0.003 to 0.019±0.003, p<0.05, n=4, and did not affect single channel conductance (139 pS). We conclude that PCL-Q as well as PCL alone activate SM cell Maxi-K channels, mainly by increasing channel open probability. While incorporation of the liposomes into the plasma membrane, by altering channel’s phospholipids environment, may directly activate Maxi-K channels, the additive action of quercetin may be due to its well known inhibition of protein kinase C.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA323

Lymphatic drainage of the brain

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The brain is an immune privileged organ due to the presence of a blood-brain barrier and the lack of lymphatic vessels within the CNS parenchyma. However, it has been clear for several decades that CNS antigens drain into regional lymph nodes. In humans, soluble amyloid\(\beta\) is produced by neurons and is deposited in the walls of ageing cerebral blood vessels as cerebral amyloid angiopathy (CAA), providing thus a natural tracer for the drainage of solutes. Our anatomical experimental studies using wild-type mice demonstrate that injection of soluble tracers in the gray matter of the brain results in diffusion and drainage of the tracers along the basement membranes of capillaries and the basement membranes surrounding smooth muscle cells of arteries, towards the surface of the brain. This pathway is effectively the lymphatic drainage pathway of the brain and is restricted to solutes, as nanoparticles of 15nm cannot enter the cerebrovascular basement membranes. Ageing, possession of apolipoprotein E4 and vascular immune complexes block this drainage pathway, resulting in CAA. The cerebrovascular basement membranes are also the pathway for convective influx of cerebrospinal fluid (CSF) into the brain. Specifically, the giall-pial basement membranes are the pathways for convective influx of CSF. Using optimally preserved canine brain tissue we have demonstrated that the structure of the wall of arteries is different in gray matter compared to white matter, providing thus a platform for better interpretation of age-related white matter hyperintensities. Our results clarify the exact pathways for lymphatic drainage of the brain parenchyma and for convective influx of cerebrospinal fluid.
Metabolic programming of human foetal umbilical artery smooth muscle cells from gestational diabetic pregnancies

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Gestational diabetes mellitus (GDM) is defined as glucose intolerance with first recognition during pregnancy (Lappas et al., 2011). Offspring from GDM pregnancies have a higher risk of cardiovascular diseases in later life, most likely as a consequence of foetal programming (Lappas et al., 2011, Barker et al., 2002). Previously we have reported that Nrf2 regulated redox signalling in foetal umbilical vein endothelial cells is impaired as a result of increased oxidative stress in GDM (Cheng et al., 2013).

In the present study, redox phenotype and gene expression were characterised using human umbilical artery smooth muscle cells (HUASMC) isolated from normal (n=63) and GDM (n=32) pregnancies. Proliferation of GDM HUASMC was slower than normal HUASMC (n=5 normal and 4 GDM donors), while the cellular redox status, as determined by mitochondria superoxide generation, intracellular glutathione (GSH) and basal protein carbonylation, were similar in normal and GDM cells (n=5-11 normal and 5-11 GDM donors). A microarray analysis of gene expression from normal (n=9) and GDM (n=7) HUASMC cultures identified 176 differentially expressed genes, associated with in utero embryonic development, lipid metabolism, proliferation, cellular responses to stresses, proteolysis, chromatin organisation, RNA processing, transcription, and other intracellular signalling pathways. Notably, as validated by qPCR, the expression of an imprinted gene cyclin-dependent kinase inhibitor 1C (CDKN1C), an inhibitor of cell proliferation and with important function in foetal growth, was increased by 3-fold in GDM HUASMC (n=32) compared to 5 days of culture in DMEM F-12 culture media (5%CO2, 37 °C with Pen-Strep) containing varying concentrations of FBS (0-10%). Vaso-motor responses (Phenyldihyprine and K+ induced constriction, Endothelium dependent- (Ach; 1nM-10 µM) and independent- (SNP; 10pM-1 µM) relaxation were recorded in a wire myograph (Danish Myotechnology 400A) on arterial rings incubated in Krebs solution. Responses (% KCl induced constriction or % relaxation of PE induced tone) were expressed as mean±SEM of n animals differences were assessed using one-way ANOVA with Bonferroni’s or Tukeys’ post-test, P<0.05 was considered statistically significant.

Culture for 3 days (DMEM+10% FBS) revealed a significant increase in sensitivity to PE constriction (logEC50 of -6.95±0.08 n=6 Day 1 Vs -7.46±0.01 day, n=3, P<0.05) with no effect on Emax. Relaxations to Ach were inhibited but SNP was unaffected. In low [FBS] (0, 0.1 and 1%) significant increases in sensitivity to PE were observed at day 2; here Ach mediated relaxation was impaired but SNP relaxations were unaffected. In Low FBS at day 5 PE induced constriction was significantly impaired (constriction to 100 nM PE; 8.92±0.99 (day 5) vs 4.75±0.1mN (day 5) in 0.1% FBS, P<0.05, n=8); relaxation responses were not possible to obtain as in addition to reduced constriction stable contractile tone was not maintained.

Initial characterisation of organ culture in the rat aorta

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Organ culture is used to study vascular function and is a potential method to reduce in vivo animal models. Organ culture potentially allows use of molecular biological techniques normally only feasible in cell cultures. Our aim is to optimise culture conditions in the rat aorta to preserve vasomotor responses over a period sufficient to utilise lentiviral transfection of shRNA. Male Wistar rats (250-300g) were killed by overdose of inhaled isoflurane, thoracic aorta was dissected and cut in to 2mm rings, that were either used immediately or cultured for up to 5 days in DMEM F-12 culture media (5%CO2, 37 °C with Pen-Strep) containing varying concentrations of FBS (0-10%). Vaso-motor responses (Phenyldihyprine and K+ induced constriction, Endothelium dependent- (Ach; 1nM-10 µM) and independent- (SNP; 10pM-1 µM) relaxation were recorded in a wire myograph (Danish Myotechnology 400A) on arterial rings incubated in Krebs solution. Responses (% KCl induced constriction or % relaxation of PE induced tone) were expressed as mean±SEM of n animals differences were assessed using one-way ANOVA with Bonferroni’s or Tukeys’ post-test, P<0.05 was considered statistically significant.

In all conditions at 5 days of culture, contractile responses were significantly impaired and it was impossible to observe. In all conditions at 5 days of culture, contractile responses were significantly impaired and it was impossible to observe. In all conditions at 5 days of culture, contractile responses were significantly impaired and it was impossible to observe. In all conditions at 5 days of culture, contractile responses were significantly impaired and it was impossible to observe. In all conditions at 5 days of culture, contractile responses were significantly impaired and it was impossible to observe.
vasomotor responses in the aorta and alteration of plasma serum concentration has no beneficial effect. As viral transfection of shRNA requires a minimum 5 days, alternative culturing conditions need to be investigated before this technique can be utilised.


British Heart Foundation
Institute of Cardiovascular and Metabolic Research (ICMR)
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**PCA326**

**Effects of nitrofurantoin on in vitro contractility of rat urinary bladder**

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Nitrofurantoin is a specific anti-bacterial drug used in urinary system and it has a key role in management of uncomplicated urinary tract infections. While most of the bacteria that cause urinary tract infections show resistance to antibiotics, many pathogens are still susceptible to this drug. The current study aims to analyse the effects of this frequently used urinary tract antiseptic, nitrofurantoin, on bladder contractions in rats. The aim of this study was to explore the possible role of nitrofurantoin on in vitro urinary bladder contraction in male rats. All experiments in this study were performed in accordance with the guidelines for animal research from the National Institutes of Health and were approved by the Local Committee on Animal Research (local committee decision number: 2016/05-38).

Bladder tissues were removed from Spraque-Dawley male rats after decapitation (n=7). Bladder tissues were attached to an organ bath containing 5 mL Krebs-Ringer bicarbonate solution with a tension of 1.5 g. Nitrofurantoin was administered to three groups with a concentration of 50, 500 and 1000 µM respectively. Contractions were examined in terms of area and peak-to-peak (p-p) values before and after treatment. The results were evaluated by applying slow voltage ramps from 80 to -120 mV. mLCaT shows prominent voltage dependent properties. Among them, modulation of the activity of ion channels is mainly mediated by TRPC4 channels [4]. Experiments were performed on single collagenase-dispersed smooth muscle cells freshly isolated from the longitudinal layer of the mouse ileum, using patch-clamp techniques. mLCaT was isolated using symmetrical Cs+ containing (125 mM) solutions with [Ca2+]i 'clamped' at 100 nM (10 mM BAPTA/4.6 mM CaCl2 mixture). The current was induced by intracellular infusion of 0.2 mM GTPγS, which activates G-proteins directly, i.e. by bypassing the muscarinic receptors. Under these conditions, mLCaT slowly reached a peak amplitude of 451±52 pA (n=9) 5-10 min after break-through. C60NPs applied at 10-6 M at the steady-state response to G-protein activation caused mLCaT inhibition by 47.0±3.5% (n=9). The current inhibition developed slowly, with the time constant of 119±16 s. C60NPs inhibited mLCaT irreversibly and in a voltage-independent manner, as examined by applying slow voltage ramps from 80 to -120 mV. mLCaT shows prominent voltage dependent properties, while its voltage dependence is additionally regulated by G-proteins [5]. Interestingly, voltage-dependent relaxations of mLCaT (deactivation during voltage steps from -40 to -120 mV and reactivation by stepping back to -40 mV) became about 5-fold faster in the presence of C60NPs, an effect opposite to that seen during increasing G-protein activation by GTPγS. Finally, specificity of the inhibitory action of C60NPs on mLCaT was tested by examining voltage-gated K+ currents. K+ current density in murine intestinal myocytes was 115±9 and 120±7 pA/pf (n=4; P=0.676) in control and in the presence of C60NPs respectively. We conclude, that C60NPs specifically

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**PCA329**

Effect of angiotensin receptor blocker with losartan on the blood pressure and peripheral vascular resistance to voluntary hand grip contraction in salt loaded normotensive and hypertensive Nigerians

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Activity of the Sympathetic and Renin-Angiotensin-Aldosterone systems play crucial roles in blood pressure response to increased salt intake. This study is on the effect of salt loading in normotensive (NT) and hypertensive (HT) Nigerian subjects and the effects of angiotensin receptor blocker (ARB) and sympathetic excitation on responses of blood pressure and peripheral vascular resistance. Salt was administered orally as 200 mmol of sodium chloride daily for five days (Elias et al, 2014), to 16 NT and 14 HT subjects, that were age matched (39.9±1.3 vs 44.1±2.1 yrs). The effect of salt loading on blood pressure and peripheral vascular resistance were then determined, before and after concurrent administration of 50 mg Losartan, an ARB. In addition the responses to 30% Maximum Voluntary Contraction (MVC) by handgrip (HG) for one minute, using a dynamometer were also determined. Blood pressure was measured with a sphygmomanometer and mean arterial blood pressure (MABP, mmHg) was computed. Finger blood flow was determined with a finger plethysmograph (Kura et al, 2008),(AD Instruments) and peripheral vascular resistance (PVR) was calculated from mean arterial blood pressure (MABP) and finger blood flow, in mmHg/mls/s. 24hr urine was also collected. Ethical clearance was obtained from the College’s Ethics Committee. Data are mean±sem, Stats by Students t-test. Urinary Na+ excretion (mmol/24hr) before salt load was 111.4±5.6 in NT and 91.9±14.0 in HT and was increased in both (P<0.05) by salt loading viz, in NT to 176.0±13.8 and in HT to 147.4±19.3. However after salt+Losartan Na+ excretion was virtually the same in NT-173.2±13.7, but in HT, Na excretion further increased to 182.2±27.0 (P<0.05). For MABP responses to sympathetic excitation by HG: In NT, HG increased MABP by 8.1±1.5% in Controls, by 9.9±1.8% after salt but fell to 7.7±2.1% after salt+losartan. In HT, the corresponding values were 7.9±1.4%, 10.8±1.0% and 8.8±1.1% respectively. HG in NT increased PVR by 25.9±8.1% in control, by 38.0±6.7% after salt and by 22.8±8.8% after salt+losartan and in HT, the corresponding values were 22.9±6.8%, 34.3±10.1% and 32.9±7.5% respectively. The PVR lowering response of Losartan to HG was significantly reduced in NT compared to salt loading (P<0.01) but this was blunted in HT. Thus Losartan ameliorates the MABP response to voluntary hand grip following salt loading in NT and HT comparatively but its effect on PVR response is significantly attenuated in NT but is not reduced in HT. However Losartan increases natriuresis in HT. This may suggest that alteration of PVR by the ARB Losartan, is not majorly responsible for its BP lowering action in salt loaded hypertensive Nigerians.

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PCA330

Uridine triphosphates analogues as inhibitors of platelet P2Y12: structure activity relationship

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Background and Aims: Platelet P2Y12 is an important ADP receptor that is involved in agonists-induced platelet aggregation and is an important target for the development of anti-platelet aggregation drugs. The aim of the present study was to characterise the effects of uridine triphosphate (UTP) and its thio-(S)-analloges on ADP-induced platelet aggregation.

Methods: The experiments were performed on platelet rich plasma freshly isolated from blood donated by healthy human volunteers. The investigation of molecular characteristics of these derivatives possibly associated with the inhibition of P2Y12 receptor was also carried out via molecular docking simulations.

Results: UTP inhibited P2Y12 receptors and antagonised ADP-induced platelet aggregation in a conc.-dependent manner with an IC50 value of ~250 µM against ADP (10 µM). A 5-fold increase in the platelet inhibitory activity was observed by adding a thio (S)- group at position 2 (2S-UTP) of the nucleotide ring with an IC50 value of 30 µM. Interestingly, a 500-fold increase in anti-platelet aggregation activity was observed when a (S)- was introduced at position 4 of the nucleotide ring (4S-UTP) with an IC50 value of 7.5 µM. However, introducing an isobutyl group at the 4S- position reduced its activity by 2-fold with IC50 of 15 µM. A modeling study using FRED docking program was performed to dock these compounds into the ligand binding site of P2Y12 receptor. An excellent correlation was observed between the experimental findings and the docking results.

Conclusion: The novel data demonstrate for the first time that thio (S)- analogues of UTP, particular 4S-UTP, are potent P2Y12 receptor antagonists and can be useful candidates for therapeutic intervention.

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PCA331

Induction of Notch signalling by perfusion of soluble Delta-like ligand 4 (sDLL4) reduces microvascular permeability

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Delta-like ligand 4 (DLL4) is a ligand for the membrane bound receptor Notch 1. DLL4 is activated during angiogenesis when VEGF induces VEGFR2 signalling, resulting in transcellular signalling to prevent hyper sprouting. Growing blood vessels are generally leaky during pathogenic angiogenesis, and VEGF induces increased vascular permeability by increasing transendothelial transport pathways. We therefore hypothesized that the induction of the DLL4/Notch signalling pathway could provide a mechanism for this increase in permeability and that activation of the DLL4 pathway with a soluble DLL4 ligand could result in vessels with increased permeability to water (Lp: hydraulic conductivity).

We used terminally anaesthetised (2% gaseous isoflurane) male Han-Wistar rats and measured hydraulic conductivity (Lp) using the modified Landis-Michel technique in post-capillary venules. Selected vessels within the rat mesentery were perfused with the soluble, extracellular portion (amino acid 26 – 524) of DLL4 recombinant protein (sDLL4; 1µg/ml) in a 1% bovine serum albumin (BSA) mammalian Ringer solution containing washed red blood cells. The vessel was occluded at regular intervals and the velocity of the red blood cells, vessel radius and the length between the measured red blood cell and the occlusion point were used to calculate the transcapillary water flow per unit area of the capillary wall (Jv/S). The difference between vessel and interstitial fluid hydrostatic and osmotic pressures (∆P) was also calculated and Lp was determined from the Starling equation: Lp = (∆v/S)/∆P

Within 120sec of perfusing with sDLL4, Lp began to decrease until it plateaued at approximately 300sec decreasing from 1.8 ± 0.542 SEM to 0.41 ± 0.055 SEM x 10⁻⁷ cm.s⁻¹.cmH₂O⁻¹. Recordings taken during this nadir response period showed a 0.35 ±0.01 SEM, n=5 fold change relative to baseline. This was significantly different from perfusing with BSA alone, (p<0.001, unpaired t-test) which resulted in no change in permeability relative to baseline (1.02 fold ±0.04 SEM, n=5). We confirmed that sDLL4 activated the Notch signalling pathway in endothelial cells in culture, by measuring the expression of Hey1, a gene known to be upregulated by Notch activation. sDLL4 resulted in a 26.4 ± 19.8 SEM fold increase in Hey1 expression relative to GAPDH in endothelial cells treated with 1µg/ml sDLL4 for 24 hours. Our data shows, in contrast with our hypothesis, that DLL4/Notch signalling reduces vascular permeability to water. This indicates that DLL4 activation does not contribute to the permeability induced during angiogenesis, and that potentially it could prevent increased permeability during normal, physiological angiogenesis.

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PCA332

Quantifying mitochondrial size and motility alterations with age, disease and proliferation

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Mitochondrial function is vital for many physiological processes, and the importance of motility, position and morphology of these organelles is starting to be appreciated. Alterations in mitochondrial position affect localised cellular events, changes in mitochondrial morphology influence metabolic responses, and the motility of mitochondria adapts to and regulates different cellular states. Quantification of the shape and motility of mitochondria is challenging due to their small dimensions, rapid velocities and crowded cellular environments. We developed live-cell image analysis tools which have revealed changes in mitochondrial dimensions, position and motility in vascular smooth muscle that occur with age, hypertension and proliferation. Flicker-assisted Localisation Microscopy (FaLM) discriminates the shape and relative
position of individual mitochondria by measuring the local covariance of potentiometric fluorophore intensity changes during stochastic “flickers” of mitochondrial membrane potential (ΔΨm). Freshly-isolated rat cerebral artery myocytes loaded with TMRE were imaged for a sufficient duration (10-20 min) to record spontaneous ΔΨm flickers. FaLM revealed that in a rat model of hypertension mitochondria were larger (SHR, mitochondrial area 0.83±0.05 µm² cf. 0.35±0.08 µm² in normotensive WKY; n=5 animals each, p<0.01; all values are mean±SEM, compared by two-sample Kolmogorov-Smirnov or t-test), occupied a greater proportion of the cell (19.7±1.2% in SHR cf. 7.0±1.4% in WKY; n=5 animals each, p<0.01) and were clustered more tightly (1.9±0.04 µm between centres of neighbouring mitochondria in SHR cf. 2.2±0.06 µm in WKY; n=5 animals, p<0.01). Mitochondria were also found to be larger in myocytes from aged cf. younger rats (1.81±0.05 µm² mitochondrial area in 18 month-old cf. 0.35±0.02 µm² in 3 month-old; n=5 animals each, p<0.01), again occupying a greater proportion of the cell (18.1±5.2% in 18 month cf. 8.5±3.2% in 3 month; n=5, p<0.01). A notable proportion of mitochondria in aged animals were highly-elongated (length:width ratio>3; 4.3% of mitochondria in 18 month cf. 0.4% in 3 month; n=5 animals, p<0.01). Mitochondria in younger animals were often motile, whereas those in aged animals were not (19% of mitochondria in 3 month cf. 0.12% in 18 month; n=5 animals, p<0.01). We have previously shown that in native cerebral artery myocytes (from 10-15 week-old guinea pigs) mitochondria were not motile but that a switch to extensive motility was involved in - and necessary for - serum induced proliferation, with bursts of motion as fast as 1 µm/s tracked by our MotionStudio analysis (1,2). Thus, mitochondrial motility within smooth muscle decreases with age but may restart during cellular remodelling to a proliferative phenotype. Concurrent with decreased motility with age, mitochondrial size increases – a change that also occurs in hypertension.


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PCA333

The effect of dual combination of Magnesium sulphate with atosiban and indomethacin on myometrial contractions of pregnant mouse

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Preterm birth is a major cause of neonatal morbidity. It currently accounts for approximately 10% of all births and about 51% of infant deaths in the UK. Several types of tocolytics (uterine relaxants) have been tested for the treatment of preterm labour: oxytocin receptor antagonists e.g. atosiban, calcium channel blockers, e.g. nifedipine, prostaglandin synthetase inhibitor e.g. indomethacin, β-adrenoceptor agonist e.g. ritodrine and Magnesium sulphate (MgSO4). The most commonly used tocolytics in the UK are atosiban, indomethacin and nifedipine. MgSO4 is not used as a tocolytic in the UK, but is given to women in threatened early preterm birth (before 30 weeks) as a fetal neuroprotectant to substantially reduce the risk of cerebral palsy. We hypothesized that MgSO4 in combination with other tocolytics may cause synergistic inhibition of myometrial contraction.

The aim of the study is to compare the tocolytic efficacy of atosiban and indomethacin, used in combination with MgSO4, to see whether the combination of these drugs, which act via different pathways, could improve the inhibition of oxytocin augmented uterine contractility in term pregnant mouse myometrium.

Longitudinal myometrial strips were obtained from term pregnant C57BL/6j mice (19 days gestation). Contractile activity was recorded using digital software (Labtrax). MgSO4 was applied in increasing concentrations between 2mM and 12mM, either alone or in combination with indomethacin or atosiban at their predetermined IC50 values. Each concentration of MgSO4 was applied for 15 minutes. Control contractile activity and activity after addition of each combination were measured using the last 10 minutes of activity and the changes expressed as a percentage of control (100%). Values were compared by one way ANOVA and Bonferroni post hoc test. P<0.05 was considered to be significant.

The IC50 values of atosiban and indomethacin were found to be 300nM and 30µM respectively and were taken forward for combination with MgSO4. 12mM MgSO4 alone reduced force integral to 44.3±8.67% of control. With the addition of indomethacin, contractions reduced to 48.43±2.69% (n=6); P = not significant. MgSO4 in combination with atosiban reduced contractions to 1.35±1.05% (n=7) of control; P<0.0001. Values are expressed as mean ± SEM.

Our preliminary in vitro data demonstrated that the combination of MgSO4 and atosiban exhibits a greater effect for mouse myometrial inhibition than MgSO4 alone or in combination with indomethacin. The greater inhibitory effect observed may be explained by atosiban’s mechanism of action as an oxytocin receptor antagonist. This suggests that atosiban may be a more promising tocolytic when magnesium has been administered for neuroprotection.

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PCA334

Vascular permeability is dose-dependently modulated by Sulforaphane treatment

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The dietary-derived isothiocyanate sulforaphane (SFN) is an electrophilic inducer of the redox-defence transcription factor Nrf2, acting on the Cys-151 residue of its cytosolic sequester partner, Keap1. In addition, sulforaphane exhibits anti-inflammatory effects through inhibition of NFκB, and at higher concentrations affects cytoskeletal polymerization. SFN pre-treatment has been shown to reduce acute plasma protein leakage into the brain parenchyma in rodent models of focal ischemia-reperfusion, implying moderation of vascular permeability. While inflammatory mediators associated with ischemia-reperfusion are known to increase vascular permeability via redox pathways in the rat (bradykinin via COX/LOX and IL1β via NOX2) the precise mechanism of vascular protection by SFN is undefined.

The ileal artery of a freshly killed Sprague-Dawley rat was cannulated orthogradely and branches that did not lead to the
cremaster muscle were ligated. The cremaster microcirculation was flushed with a high MgCl2 & K+ solution containing heparin. The muscle was spread over a transparent support and superfused with Krebs buffer at 37°C and perfused with a buffer containing albumin (5 mg.ml⁻¹) and FITC-albumin (5 mg.ml⁻¹). Permeability was obtained from the rate of change in fluorescent signal across the wall of a selected venule (k) and its diameter (d): P = kd/4 when perfusion was stopped. Bk (0.1µM in the presence of 10µM each of captopril and thiorphan) was applied in the superfusate for 1 min, and IL-1β (30pm) was applied topically for 10 min before being washed off. The effects of sulforaphane were assessed by both acute superfusion (1nM to 100µM) and sulforaphane pre-treatment (5mg.kg⁻¹ i.p. 24h before surgery. The permeability response to bradykinin (0.20 ± 0.08 10-6 cm s⁻¹; mean ± sem, n =5) was unaffected by SFN pre-treatment (0.20 ± 0.19). IL1β pretreatment resulted in an increased response to Bk (1.64 ± 0.55). Both responses were abolished by scavenging with and superoxide dismutase and catalase (0.1 ± 0.2; S&G 100U/ml each). SFN pre-treatment had no effect on the response to Bk, but abolished the IL1β potentiated Bk response (0.13 ± 0.06). Acute SFN application had little effect on permeability at doses below 0.1µM, but SFN 1µM and 10µM resulted in a significant permeability response (0.60 ± 0.05 and 0.60 ± 0.15). Intriguingly higher SFN doses resulted in much lower permeability increases, and these high doses blocked the permeability response to Bk. This novel finding of a direct permeability response to SFN, in combination with an ability to acutely block the Bk permeability response, implies additional Nrf2-independent effects may underlie physiological cerebrovascular protection.

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These results suggest that implanting grafts seeded with donor piglet blood MSC does not cause any excessive or adverse immune responses in the receiver piglet. The lower expression of MHC II in the spleen of cell-seeded group animals suggests that perhaps MSCs help modulate inflammation following this procedure.

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PCA337

Potential use of Sulforaphane to reverse deficits in Nrf2-mediated redox defences and protect the fetal vasculature following in utero exposure to adverse pregnancy

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Gestational diabetes (GDM) is classified as first onset of diabetes during pregnancy and its prevalence is increasing due to the growing obesity epidemic. Several studies highlight a strong association between in utero exposure to GDM and increased risk of later-life insulin resistance and vascular dysfunction. In fetal GDM endothelial cells challenged with endogenous oxidative stimuli such as 4-hydroxynonenal (HNE), we previously identified deficits in protective Nrf2-mediated redox defences which parallel markers of oxidative damage including DNA fragmentation, protein oxidation and enhanced mitochondrial ROS generation. Here we examined whether (I) Nrf2 activation in response HNE could be restored using the dietary Nrf2 activator Sulforaphane (SFN) in GDM human fetal endothelial cells, (II) whether Nrf2 redox defences are compromised in a murine model of insulin-resistant obesogenic pregnancy and (III) whether SFN can cross the placenta to induce fetal Nrf2 gene expression in vivo.

Fetally derived normal and GDM umbilical vein endothelial cells (HUVEC) were pre-treated with or without SFN (2.5µM 24h) before challenge with HNE (20µM 12h) and assessment of downstream gene expression by immunoblot or as an index of oxidative damage, protein carbonylation by oxyblot. In other experiments, fetals hearts taken from high-fat diet (HFD) insulin-resistant C57BL/J6 dams mice were used to assess basal Nrf2 signalling by qPCR. Similarly, maternal and/or fetal hearts were isolated from normal chow fed dams given SFN (I.P 5mg/kg 24h or diet 0.5mg/kg 5days) before assessing Nrf2 and target gene expression by qPCR or immunoblot. In GDM HUVEC, SFN pre-treatment restores Nrf2 target gene NQO1 and GCL induction in response to HNE and prevents enhanced protein oxidation of GDM HUVEC. To examine whether SFN may restore Nrf2 signalling in vivo, we examined basal Nrf2 signalling in murine fetal heart tissue taken from HFD insulin-resistant pregnancies. Despite exposure to a HFD, no basal compensatory increase in Nrf2 target genes such as NQO1, HO-1 or GCL was observed in HFD fetal hearts. In normal chow fed animals, IP or dietary SFN administration could increase Nrf2 and downstream target genes such as HO-1 and GCL in maternal heart, placenta and fetal heart.

In summary, our findings suggest exposure to SFN may alleviate oxidative stress-induced fetal vascular damage associated with human GDM pregnancy. Our findings in normal chow fed mice demonstrating SFN can induce maternal and fetal Nrf2 antioxidant gene expression in vivo further support the therapeutic potential of Sulforaphane which we will explore in our murine model of obesogenic insulin-resistant pregnancy. Tam et al., (2012) The association between in utero hyperinsulinaemia and adolescent arterial stiffness Diabetes Res.Clin.PRACT. 95:169-169

Cheng et al., (2013) Gestational Diabetes Mellitus Impairs Nrf2-Mediated Adaptive Antioxidant Defenses and Redox Signaling in Fetal Endothelial Cells In Utero Diabetes 62:4088-4097


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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA338

Cu(II)ATSM protects against the pro-oxidant effects of angiotensin II via DJ-1

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Oxidative stress contributes to many cardiovascular pathologies, including hypertension, atherosclerosis and heart failure. The copper-bis(thiosemicarbazone) complex Copper(II)dicyclohexyl-tert-bis(N4-methylthiosemicarbazone) [Cu(II)ATSM] was developed as a hypoxia selective positron emission tomography imaging agent, however, recent studies have highlighted the therapeutic properties of Cu(II)ATSM against oxidative stress in the brain. In this study we determine whether Cu(II)ATSM affords protection against the pro-oxidant effects of angiotensin II (Ang II) in human coronary artery smooth muscle cells (HCASMC) via the multifunctional Parkinson’s-associated protein DJ-1 and the redox sensitive transcription factor NF-E2 related factor 2 (Nrf2). Acute treatment with Cu(II)ATSM (1µM, 30 min) attenuated Ang II (200nM, 4h)-induced superoxide generation (Ang II 1.89 x10\(^5\) ± 0.13; Ang II + Cu(II)ATSM 0.77 x10\(^5\) ± 0.06 mean light units (MLU)/mg protein; p<0.001, n=4) as assessed by L-012 chemiluminescence. Cu(II)ATSM treatment alone (0.88 x10\(^5\) ± 0.13) did not affect basal superoxide levels (1.02x10\(^5\) ± 0.11). As DJ-1 acts as a copper chaperone protein for cytosolic superoxide dismutase (SOD1), we determined the intracellular levels of Cu and DJ-1/SOD1 protein interaction. Cu levels, determined by inductively-coupled plasma mass spectrometry, were significantly increased following Cu(II)ATSM treatment (1mM, 30 min, 2.395 ± 0.12 µg/L) compared to vehicle (0.89 ± 0.26, P<0.01, n=4), whilst immunoprecipitation demonstrated increased protein interaction between DJ-1 and SOD1 following Cu(II)ATSM (1mM, 1h) treatment (n=6). Furthermore, Cu(II)ATSM treatment (1mM, 1h) significantly increased in SOD1 activity (22.94 ± 3.10 U/mg protein) compared to vehicle (11.75 ± 1.38). Pre-treatment of HCASMC with Cu(II)ATSM (1mM, 12h) attenuated Ang II (200nM, 12h)-induced superoxide generation (0.75 x10\(^5\) ± 0.02 MLU/mg protein) compared to Ang II alone (1.80 ± 0.13, p<0.001, n=4). Cu(II)ATSM treatment (1mM, 1-4h) time dependently increased Nrf2 nuclear localisation determined by immunofluorescence, and induction of the antioxidant.
protein heme oxygenase-1 (HO-1) after 12h. However, HO-1 protein expression was attenuated in cells deficient for Nrf2 or Dj-1 following siRNA knockdown. Our findings suggest that Dj-1 plays an important role in mediating protection in HCASMC afforded by Cu(II)ATSM, via upregulation of SOD1 activity, and induction of NrF2-regulated antioxidant proteins. Hence, Cu(II)ATSM may represent a novel therapeutic strategy against oxidative stress in cardiovascular disease.

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PCA340

Role of L-type Ca\(^{2+}\) channels, Ca\(^{2+}\)-activated Cl\(^{-}\) channels and TRPC in mediating contractile responses to carbachol and histamine in rabbit bronchi

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Airway smooth muscle cells express a variety of ion channels, e.g. L-type Ca\(^{2+}\) channels, Ca\(^{2+}\)-activated Cl\(^{-}\) channels (CaCC) and transient receptor potential channels (TRP), but unlike vascular smooth muscles, their role is still controversial (Janssen, 2012). The aim of this study was to investigate if these channels contribute to the contractile responses to carbachol (CCh) and histamine (His) in rabbit bronchial rings. New Zealand white rabbits were euthanized and the respiratory tree removed. Rings (length 2-4mm) from 3rd and 4th order bronchi were isolated and mounted in organ baths for isometric tension recording. Stock solutions of drugs were added directly to the bath and diluted to final concentrations. Data are presented as the mean±S.E.M, and statistical differences compared using Student’s paired t-test, taking p<0.05 as significant.

When cumulative concentrations of CCh (0.1–10\(\mu\)M) were added, there was a concentration-dependent increase in tension (EC\(_{50}\)=0.13\(\mu\)M; maximum response, Max=7.0±3.1 mN, n=6). When this was repeated in the presence of nifedipine (100\(\mu\)M), the responses were reduced throughout the CCh concentration range (Max=1.2±1.6 mN, n=6, p<0.05), and EC\(_{50}\) was increased to 1.2\(\mu\)M. Similarly, His (0.1–10\(\mu\)M), caused a concentration-dependent increase in tension (EC\(_{50}\)=1.3 \(\mu\)M; Max=9.2±2.1 mN), which was also reduced by nifedipine (EC\(_{50}\)=1.1\(\mu\)M; Max=3.1±0.4 mN, n=6, p<0.05). In contrast, the CaCC channel blocker, CaCCinh-A01 (10\(\mu\)M), had very little effect on CCh responses but markedly reduced His responses. For CCh it reduced Max slightly from 7.5±1.7 to 6.0±2.2 mN (n=6, p<0.05), while EC\(_{50}\) was unaffected. However, for His, Max was reduced by CaCCinh-A01 from 7.1±1.9 mN to 3.6±0.8 mN (n=6, p<0.05) and EC\(_{50}\) increased from 1.3\(\mu\)M to 2.5\(\mu\)M. The fact that the novel TRPC4/5 blocker ML204 also reduced CCh responses, but not CCh, suggests that more than one depolarising pathway was available to activate the L-type Ca\(^{2+}\) channels. To investigate if this could have been via TRPC channels, we decided to test the effect of ML204, which blocks TRPC4 and TRPC5, selectivity compared to other TRP channels (Miller et al., 2011). ML204 (10\(\mu\)M) significantly reduced responses to both His and CCh. For CCh, it shifted the EC\(_{50}\) from 0.5\(\mu\)M to 3.1\(\mu\)M and reduced the response to 10\(\mu\)M from 10.2±1.5 to 6.6±1.1 mN (n=6, p<0.05). The effects on His were greater, with EC\(_{50}\) shifting from 1.8 to 4.0\(\mu\)M and the response to 10\(\mu\)M decreasing from 4.9±0.6 to 1.7±2.0 mN (n=6, p<0.05).

In conclusion, L-type Ca\(^{2+}\) channels are likely to play a part in mediating responses to both His and CCh in rabbit bronchus, while CaCC seem to be involved only in the His responses. The fact that the novel TRPC4/5 blocker ML204 also reduced the responses to both agonists, provides preliminary evidence that TRPC4/5 may also be involved.


PCA339

Effects of bradykinin on calcium signalling and force of ureteric smooth muscle

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Although bradikinin (BK) was reported to be present in urine and cause stimulant action on ureter more than 30 years ago (Marin-Grez et al., 1980), the mechanisms of it’s action on ureteric smooth muscle have not been studied. Thus, the aim of the present study was to investigate the effects of BK on calcium (Ca\(^{2+}\)) signalling and force of rat ureteric smooth muscle using combined confocal imaging and force measurements. Resting rat ureter always responded to BK with a complex Ca\(^{2+}\) transient which consisted of an initial phasic component (seen as propagating intracellular Ca\(^{2+}\) waves), followed by a sustained plateau component superimposed by Ca\(^{2+}\) oscillations which appeared as propagating intercellular Ca\(^{2+}\) waves. This complex Ca\(^{2+}\) response was accompanied by a brief phasic followed by sustained tonic contraction superimposed by brief phasic contractions. Inhibition of voltage gated L-type Ca\(^{2+}\) channels by Nifedipine (10\(\mu\)M) selectively blocked intercellular Ca\(^{2+}\) oscillations and phasic contraction, but had no effect on initial phasic and sustained components of Ca\(^{2+}\) transient and force, respectively. In Ca\(^{2+}\) free solution, BK showed a transient increase in Ca\(^{2+}\) and force which were blocked by Cyclopiazonic acid (CPA) but not Ryanodine. There was a large increase in base-line Ca\(^{2+}\) and force superimposed by intercellular Ca\(^{2+}\) oscillations associated with phasic contraction upon addition of external Ca\(^{2+}\) to Ca\(^{2+}\) free solution, following Ca\(^{2+}\) depletion of the SR either by BK or CPA. The data obtained suggest that in rat ureteric smooth muscle, SR Ca\(^{2+}\) release is coupled to marked Ca\(^{2+}\) entry via store operated Ca\(^{2+}\) channels, leading to depolarization and activation of spontaneous action potentials associated with intercellular Ca\(^{2+}\) oscillations and phasic contractions.

Ureteral contractions induced by rat urine in vitro: probable involvement of renal kallkrein Marin-Grez M., Bonner G and F.Gross et al., Experientia, 1980, 36, 865-866

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
AMP-activated protein kinase couples K\textsubscript{1.5} channel function to inhibition of mitochondrial metabolism in pulmonary arterial myocytes

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Background. The AMP-activated protein kinase (AMPK) is intimately coupled to mitochondrial metabolism through changes in the AM(D)P:ATP ratios induced by metabolic stresses such as hypoxia [1, 2]. Of the various known Kv channel types it has been established that K\textsubscript{1.5} contributes the majority of the macroscopic voltage-gated potassium currents recorded from myocytes of near resistance-sized pulmonary arteries, that exhibit the greatest response to hypoxia [3]. Kv\textsubscript{1.5} current suppression during hypoxia [3] occurs as a consequence of inhibition of mitochondrial oxidative phosphorylation [4] and mediates, in part, progression of hypoxic pulmonary hypertension [3]. However, the nature of the signalling pathway that couples mitochondrial function to K\textsubscript{1.5} channels has been unclear. We sought to determine the role of AMPK in this process.

Methods. Myocytes were enzymatically isolated from resistance pulmonary arteries (<200 \mu m i.d.) of male Sprague Dawley rats. Kv\textsubscript{1.5} currents were recorded using whole-cell patch clamp in voltage-clamp mode with a holding potential of -80 mV. Kv\textsubscript{1.5} currents were assessed by voltage ramps (-100 to +40 mV), single voltage steps (-80 to +40 mV), and by acquisition of full current-voltage relationships for steady state activation (200 ms steps from -80 mV to +40 mV in 10 mV increments). Isoform-specific AMPK activities were determined by immunoprecipitating tissue lysate with antibodies raised against \alpha\textsubscript{1} or \alpha\textsubscript{2} subunits bound to protein G-Sepharose beads and quantified using the AMARA peptide and [\gamma-32P]ATP substrates. Phosphorylation assays were performed as described previously [5].

Results. Inhibition of the mitochondrial electron transport chain using phenformin activated AMPK and inhibited K\textsubscript{1.5} currents in pulmonary arterial myocytes, consistent with previously reported effects of mitochondrial inhibitors [4]. Myocyte K\textsubscript{1.5} currents were also markedly inhibited by application of three AMPK activators with distinct mechanisms of action, i.e., A769662, AICAR and C13. Hypoxia and inhibitors of mitochondrial oxidative phosphorylation (phenformin and antymycin A) reduced K\textsubscript{1.5} currents and blocked further inhibition by AMPK activators, as did the selective K\textsubscript{1.5} blocker DPO-1. Moreover, recombinant human K\textsubscript{1.5} channels were phosphorylated by AMPK in cell-free assays, suggesting direct regulation of the channel by AMPK.

Conclusion. These results suggest that AMPK is the primary regulator of reductions in K\textsubscript{1.5} channel availability in pulmonary arterial myocytes following inhibition of mitochondrial oxidative phosphorylation during hypoxia, and that AMPK effects this change, at least in part, through phosphorylation of K\textsubscript{1.5} and/or an associated protein.
Expression of Endothelin-b Receptor in the heart muscle of Diabetic rats induced by Streptozotocin

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Background: Diabetes mellitus is a degenerative disease which prevalence is increasing in developing country. Diabetes mellitus has some acute and chronic complications. Those affect blood vessels trough microvascular and macrovascular complications. In a state of chronic hyperglycemia, oxidative stress can occur. The condition affected endothelin-B (ETB) receptors in the heart organ.

Objective: This research aimed to see the changes in the expression of ETB receptors on heart muscle and endothelial blood vessels of heart in a streptozotocin-induced diabetic rat model.

Method: Rats were divided into two groups, one group as a control, and one group of diabetic rats model. Each group consist of 6 rats. Diabetic rat model obtained by streptozotocin injection dose 35mg/kgbw intra peritoneal to Sprague Dawley male rats aged 11-12 weeks. After 8 weeks of experiments, all the rats were terminated. The heart muscle was stained with immunohistochemical staining. Antibody of ETB was used to stain the heart muscle.

Result: Immunohistochemical stain showed 18.32% ETB receptor in the heart muscle of control group and in 8.72% in diabetic group (p value = 0.001). Meanwhile, on endothelial blood vessels of heart there was 19.75% ETB receptor in control group and 10.39% ETB receptor in diabetic group (p value = 0.004).

Conclusion: This study showed that the number of ETB receptors in heart muscle and endothelial blood vessels of the heart was reduced in diabetic group.

Keywords: Diabetes Mellitus, Heart, Endothelin-B receptor, Streptozotocin

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PCA344

Effect of asiatic acid and captopril on hemodynamics and renin-angiotensin system in 2K-1C hypertensive rats

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Renin-angiotensin system (RAS) plays a major role in the development of hypertension in two-kidney, one-clip (2K-1C) hypertensive rats. Asiatic acid exhibits antioxidative, anti-inflammatory and antihypertensive properties in L-NAME hypertensive rats (1). Moreover, Centella asiatica extract, a major source of asiatic acid, inhibits angiotensin converting enzyme (ACE) activity (2). This study, we investigated the effect of asiatic acid and captopril, an ACE inhibitor, on hemodynamic parameters and RAS activity in 2K-1C hypertensive rats. Male Sprague-Dawley rats were anaesthetized with intraperitoneal injection of pentobarbital-sodium (60 mg/kg) and a silver clip (0.2 mm width) was placed on the left renal artery to induce 2K-1C hypertension. 2K-1C hypertensive rats (n = 7/group) were intragastrically administered with vehicle or asiatic acid (30 mg/kg/day) or captopril (5 mg/kg/day) or asiatic acid (30 mg/kg/day) plus captopril (5 mg/kg/day) for six weeks while sham-operated rats (n = 7/group) were treated with vehicle. Systolic blood pressure (SP) was measured weekly using the tail-cuff plethysmography. Rats were anaesthetized as above and then SP, diastolic blood pressure (DP), mean arterial pressure (MAP), heart rate (HR), hind limb blood flow (HBF), and hind limb vascular resistance (HVR) were measured. Angiotensin II type 1 receptor (AT1R) and Angiotensin II type 2 receptor (AT2R) expression in mesenteric arteries and serum angiotensin II (AngII) were examined. All procedures are complied with the standard for the care and use of experimental animals and approved by Animals Ethics Committee of Khon Kaen University. Data are express as mean ± S.E.M which compared by ANOVA. SP was significantly high in 2K-1C hypertensive rats compared to sham-operated rats (201.2 ± 5.7 mmHg vs. 123.4 ± 3.9 mmHg, p<0.001). There were increases in SP, MAP, HR, HVR and a decrease in HBF in 2K-1C hypertensive rats (p<0.001). High serum Ang II level, upregulation of AT1R expression and downregulation of AT2R expression were also seen in hypertensive rats (p<0.05). Asiatic acid, captopril or asiatic acid plus captopril significantly reduced SP (171.1 ± 4, 158.3 ± 5.2 and 151.5 ± 4.8 mmHg, respectively) and improved hemodynamic parameters as well as RAS activation in 2K-1C hypertensive rats (p<0.05). Combined treatment with asiatic acid and captopril showed a greater effect than asiatic acid or captopril alone on hemodynamic parameters (p<0.05). These data suggest that asiatic acid exhibited anti-hypertensive effect in renovascular hypertensive rats that may associate with their abilities to reduce RAS activation in 2K-1C hypertensive rats. Combined therapy with asiatic acid and captopril produced a synergistic antihypertensive effect.

Background: The mechanism of vasodilation, caused by acetylcholine (ACh) stimulates the release of different substances from endothelial cells: endothelium-derived hyperpolarizing factor and endothelium-derived relaxing factor - nitric oxide (NO) and prostacyclin. The deficiency of NO may be the result of reduced expression or impaired activity of the enzyme eNOS, deficiency or reduced cellular uptake of L-arginine, elevated arginine activity, increased degradation of NO by endogenous inhibitors or by reactive oxygen species. Some of those processes are associated with aging and might lead to the development of cardiovascular diseases. The aim of the present study was to determine changes of endothelium-dependent and endothelium-independent vasodilation in the course of aging.

Methods: We measured laser Doppler (LD) flux of cutaneous microvessels on the ventral side of the forearm. The endothelium-dependent and endothelium-independent vasodilation was determined in 81 healthy subjects divided into four age groups. In the first group there were 32 subjects aged 20-28 years, in the second 19 subjects aged 29-39 years, in the third 19 subjects aged 40-49 years and in the fourth 17 subjects aged 50-74 years old. The endothelium-dependent vasodilation was assessed by ACh iontophoresis and the endothelium-independent vasodilation by sodium nitroprusside iontophoresis. Data were analysed using ANOVA followed by Dunnett’s test. The study was approved by the National Medical Ethics Committee; written informed consent was obtained from each subject.

Results: With aging maximal LD flux during endothelium-dependent vasodilation in three groups did not differ. In response to ACh application LD flux increased 9.94 ± 1.1 times in the group aged 20-28 years; 10.7 ± 1.3 times in the group aged 29-39 years, and 10.5± 1.7 times in the group aged 50-74 years. In the group of subjects aged 40-49 years LD flux increased significantly less (5.38 ± 1.0 times). On the contrary maximal LD flux during endothelium-independent vasodilation decreased with aging (LD flux increased 11.5 ± 1.4 times in the group aged 20-28 years, 6.12 ± 8.3 times in the group aged 29-39 years, 6.43 ± 8.9 times in the group aged 40-49 years and 3.85 ± 7.4 times in the group aged 50-74 years).

Conclusion: The endothelium-dependent vasodilation is mainly independent of age. The group of subjects, where endothelium-dependent vasodilation is smaller is the group in the period of greatest hormonal changes. The endothelium-independent vasodilation decreases with aging. From the observed results it can be concluded that other mechanisms that substitute diminished effect of NO as, for example, an increased release of prostacyclin or EDHF might be involved in endothelium-dependent vasodilation of aged subjects.
between disease states. This may lead to a safer, less expensive, and more reliable method for predicting the onset of early coronary microvascular disease.

Figure 1. Definitions of coronary flow patterns over the course of one cardiac cycle.

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PCA347

Ankrd23: Novel modulator of skeletal muscle vascular adaptation following femoral artery occlusion

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Ankrd23 is a member of a conserved family of genes containing four Ankyrin repeats and a binding site for the N2A region of titin1, 2. Ankrd23 is a member of this family of genes and a novel protein discovered in muscle and adipose tissue of diabetic mice and Obese Zucker rats3. Ankrd1, in cardiac muscle and Ankrd2, in skeletal muscle are well known as muscle stretch proteins that are induced under stress conditions and have gene expression regulatory functions1, 5. Ankrd1 and Ankrd2 appear to serve as both nuclear transcriptional regulators in vascular smooth muscle, similar to Ankrd and titin-associ-ated proteins that are pro-angiogenic and/or involved in vascular remodeling, including Ankrd23. 6 We compared the ability of wild type (WT) and Ankrd23−/−mice to form collateral arteries following femoral artery occlusion; we found very limited collateralization was present in the Ankrd23−/−mice (angioscore WT = 0.18 ± 0.03; Ankrd23−/− = 0.04 ± 0.01). Also, femoral occluded Ankrd23−/−mice exhibited less contraction induced hyperemia in the soleus and gastrocnemius muscles than in WT. These results are consistent with our hypothesis that Ankrd23 plays an important role in mechanically-induced vascular remodeling of the arterial tree. Linke WA. Sense and stretchability: The role of titin and titin-associated proteins in myocardial stress-sensing and mechanical dysfunction. Cardiovasc Res. 2008;77:637-648


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PCA348

Effects of fluid shear stress and the glycocalyx on redox signalling in human endothelial cells

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Fluid shear stress (FSS) elicits a haemodynamic force on endothelial cells (EC) which can modulate their redox phenotype. Arterial branch points or curvatures where FSS is low and oscillatory (OS) are associated with an ’atheroprone’ EC phenotype whereas high unidirectional FSS (US) is ’atheroprotective’ 1. Nuclear factor E2-related factor 2 (Nrf2), a FSS-responsive transcription factor that regulates antioxidant gene expression and the GCX and also the role of the GCX as a mediator of Nrf2 signalling by US. Human umbilical vein EC (HUVEC) were subjected for 48h to either US (15 dyn/cm²) or OS (±5 dyn/cm², 1Hz) using microfluidic slides (ibidi, GmbH) or grown in static conditions. Cells were then fixed (4% formaldehyde) and heparan sulphate (HS),
enalapril (10 mg/kg/day in drinking water) from 5-9 weeks old male and female SHR were studied. Rats pre-treated with cyte number in male spontaneously hypertensive rats (SHR) we adapted a method previously reported to reduce peri-
tigation within the renal medulla following periods of ischemia of renal medullary pericytes is to help prevent RBC aggrega-
tion reperfusion (IR) injury. We hypothesize a novel function unclear. Red blood cell (RBC) stasis is a hallmark of renal isch-
emia capillaries and are most densely populated in the outer

**Reduced pericyte density promotes renal peritubular capillary red blood cell aggregation following ischemia reperfusion in rats**

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Pericytes are contractile cells that surround the renal vasa recta capillaries and are most densely populated in the outer medulla. Recent clinical trials have linked pericyte number to improved outcomes following transplant, however, why pericytes are associated with improved graft survival remains unclear. Red blood cell (RBC) stasis is a hallmark of renal ischemia reperfusion (IR) injury. We hypothesize a novel function of renal medullary pericytes is ‘to help prevent RBC aggregation within the renal medulla following periods of ischemia and that reduced pericyte number will result in greater RBC aggregation following IR’. In order to test this hypothesis, we adapted a method previously reported to reduce pericyte number in male spontaneously hypertensive rats (SHR) involving transient treatment with an ACE inhibitor. 13 week old male and female SHR were studied. Rats pre-treated with enalapril (10 mg/kg/day in drinking water) from 5-9 weeks of age and controls (untreated) were subject to 45 min of warm (37°C) bilateral renal ischemic clamping prior to reper-

**Impaired hypoxic vasoconstrictive responses of femoral artery in eNOS-deficient mice; a regulatory role of eNOS expressed in the smooth muscle of skeletal arteries**

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Hypoxia augments alpha-adrenergic contraction (HVC) response was consistently observed in deep femoral arteries (DFA) of rats. We previously suggested that hypoxic inhibi-
tion of eNOS expressed in skeletal artery smooth muscle cells could be an important key on mechanism of HVC (Han et al., 2013). To investigate the role of muscular eNOS in skeletal arteries, we tested HVC response in femoral arteries (FAs) used wild type (WT) mice compared with eNOS hetero (H2) and eNOS knockout (KO) mice. In analysis of immunohisto-
chemistry, eNOS is expressed in medial layers of WT FAs as
Cerebral autoregulation in healthy humans: An MRI study


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Background: Cerebral autoregulation is the mechanism that maintains adequate cerebral blood flow despite fluctuations in systemic blood pressure (BP) and/or volume. To date, this has primarily been assessed using transcranial Doppler of the middle cerebral artery, which provides measures of blood velocity in one vessel. The aim of this study was to use high-resolution magnetic resonance imaging (MRI) to examine whether autoregulation of whole brain blood flow is maintained during shifts in blood volume due to lower body negative pressure (LBNP) in healthy middle-aged volunteers. This is the first study to use MRI to measure brain blood flow during LBNP.

Methods: 11 healthy volunteers (4 females), age 50 ± 0.7 years (mean ± SEM), body mass index 26.3 ± 0.2 kg/m², ambulatory day time blood pressure 122/80 ± 6/0.6 mmHg were recruited. Cardiac-gated phase contrast MRI at 1.5T (Siemens Avanto) was completed to measure cerebral blood flow. Images were obtained in the transverse plane perpendicular to the internal carotid arteries at the level of the basilar artery. Passive changes in central blood volume were induced by graded LBNP at -20, -40 and -50 mmHg. Participants were maintained at each level of LBNP for 1 minute before imaging. The internal carotid and basilar arteries were contoured in each image; mean flow velocity and blood flow were quantified in each vessel using semi-automated software (Argus, Siemens Healthcare, UK). The data was analysed using repeated measures one-way analysis of variance (ANOVA) and post-hoc using Bonferroni multiple comparisons with respect to resting values. Correlations were calculated using Pearson’s correlation coefficient.

Results: Brain blood flow and mean velocity were decreased during lower body negative pressure greater than -20 mmHg (Figure 1a and 1b, p < 0.0001 for both, ANOVA). Diastolic BP (Figure 1c, p = 0.0025) and heart rate (Figure 1d, p < 0.0001) showed an increase at high levels of LBNP, while there was no difference in the systolic or mean BP during LBNP (p = 0.07, p = 0.48, respectively). Importantly, there were no reported alterations in the state of consciousness or development of presyncopal symptoms in any of the participants. There were no correlations of BMI (p > 0.05), ambulatory systolic BP (p > 0.05) or age (p > 0.05) to brain blood flow at any level of LBNP.

Conclusion: Total cerebral blood flow and blood flow velocity is reduced in response to central hypovolemia before changes in systemic blood pressure occur. This indicates that cerebral blood flow is not controlled within narrow limits during simulated hypovolaemia in healthy middle-aged participants, despite well-regulated blood pressure.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
PCA352

Investigation into the bimodal effects of Niflumic acid and Anthracene-9-Carboxylic acid on TMEM16A currents expressed in Human Embryo Kidney cells


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Calcium-activated chloride channels (CaCCs) are involved in a range of physiological functions including fluid secretion and smooth muscle contraction. CaCCs are now thought to be encoded by TMEM16A (ANO1) [1]. Both niflumic acid and anthracene-9-carboxylic acid (A9C) have been used to characterise CaCC/TMEM16A activity in a range of cell types. However, the effects of these agents are complex, as they both produce a paradoxical potentiation of native calcium-activated chloride currents and TMEM16A currents (I_{TMEM16A}) in HEK cells [2,3]. The mechanisms underlying this effect are still unclear. The purpose of this study was to investigate if the potentiating effects of niflumic acid and A9C on I_{TMEM16A} were related to intracellular Ca^{2+} concentration ([Ca^{2+}]_i).

Currents were recorded from Human Embryo Kidney (HEK) 293 cells stably expressing human TMEM16A (transcript 1), at room temperature using the whole cell patch clamp technique. The effects of niflumic acid and A9C were compared on I_{TMEM16A} recorded with pipette solutions containing either 58 or 335 nM [Ca^{2+}]_i.

In pipette solution containing 58 nM [Ca^{2+}], niflumic acid and A9C inhibited I_{TMEM16A} evoked by a step to +80 mV, with IC_{50} values of 7.2 and 293 µM respectively (n=8). When repeated in solution containing 335 nM [Ca^{2+}], the IC_{50} values decreased to 2.6 and 174 µM, for niflumic acid and A9C respectively (n=6–10). In the presence of 58 nM [Ca^{2+}], tail currents, evoked by a step back to -140 mV, were enhanced in amplitude. For example, 10 µM niflumic acid increased the amplitude of tail currents from -1.2 ± 0.2 nA to -2.5 ± 0.3 nA (p<0.01, n=5). When repeated in 335 nM [Ca^{2+}], this potentiation effect was abolished and a slight inhibition was observed (-4.5 ± 0.7 nA under control conditions versus -4.2 ± 0.7 nA in 10 µM niflumic acid, n=6 p<0.05). Similar potentiating effects were observed with A9C in 58 nM [Ca^{2+}], A9C (1 mM) increased the amplitude of TMEM16A tail currents, at -140 mV, by approximately 3-fold from -1.5 ± 0.7 nA to -5.5 ± 1.1 nA (p<0.01, n=5). When repeated in 335 nM [Ca^{2+}], this enhancement effect was reduced, whereby at -140 mV an approximate 2-fold increase from -2.5 ± 0.4 nA in control, compared to -5.4 ± 0.8 nA in 1 mM A9C was observed (p<0.05, n=8).

In summary, in 58 nM [Ca^{2+}], both niflumic acid and A9C produced a bimodal effect on TMEM16A currents, causing inhibition at positive potentials and potentiation at negative potentials. However, in 335 nM [Ca^{2+}], the paradoxical stimulatory effects of niflumic acid were abolished and the stimulatory effects of A9C were reduced. These data suggest that the paradoxical stimulatory effects of niflumic acid and, to a lesser extent, A9C on TMEM16A currents, observed at negative potentials, is reduced when the intracellular Ca^{2+} concentration is increased.


PCA353

Human monocytes adhere to vascular smooth muscle cells in a proinflammatory environment

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Endothelial denudation following angioplasty in occluded blood vessels increases neointimal growth and leads to re-occlusion. Increased inflammation via the adhesion of circulating monocytes to exposed vascular smooth muscle cells (VSMCs) in denuded vessels may be involved. The aims of this study were to determine if human monocytes can adhere to human VSMCs and examine mediators which might regulate this process.

VSMCs were cultured from saphenous veins obtained from patients undergoing coronary artery bypass surgery and monocytes were isolated from blood of healthy volunteers. A static adhesion assay was established using monocytes co-cultured for 1 hour with VSMCs. Firstly, monocytes and VSMCs were preincubated separately with 100ng/mL tumour necrosis factor (TNF-α) for 24 hours. Following co-culture, monocyte adhesion was significantly increased (3.9±0.9 fold increase compared to untreated, n=18, p<0.05). To determine which cell type was responsible for this adhesion, either VSMCs or monocytes were preincubated with TNF-α for 24 hours. In co-cultures where only monocytes were preincubated with TNF-α, there was no increase in adhesion (n=7). However, in co-cultures with only VSMCs preincubated with TNF-α, there was a significant increase in VSMC-monoocyte adhesion (3.9±0.5 fold increase compared to untreated, n=7, p<0.05). To mimic the physiological environment, an adhesion assay under flow conditions was developed. Using this model, VSMCs preincubated with 100ng/mL TNF-α for 24 hours demonstrated significantly increased binding to untreated monocytes over 1 hour (5.3±1.4 fold increase compared to untreated, n=7, p<0.05). To examine the mechanisms involved, expression of adhesion molecules, intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 in VSMCs was determined by immunoblotting. In TNF-α-treated human VSMCs, expression of both ICAM-1 and VCAM-1 were significantly increased compared to untreated VSMCs (n=4). To examine factors which, in addition to TNF-α, regulate monocyte-VSMC adhesion in cardiovascular disease, we incubated VSMCs with glycated albumin (an advanced glycation end-product linked with diabetes). Monocyte adhesion to VSMCs was increased 1.5±0.1 fold (n=7, p<0.05) under static conditions following incubation of VSMCs with 200µg/mL glycated albumin for 24 hours compared to normal albumin, and this was reflected under flow conditions. Glycated albumin also upregulated ICAM-1 and VCAM-1 expression in VSMCs (n=3). Values are mean ± S.E.M., compared by t test.
In conclusion, human monocytes can adhere to human VSMCs in vitro under physiological conditions. This adhesion is upregulated by the cytokine TNF-α and advanced glycation end-products. These results indicate that increased monocyte adhesion to VSMCs can therefore play a role in initiating restenosis, especially in pro-inflammatory conditions and in diabetes.

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stabilized bladder and had no effect in NDO bladders. For stable bladders, tension was reduced by 12.2±2.6%, 12.2±2.6% and 13.9±2.4% for CCh, T40 and T4 contractions (n=30,14,14). The actions of NECA were abolished by the A2α-receptor antagonist alloxazine (1 μM). The A1-selective agonist, CPA had no effect on stable detrusor but attenuated T40 and T4 contractions in NDO detrusor (31.6±10.5, 72.3±24.0 respectively, n=17,17). AR subtype transcription was similar in stable and NDO bladders, except reduced A2α levels in NDO bladders. Ado reduced nerve-mediated and CCh-induced contractions, only partially mirrored by an A1/2-selective agonist NECA, through A2α receptors (A3-receptor agonists had no effect, not shown). The A1-receptor agonist CPA affected only nerve-mediated contractions, especially at low frequencies in NDO bladders. We propose that Ado attenuated directly nerve-mediated contractions, especially at low frequencies (not shown). The A1-receptor agonist CPA affected only nerve-mediated contractions, especially at low frequencies in NDO bladders. Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

**PCA356**

**Insulin resistance is associated with lower arterial blood flow and cortical hyperperfusion in cognitively healthy middle-aged adults**


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Insulin resistance (IR) is associated with poor cerebrovascular health and an increased risk for dementia. Little is known about the unique effect of IR on both micro and macrovessel flow particularly in midlife when interventions against dementia may be most effective. We examined the effect of IR as indexed by the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) on cerebral blood flow in both macrovessels and microvessels utilizing magnetic resonance imaging (MRI) among cognitively healthy middle-aged individuals. We hypothesized that higher HOMA-IR would be negatively associated with blood flow in macrovessels supplying the brain, which in turn would be associated with lower cortical perfusion at the level of the microvasculature. 120 cognitively healthy middle-aged adults (57±5 yrs, cognitive health determined by mini-mental state examination (MMSE) and immediate and delayed memory testing, Rey Auditory Verbal Learning Test (RAVLT)) underwent fasting blood draw, phase Contrast (PC) MRI and arterial spin labeling (ASL) perfusion MRI. HOMA-IR was calculated from fasting insulin and glucose measures (HOMA-IR = [Insulin] (μU/mL) x [Glucose] (mg/dL) / 405). Mean flow (ml/min) was measured in the internal carotid arteries (ICA, right and left) and the basilar artery (BA). CBF blood flow was then calculated as the sum of bilateral ICA and BA flow. HOMA-IR was regressed against CBF and perfusion using linear regression controlling for several co-morbid risk factors, as well as interactions between IR and CBF on perfusion. All subjects had normal cognitive function (MMSE = 29±0.7, immediate memory RAVLT = 10±2.1. Delayed Memory RAVLT = 10±3.2). Higher HOMA-IR was associated with lower CBF (F1,122 = 4.76, p = 0.031), particularly within the ICAs (right ICA, F1,122 = 4.87, p = 0.029, left ICA, F1,122 = 5.65, p = 0.019). Voxel-wise analyses of ASL data revealed a main effect of HOMA-IR, highlighting hyperperfusion in the right anterior cingulate (t = 3.5, p = 0.0001) and the left middle frontal gyrus (t = 3.7, p = 0.0001). Further, high ICA flow was predictive of higher cortical perfusion in the right superior frontal gyrus only in individuals with low HOMA-IR (p = 3.8, p = 0.0001); This relationship was not found in individuals with high HOMA-IR. Our findings provide novel evidence for an uncoupling of macrovessel flow and microvessel perfusion among individuals with higher IR and emphasize the importance of normal insulin function on cerebrovascular health in midlife. Furthermore, the observed relationships have implications for interventions aimed at promoting normal insulin and cerebrovascular function in midlife to attenuate risk for pathological aging.

**PCA357**

**Apelin causes endothelium-dependent relaxation of rat coronary, but not cerebral arteries**

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Increasing evidence supports a role for the adipokine, apelin, and its receptor (AP), in the regulation of cardiovascular function. Activation of apelin/AP receptor signaling pathways causes vasodilation in most peripheral arteries studied thus far. Apelin-induced vasodilation may be mediated, at least in part, by nitric oxide (NO) or by prostanoids derived from cyclooxygenase metabolism of arachidonic acid. The effects of apelin on cerebral arteries are presently unknown, and although apelin increases blood flow in the coronary circulation, the underlying mechanism(s) have not been elucidated. Here, we investigated the vasorelaxant effects of apelin in coronary and middle cerebral arteries isolated from rats (Sprague-Dawley, male, 275-325 g). Immunoblot analysis established that AP receptor protein was present in both arteries and immunofluorescence studies using confocal microscopy demonstrated AP receptor expression on endothelial cells (n=4). In isolated arteries suspended in myographs for isometric tension recording, concentration-response curves to apelin (10^-8 – 10^-6 M) were obtained in arterial rings contracted with 5-HT (10^-6 M). Values are means ± SEM, compared by ANOVA. In cerebral arteries, apelin had no effect on vascular tone in rings with or without endothelium; however, the endothelium-dependent vasodilator, bradykinin (10^-8 – 10^-6 M) caused relaxations (pDEmax=8.02 ± 0.3; Emax=83 ± 4% relaxation; n=5) that confirmed the presence of functional endothelium in these.
In contrast to cerebral arteries, apelin caused relaxation in endothelium-intact coronary arteries ($pD_{2}=6.91 \pm 0.1$; $E_{\text{max}}=45 \pm 6$% relaxation; $n=9$), but had no effect in those without endothelium. The endothelium-dependent response to apelin was completely abolished by the nitric oxide synthase inhibitor, nitro-l-arginine (3 x 10$^{-5}$ M; $P<0.05$, $n=6$) but was unaffected by the cyclooxygenase inhibitor, indomethacin ($10^{-5}$ M) ($pD_{2}=6.72 \pm 0.3$ vs 6.85 $\pm 0.5$; $E_{\text{max}}=38 \pm 5$ vs 40 $\pm 9$% relaxation, in the absence and presence of indomethacin, respectively; $P>0.05$, $n=6$). Apelin-induced relaxation was also abolished by the large conductance, calcium-activated $K$ channel (BK$_{Ca}$) blocker, iberiotoxin ($10^{-7}$ M; $P<0.05$, $n=6$). Thus, APJ receptors are expressed on endothelial cells in both coronary and cerebral arteries; however, apelin causes endothelium-dependent relaxation of coronary arteries but has no vasorelaxant effect in cerebral arteries. Apelin-induced relaxation of coronary arteries is likely mediated by NO rather than prostanooids and requires activation of BK$_{Ca}$ channels. The findings also suggest that the putative beneficial vasodilator effect of apelin in the coronary circulation may not occur in cerebral arteries, despite the presence of APJ receptors in the cerebrovascular endothelium.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCA358

The influence of high salt intake on carotid arteries responses to acetylcholine and changes in flow in whild type (WT) 129/Sv and TFF3/-/- mice

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High salt (HS) diet leads to endothelial dysfunction and impaired vascular reactivity to various stimuli. Different strains of rats, including genetically altered are the most frequent experimental models used for HS vascular functional studies (1,2,3) Hereby we introduce new model of transgenic TFF3/-/- mice which have favorable ratio of $\omega$-6/$\omega$-3 free fatty acids and modified metabolism of arachidonic acid (AA) (4,5). Considering the importance of metabolites of AA in vascular responses to stimuli, TFF3/-/- mice may give valuable information on the impact of HS diet of vascular function related to AA metabolism.

The aim of this study was to assess the effects of 1 week HS diet on acetylcholine (10$^{-6}$ M), SNP (10$^{-6}$ M) and flow-induced response (FIR) in isolated, pressurized carotid arteries of TFF3/-/- and their wild type controls, WT 129/Sv mice.

9-weeks old TFF3/-/- and WT 129/Sv mice (both sexes) were divided in LS group (N=4 + 4) fed with standard rat chow (0.4% NaCl) and HS group (N=5 + 4) fed food containing 4% NaCl for 1 week. Mice were anesthetized with ketamin-klorid (100 mg/kg) and midazolam (5 mg/kg) and decapitated. Carotid arteries were isolated, cannulated and pressurized for 60 at 100 mmHg to assess basal diameter and than subjected to flow at pressure gradients from $\Delta$10-$\Delta$180 mmHg (DMT pressure myograph, Danmark). To test differences among groups Two-way ANOVA was used, $p<0.05$ considered significant.

All experimental procedures conformed to the EU Guidelines (Directive 86/609) and national laws were approved by local and national Ethical Committee (Class:UP/I-322-01/14-01/36, No. 525-10/0255-15-6).

In each group (LS or HS) FIR at pressure gradients from $\Delta$10-$\Delta$60 mmHg leads to constriction of vessels while FIR at pressure gradients from $\Delta$100-$\Delta$180 mmHg leads to dilation of vessels compared to baseline ($\Delta$0 mmHg). TFF3/-/- HS group did not significantly differ in FIR compared to TFF3/-/- LS group. Carotid arteries of WT 129/Sv HS mice exhibited increase in constriction at $\Delta$60 mmHg ($p<0.05$) and reduced dilation at pressure gradients from $\Delta$100-$\Delta$160 mmHg ($p<0.05$) compared to its LS control group. Both, TFF3/-/- HS and WT 129/Sv HS groups had reduced response to ACh compared to their respective controls. SNP response was preserved in all tested groups. FIR of TFF3/-/- LS vs. 129/Sv LS mice and TFF3/-/- HS vs. 129/Sv HS mice did not show significant differences ($p>0.05$). These preliminary results show that FIR differs from ACh-induced responses. ACh-induced dilation was abolished, while FIR was preserved in TFF3/-/- HS groups. HS intake seems to have smaller impact on FIR in TFF3/-/- mice compared to their WT controls possibly due to favorable fatty acid composition and their direct or indirect role in production of vasoactive metabolites mediating FIR.


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PCA359

The role of enzymes matrix metalloproteinases-2 and 9 in the pathogenesis of atherosclerosis

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Atherosclerosis represents a complex, chronic multifactor disease with inflammatory, metabolic and autoimmune components that affects arteries and is characterized by atherosclerotic plaques and phenotypic changes of vascular cells. The members of the matrix metalloproteinase (MMP) family play a crucial role in angiogenesis and vascular remodeling and are involved in the pathogenesis of different vascular diseases such as atherosclerosis, varicose veins, hypertension, abdominal aortic aneurysm, preeclampsia, etc. Alterations in vascular tone are usually a result of changeable endothelial cell function, as well as increasing activity of neurohormonal stimuli or altered sensitivity of vascular smooth muscle. Chronic changes in vascular function lead to structural changes in the blood vessel architecture.

The aim of our study was to examine the values of enzymes matrix metalloproteinase-2 and 9 in urine from patients with atherosclerosis of carotid arteries, who underwent surgery, and compared them to controls (healthy volunteers). Patients and methods: we analyzed 40 patients with atherosclerosis who were undergoing the surgical procedure. The study protocols was approved by Ethics Committee of the Medical Faculty, University of Rijeka and written informed consent was obtained for each patient included in the study. Patients’ data and tissue samples were acquired in accordance with the published International Health Guidelines outlined in the declaration of Helsinki. Patients were classified into three groups (patients with symptomatic carotid artery disease, asymptomatic patients in whom surgery is necessary, and blood donors as healthy controls). The method of enzyme immunosay (ELISA) was used to determine enzymes expression of matrix metalloproteinase-2 and 9 (MMP-2 and 9). Immunohistochemistry, we determined the expression of heat shock protein 70 (HSP 70) on paraffin sections of atherosclerotic carotid arteries. Statistical data were calculated using the computer program Statistica 10.1.

Results: The patients with atherosclerosis had a statistically significantly increased level of MMP-2 and 9 in the urine in comparison with healthy volunteers, as well as more intensive expression of heat shock protein 70. Results of MMP 2 and 9 correlated with clinical symptoms.

Conclusion: Our data has showed a large increase in the enzyme MMP-2 and 9 in the urine of atherosclerotic patients, which can be an easy and economic marker for the monitoring of the development of atherosclerosis. Upregulation of enzymes MMP 2 and 9 in patients with atherosclerosis underline the crucial role of MMP in pathogenesis of atherosclerosis.


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PCA360

Tempol in vivo restores impaired relaxation of middle cerebral arteries in Sprague-Dawley rats on high salt diet

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Objective: It is known that increased dietary NaCl intake leads to increased vascular oxidative stress, related to impaired vascular responses to various stimuli. Our previous studies have shown that high salt (HS) intake reduces vascular responses to flow-induced dilation (FID) of middle cerebral artery (MCA) in Sprague-Dawley (SD) rats, and in vitro application of TEMPOL restores FID while it had no effect in rats on low salt (LS) diet (1). The aim of the present study was to determine the effect of simultaneous TEMPOL in vivo consumption with HS diet on MCA responses to FID in SD rats.

Design and method: 11-weeks old healthy male SD rats were divided in 4 groups: low salt group (LS) fed with standard rat chow (0.4% NaCl, N=16); LS+TEMPOL (0.4% NaCl+1mM; dissolved in tap water, N=10); high salt group (HS) (4%NaCl, N=15); HS+TEMPOL (4% NaCl+TEMPOL (1mM; dissolved in tap water, N=16) for 7 days. Prior to decapitation, rats were anesthetized with 75 mg/kg ketamine+2.5 mg/kg midazolam. MCA were isolated and cannulated (DMT pressure myograph) for vascular reactivity measurements in response to stepwise increase in pressure gradient (Δ100), in the absence/presence of the NOS inhibitor L-NAME, COX-1,2 inhibitor indomethacin (INDO) and selective inhibitor of microsomal CYP450 epoxidase activity MS-PPOH. To test differences among groups Two-way ANOVA was used, p<0.05 considered significant. All experimental procedures conformed to the European Guidelines for the Care and Use of Laboratory Animals (directive 86/609) and were approved by the local and national Ethical Committee (Class: 602-04/14-08/06, No 2158-61-07-14-04).

Results: FID was reduced in HS group at Δ40, Δ60 and Δ100 compared to LS group, at Δ20, Δ40 and Δ60 compared with HS+TEMPOL group, and at Δ10, Δ40 and Δ60 compared to LS+TEMPOL group (p<0.05). L-NAME, INDO and MS-PPOH reduced FID in LS and LS+TEMPOL compared with baseline, independently and in combination. In HS group only L-NAME statistically reduced FID at Δ20-Δ60 pressure gradient. In HS+TEMPOL group FID was reduced in the presence of L-NAME and MS-PPOH at Δ20-Δ100 pressure gradient, independently and in combination (p<0.05).

Conclusions: These results demonstrate that high salt intake impaires vascular responses to FID and SOD mimetic TEMPOL.
Antiphospholipid syndrome (APS) denotes antibody-induced thrombophilia with recurrent thrombosis and/or complications in pregnancy. One of the possible mechanisms of thrombosis in APS may be linked to diminished production of vasodilator nitric oxide (NO). It is known that reduced availability of NO leads to dysfunction of the endothelium and increased platelet aggregation. Local heating of the skin to temperatures below pain threshold (42°C) induces a biphasic increase in skin blood flow that is mediated by two independent mechanisms. The initial rapid increase is caused by a sensory axon reflex followed by a brief nadir and the secondary slowly rising plateau that is predominantly dependent on NO mechanisms. The aim of our study was to compare vasodilator response of cutaneous microvasculature to local heating in APS patients and healthy controls of the same sex and age.

METHODS. In our prospective study we included 34 patients and 34 healthy controls. Subjects (48 females and 20 males) were divided into three age groups: 22 to 38, 39 to 49 and 50 to 74 years. We measured electrocardiogram, arterial blood pressure and laser Doppler (LD) flux of cutaneous microvessels in response to local warming of the skin to 42°C. Analysis was conducted using ANOVA followed by Dunnett's test. The criterion selected for a statistically significant difference was p-value less than 0.05.

The study was approved by the National Medical Ethics Committee; written informed consent was obtained from each subject.

RESULTS. There were no differences in RR-intervals, systolic and diastolic blood pressure between patients and controls. At rest LD flux was statistically significantly higher in the group of 39-49 years old healthy control subjects than in APS patients of the same age. Vasodilatory response to local heating was significantly smaller in patients than in healthy subjects in the age group of 22 to 38 years (44.4±16.1 PU in patients vs. 55.4±20.2 PU in age matched controls) (t-test, p<0.05). In both older age groups (39-49 and 50-74 years) of APS patients and controls vasodilatation was approximately the same (59.3±9.3 PU in patients aged 39 to 49 vs. 57.7±9.5 PU in age matched controls and 43.1±11.3 PU in patients aged 50 to 74 vs. 55.6±18.9 PU in age matched controls).

CONCLUSIONS. From our research it can be concluded that the microcirculation in patients with APS is diminished. For more precise definition of these changes further research is required, which should take into account other characteristic of patients and health controls, as for instance the way of living, training, treatment of APS and other diseases such as hypertension etc.

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age groups of patients did not differ from the control group (35.3 ± 8.2 PU in patients aged 22 to 38 years vs. 66.2 ± 22.2 PU in age matched controls; 33.1 ± 10.5 PU in patients aged 39 to 49 years vs. 49.0 ± 11.7 PU in age matched controls, 27.3 ± 3.7 PU in patients aged 50 to 74 years vs. 33.5 ± 8.1 PU in age matched controls).

Conclusion. Vasodilation capability of patients with APS is altered. For more precise determination of these alterations further research is required, which should take into account the impact of certain drugs that are widely used for the treatment of APS patients on vasodilation.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Is the guinea pig a full negative model to study the carotid body mediated Chronic Intermittent Hypoxia effects?

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Chronic Intermittent Hypoxia (CIH) is considered to be one of the main causes of systemic arterial hypertension observed in the obstructive sleep apnea syndrome. It is thought that repetitive episodes of hypoxia/re-oxygenation produce oxidative stress, inflammation and sympathetic hyperactivity, generating endothelial dysfunction and systemic hypertension. It has been proposed that the repeated carotid body (CB) stimulation produced by CIH would induce CB sensitization, increasing chemoreceptor input to the brainstem that originates an exaggerated sympathetic reflex with a rise of circulating catecholamine (CA) and hypertension. Unlike other rodents, preliminary data show a lack of CB sensitivity to acute hypoxia in guinea pigs, in which case, CIH would not induce CB sensitization and the effects derived from the CB hyperactivity would not be observed. Therefore, guinea pigs could be a negative control model to study the effects of CIH mediated by CB. In this study, experiments were performed on adult male Hartley guinea pigs (475±10 g; n=32) control or exposed to CIH (21% O₂-80±5% O₂-40±5 8h/day; 30 days). Ventilatory parameters (tidal volume and respiratory rate) were measured in freely moving animals by whole body plethysmography; other measurements were performed on animals anaesthetized with a mixture of ketamine (100 mg/Kg) and diazepam (2 mg/Kg; ip.). Control and CIH guinea pig respiratory minute volume exhibited similar changes when acute hypoxic test was applied (399±5 vs 411±5 in air and 417±15 vs 456±17 ml/min/Kg in 10% O₂; n=16). Values are means ± S.E.M. compared by ANOVA. There were no in vitro CB responses to acute hypoxia (CA secretion or Ca²⁺ changes) in either group of animals. No differences were found in mean arterial blood pressure (37±2 vs 43±3 mmHg; n=8), or in plasma and tissue CA levels (CB, adrenal medulla, renal artery) measured after exposure to CIH. The fact that the guinea pig CB is hypo-functional and is not sensitized by CIH would reinforce our working hypothesis: systemic effects associated to CIH in other species and absent in guinea pigs are due to the CB hyperactivity induced by CIH. However several unexpected results would indicate hypoxic activation of the sympathetic system during acute hypoxic test as increased arterial pulse pressure (20±1 mmHg in air and 28±2 mmHg in 10% O₂; p<0.01; n=8), rise of heart rate (25%; p<0.01), renal artery CA synthesis (32%; p<0.05) and CB hypertrophy (50%; p<0.05) after CIH treatment. These results suggest that guinea pigs possess O₂-sensitivity responsible for the sympathetic cardio-circulatory reflex, probably through CB stimulation, the main oxygen sensing structure mediating this reflex. The mechanisms are being studied.

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PCB001

Is the guinea pig a full negative model to study the carotid body mediated Chronic Intermittent Hypoxia effects?

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Background: Previous studies report conflicting relationships between changes in blood pressure (BP), muscle sympathetic nerve activity (MSNA) and sympathetic baroreflex sensitivity (BRS) following renal denervation (RDN)1-4. The mechanisms underlying the anti-hypertensive effect of RDN remain unclear. Methods: We investigated changes in office BP, MSNA and BRS following RDN. Patients were assessed at 0, 1, 3, 6 and 12 months with measurement of MSNA (peroneal microneurography) and beat-to-beat BP (Finapres) over 5-10 min. Diastolic (D)BP readings were collated into 1 mmHg bins and the percentage of cardiac cycles containing an MSNA burst was calculated. The weighted linear fit to these data yields BRS5. Data analysed with ANOVA/Friedman test with Bonferroni/Dunn’s multiple comparison tests post hoc, and Pearson correlation, and presented as mean ± SEM.

Results: 13 patients (8 men), aged 52±3 years, office BP 193±5/106±7 mmHg, taking 5±0.4 antihypertensives underwent RDN (10.8 ± 0.5 ablations). Mean baseline MSNA 60±6 bursts/100 heartbeats and BRS -1.4±0.3 %/mmHg. Systolic (S) BP reduced post RDN (see Figure, p=0.006: 1 month, n=12, -15±9/6±7 mmHg; 3 months, n=10, -21±10/8±7 mmHg; 6 months, n=13, -18±11 mmHg; 12 months, n=10, -30±12 mmHg). There was no change in MSNA or BRS (p=0.72 and p=0.63 respectively). At 1 and 6 months post RDN, those with a higher baseline SBP had a greater reduction in SBP (R=−0.68, p=0.01; R=−0.69, p=0.008), but there was no correlation between baseline MSNA and the change in SBP. There was a trend towards a positive correlation between baseline BRS and change in SBP at six months (R=0.52, p=0.07). Contrary to previous findings, those with a higher baseline SBP had lower MSNA at baseline (R=−0.67, p=0.01), this is likely because within this small cohort those with the highest baseline SBP were younger females with lower MSNA. 62% (8/13) patients responded to RDN with a ≥10 mmHg reduction in SBP at 6 months. Amongst these responders, BRS showed a strong trend towards a temporal pattern (p=0.06), increasing at 1 month and then returning to (at least) baseline levels by 12 months post RDN (see Figure), however, there was no change in MSNA (p=0.25).

Conclusion: Patients who respond to RDN appear to have a short-term increase in BRS which regresses by 12 months. In
contrast, the reduction in SBP post RDN is progressive and sustained, suggesting that temporary baroreflex modulation may be sufficient to reset BP to a lower operating level, although this is independent of MSNA, perhaps indicating post-junctional remodelling. These pilot data provide important insights into the autonomic mechanisms underlying RDN and will assist in directing future research.

Figure. Change in SBP, MSNA and BRS in all patients (left column) and those patients responding at 6 months (right column) over 12 months post RDN. P values across all data represent 1-way ANOVA/Friedman test, other statistical results are from Bonferroni/Dunn’s multiple comparison tests.


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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements. 

PCB003

Consecutive treatment with isoproterenol and adenosine protects adults but not immature heart against ischaemia and reperfusion

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Background
Ischaemia and reperfusion injury remains a critical issue in many clinical contexts, especially cardiovascular surgery. Our previous work (1) using normal and diseased adult rat heart has shown that sequential activation of Protein Kinase A (by isoprenaline (Iso)) followed by activation of Protein Kinase C (with adenosine (Ade)) confers marked protection against ischaemia/reperfusion injury.

Purpose
The protective effect of this intervention in the immature heart against ischaemia/reperfusion injury is unknown. The aim of this work was to investigate the cardioprotective efficacy of this novel intervention during postnatal myocardial development.

Methods
Hearts from 14-day postnatal (interventions n=6, controls n=5) and from adult male (interventions n=7, controls n=8) Wistar rats were perfused in the Langendorff mode. The coronary perfusion pressures were held constant at 65 mmHg for the 14-day group and 80 mmHg in the adult group. After a 30 minute equilibration period, hearts were subjected to 30 minute global ischaemia (37°C) and 2 hours reperfusion. In the intervention group, hearts were perfused with 5nM isoprenaline for 3 min followed by 0.3µM adenosine for 5 min prior to the onset of global ischaemia. Cardiac function through the experiment until 2 hours of reperfusion was assessed by measuring left ventricular developed pressure using a pressure transducer through a latex balloon inserted in the left ventricle and calculating the rate-pressure product (RPP).

Results
Pre- and post- ischaemia function was compared for each animal using the paired t-test. Consistent with earlier work, consecutive treatment of adult heart with isoprenaline and adenosine (Iso/Ade) was associated with marked and significant (p=0.03) improvement in percentage recovery in RPP after 2 hr reperfusion (20% recovery in controls, 45% in the intervention group). In contrast, the treatment did not improve recovery of RPP in postnatal heart (p=0.76; controls had a 25% recovery and interventions had a 26% recovery).

Conclusion
This work shows that the strong cardioprotective efficacy of Iso/Ade treatment is only seen in adult but not in postnatal heart. This difference is likely to be due to developmental changes in isoprenaline-induced signalling pathways through cAMP and downstream mediators. Our observations highlight the need for further work regarding the fundamental cell signalling differences in the developing myocardium to develop effective cardioprotective interventions.
Hypoglycaemia alters the ventilatory responses to hypercapnia but not to hypoxia

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Hypoglycaemia (HG) evokes a highly integrated counter-regulatory response in order to restore normal blood glucose levels. HG also stimulates a carotid body-mediated hyperpnoea via an increase in peripheral CO2 sensitivity, thus enabling normocapnia to be maintained despite the increased metabolism. The present study aimed to investigate whether HG changes the pattern of ventilation evoked by hypercapnia and also whether HG modulates the response to hypoxia in a similar manner.

Whole body plethysmography (WBPG) was used to measure ventilation in air, acute hypoxia (10% O2) and hypercapnia (8% CO2), in 7 conscious male Wistar rats. Ventilatory responses were measured both in normoglycaemic (NG) and after HG was induced by insulin (1.5 IU kg^-1 ip). All animals had free access to food and water until they were fasted for 8 hours prior to experiments.

Blood glucose levels, sampled by tail prick, tended to be lower after fasting but not significantly (8.0±0.8 vs 6.8±0.5 mmol l^-1). NG hypercapnia and hypoxia increased ventilation (V_e) from 100±9 to 265±32 ml min^-1 and from 104±13 to 223±21 ml min^-1 respectively. Insulin induced a significant HG (3.8±0.5 mmol l^-1). HG increased the response to hypercapnia (ΔV_e from 165±37 to 232±51 ml min^-1) by having a greater effect on tidal volume (ΔVT from 0.65±0.14 to 0.99±0.27 ml). During HG, hypercapnic T_e was reduced to a similar level as seen in NG (185±21 vs 200±29 ms). In contrast, the decrease in T_e in hypoxia was not affected by HG. Both hypercapnia and hypoxia increased the T_e/T_r ratio (from 0.58±0.06 to 0.91±0.10 and 0.58±0.05 to 0.78±0.08 respectively). Only the magnitude of the hypercapnia-induced increase was dependent on glycaemic state. HG induces changes in baseline ventilation but also interacts with the pattern of the ventilatory response to hypercapnia. This is not the case during hypoxia. Central glucose sensors located within hypothalamic and medullary nuclei, may have the potential to interact with respiratory control to modulate the pattern of response to CO2 during HG, particularly influencing expiratory drive. The dependency of the increase in T_e/T_r on blood glucose levels during hypercapnia may reflect the coordinated neuroendocrine responses seen during the counter-regulatory response to HG, allowing ventilation to be matched to the increased metabolic rate.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Banana pudding flavored e-liquid alters cell proliferation and Ca2+ signaling in lung epithelia

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With over 7,000 flavors available in the US, e-cigarettes have grown increasingly popular. However, little is known about their individual constituent chemicals or their effects on lung epithelia. We purchased flavored e-liquids from the Vapor Girl (http://www.thevaporgirl.com/) to characterize both the chemical constituents and biological effects of different flavors on human bronchial epithelial cultures (HBECS) and CALU3 lung epithelia. We screened a range of e-liquid flavors and doses for effects on cell proliferation/viability as well as their ability to alter cell signaling (e.g. Ca2+ signaling). Ca2+ signaling regulates cell division, mucus secretion, ciliary beat frequency, and Cl-/fluid secretion in airway epithelia and is disrupted by cigarette smoke. Altered Ca2+ signaling could cause changes in cell homeostasis. Thus, we measured changes in cytosolic Ca2+ following acute e-liquid exposures as well as other components of Ca2+ signaling pathways (i.e. STIM1 puncta formation, kinase phosphorylation, IP3 generation). Cells were plated on glass coverslips or in multiwell plates and exposed to a range of e-liquid doses (v/v %) diluted into media either acutely (10 min) or over 24h. Cell proliferation/viability was measured using the MTT assay. Cytosolic Ca2+ changes were measured using Fura-2-AM. Relative kinase phosphorylation was measured using a Human Phospho-Kinase Array. IP3 generation was measured using a competitive ELISA. All assays were completed with HBECS and/or CALU3 cells except STIM1 puncta visualization, which used transiently transfected STIM1-mCherry HEK29293 cells.

We found that several flavors inhibited cell proliferation in a dose-dependent manner, including Banana Pudding Southern Style (BPSS). BPSS also elicited an acute cytosolic Ca2+ signal involving both the endoplasmic reticulum (ER) and store-operated Ca2+ entry (SOCE), formed STIM1 puncta, and altered phosphorylation of ERK1/2, which regulates STIM1 phosphorylation. Our data demonstrated that the BPSS-flavored e-liquid altered the ER/SOCE Ca2+-signaling mechanism in airway epithelia, which could have biological consequences to Ca2+-mediated innate defenses of HBECS exposed to BPSS e-cigarette aerosol long-term. This also suggested that other flavors may have the ability to alter cell signaling (e.g. Ca2+, etc.) and other important lung epithelial functions. Investigations into the effects of flavored e-cigarette aerosol on Ca2+-dependent aspects of innate defense are ongoing. We have also identified specific chemical constituents from BPSS using mass spectrometry and we aim to identify the individual constituents that alter cell Ca2+ signaling in flavored e-liquids.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Do rat and/or human primary pacemaker cells have t-tubules?

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The sinus node (SN) is located in the area between the superior caval vein and the right atrium. It is the first component of the cardiac conduction system where the initiation and propagation of the electrical activity in the heart begins. Transverse tubules (t-tubules) are invaginations in the cell membrane of mammalian ventricular myocytes. L-type Ca\textsuperscript{2+} channels are located on these membranes, in close proximity to the sarcoplasmic reticulum (SR), and trigger, Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release. T-tubules are not present in the atrial and nodal myocytes of small animals such as rats; however, atrial myocytes of larger animals such as sheep do have t-tubules. This raises the question: are these structures present in the primary pacemaker cells? The aims of this study were to investigate (1) if t-tubules are present in rat and/or human SN myocytes and (2) is ryanodine receptor (RYR2) organization similar to that observed in ventricular myocytes.

Ventricular and nodal myocytes were isolated by enzymatic digestion from male Wistar rats. All procedures were in accordance with the Animal (Scientific Procedures) Act 1982. Immunocytochemistry and confocal microscopy was performed on isolated myocytes (n=29 nodal and n=12 ventricular cells; from n=8 rats). Cells were labelled with wheat germ agglutinin (WGA) as cells membrane and its invagination marker and an RYR2 antibody as specific marker for RYR2. Immunohistochemistry and confocal microscopy was also performed on tissue sections from frozen human SN and its surrounding atrial muscle (n=3 healthy specimens). Specimens were stored under the Human Tissue Act 2004. Tissue sections were labelled with: (1) WGA, (2) RYR2, (3) alpha-actinin (a marker of Z-lines of myofibrils), (4) HCN4 (responsible for funny current that contributes to the pacemaker potential), (5) Cx43 (a major gap functional channel responsible for electrical activity between working myocardial cells) as a positive marker and negative marker of the SN respectively and (6) Caevolin3 (a cardiac cell membrane marker).

Our results showed that rat isolated nodal cells lack t-tubules (by the absence of a striated pattern of WGA labeling) but RYR2 expression in the SN was striated. In ventricular cells both WGA and RYR2 expression [MLM3] pattern was striated and signal pattern was identical with clear t-tubular labelling. In tissue sections, we observed that some atrial and nodal cells contained t-tubules. The SN cells did not contain t-tubules and the striated pattern of RYR2 expression was co-localised with alpha-actinin. HCN4, Cx43 and Caevolin3 expression was the same as previously reported by Chandler et al.,. HCN4 was present and Cx43 was absent in the SN and Caevolin3 was expressed in all atrial and nodal myocytes. We conclude that a striated pattern of RYR2 expression and its co-localisation with alpha-actinin is important for efficient Ca\textsuperscript{2+} signalling in these specialised cells.


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Acute hypoxic stress causes diaphragm muscle weakness in mice and rats


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Diaphragm weakness is a strong predictor of poor outcome in patients. Acute hypoxia (AH) is a feature of respiratory conditions such as acute respiratory distress syndrome and ventilator-associated lung injury. However, the effects of AH on the diaphragm are largely unknown despite the potential clinical relevance.

Adult male C57BL6/J mice (n=8 per group) were exposed to 8hr of AH (P<0.10) or normoxia. A separate group of mice (n=8) were administered N-acetyl cysteine (NAC, 200mg/kg, I.P.) immediately prior to AH. Ventilation was assessed using whole-body plethysmography. Oxygen consumption and carbon dioxide production were measured. Diaphragm muscle contractile performance was determined ex-vivo. Gene expression was examined at 1, 4, and 8hrs using qRT-PCR. Citrate synthase activity was measured following AH exposure using a spectrophotometric assay. Ventilation, metabolism and diaphragm contractile function was also determined in adult male Sprague Dawley rats exposed to normoxia (n=8) and AH (n=8). Data are mean±SD and were compared by ANOVA or Student t test; p values are reported.

In mice, minute ventilation during AH was initially increased (p<0.001, ANOVA at 10mins), but quickly returned to normoxic levels for the duration of gas exposure. VCO\textsubscript{2} production was reduced throughout AH exposure (p<0.0001). AH decreased diaphragm peak force (30±3 vs. 21±2 N/cm\textsuperscript{2}, p=0.0334) and force-frequency relationship (p=0.0112), but increased endurance (p<0.0001). AH increased PGC1α (p<0.05), UCP-3 (p<0.01), FOXO-3 (p<0.05) and MuRF-1 (p<0.05) gene expression. Citrate synthase activity was increased (p<0.05) following AH. NAC pre-treatment prevented the AH-induced diaphragm weakness. In rats, AH increased minute ventilation (p<0.0001) throughout the 8hr exposure associated with a decline in VCO\textsubscript{2} production (p<0.0001). AH caused diaphragm weakness in rat similar to mouse.

Diaphragm weakness is reported in mechanically ventilated patients, which is primarily attributed to inactivity (unloading) of the muscle, although this is controversial. The potential role of hypoxia in the development and/or exacerbation of ICU-related weakness is unclear and perhaps underestimated. Our data reveals that AH is sufficient to cause diaphragm muscle weakness, which may relate to atrophy, as evident from increased pro-atrophy (MuRF-1) gene expression. Muscle...
The Allies planned for a 40,000 foot air war in World War II (WWII): development and testing of the demand type pressure breathing O₂ equipment used by the U.S. Army Air Force (USAAF), 1942-45

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In the early days of WWII, Sir Frederick Grant Banting, 1923 Nobel Laureate (co-discovery of insulin), pioneering aviation physiologist, and Major in the Royal Canadian Air Force stated ‘...whichever power gets up to 40,000 feet first and stays there longest with the heaviest guns will win the war (1).’ At 40,000 ft, however, air is only 1/5 as dense as it is at sea level, inspired pO₂ has decreased to ~1/5 its sea level value, and ambient temperature drops to -60° to -100°F! One way to survive in the stratosphere for 1.5-8 hr during flight operations without suffering “oxygen want” or “anoxia” was to fly in a pressurized aircraft; however, pressurized flight was rare in WWII until America deployed the B-29 Superfortress bomber in April 1944 in the Pacific war. The alternative, which was pursued by the USAAF aero physiologists beginning in January 1942 (2), was an accelerated research and testing program to develop the demand type pressure breathing (PB) O₂ mask and regulator (3). The PB mask works like a small hyperbaric chamber strapped to the aviator’s face, increasing ambient pressure by 2-12 inches of water pressure (in H₂O) so that airman’s “ceiling” on pure O₂ was extended from 33,000 ft to as high as 39-50,000 ft for short periods of time in unpressurized aircraft (3). For example, arterial O₂ saturation (%O₂ sat.) started decreasing above 33-41,000 ft using the continuous flow O₂ mask (A-8 & A-9) and conventional demand type O₂ mask (A-14). Using the demand type PB O₂ mask (A-13 & A-15) and A-14 regulator, however, arterial %O₂ Sat. remained in the near normal to normal range (high 70s-low 90s) depending on the altitude (2-5). PB equipment underwent a rapid metamorphosis during 1942-43 (Fig. 1a-c), finally being deployed operationally in Feb. 1944 in photographic reconnaissance squadrons and later, fighter and bomber groups (Fig. 1c). Using PB equipment, the altitude record for an unpressurized aircraft was set at 43,299 ft by members of Boeing’s Flight Test Unit, July 1945. In the end, Sir Frederick C. Banting had overestimated the altitude at which the Allies would have to fly and fight to win air superiority. The air war of 1939-1945 was waged mostly at 25,000 to 35,000 ft with limited numbers of missions penetrating higher, but when they did, PB was available and used. Despite the mostly stratospheric air war, Major Banting’s goal of 40,000 ft had shaped the goals of the aero medical research and altitude indoctrination programs in America for the duration of the war. Consequently, the availability of PB equipment made for an easy transition to the jet age with it’s higher operational altitudes in the post-war period.

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spinal cord stimulation (conventional SCS, 50 Hz, 15mA) results in large positive airway pressure generation, and is a useful method to restore an effective cough mechanism. Unfortunately, activation of the expiratory muscles via SCS requires high stimulus amplitudes, which may also cause unwanted side effects including stimulation of sensory fibers, and cannot be applied in patients with intact sensation.

Objective: The purpose of the present study was to evaluate our hypothesis that lower thoracic SCS with high stimulus frequencies and low stimulus currents will result in sufficient activation of the expiratory muscles to produce large positive airway pressures and high peak expiratory airflow rates sufficient to generate an effective cough.

Methods: Studies were performed on 5 dogs, anesthetized with pentobarbital sodium (25 mg/kg IV, initially) and intubated. Additional doses of pentobarbital sodium were given, as required (1-3 mg/kg IV). The effects of varying stimulus amplitudes and frequencies on positive airway pressure generation produced by SCS were evaluated following hyperventilation-induced apnea. SCS was applied after tracheal occlusion, at functional residual capacity (FRC) and also over a wide range of lung volumes (0.3L below to 1.3L above FRC) via a disc electrode positioned epidurally at the T9 spinal level. Given our previous success with conventional stimulus parameters (50Hz, 15mA), these were used as our gold standard to which all comparisons were made.

Results: At any given level of stimulus current, at FRC, mean expiratory airway pressure generation was largest at 500Hz, compared to all other stimulus frequencies. For example, with stimulation at 1 mA and frequencies of 50, 200, 300, 500 and 600Hz, expiratory airway pressures were 12±2, 26±2, 39±2, 60±5 and 51±6cmH2O, respectively. In comparison, the mean airway pressure generation measured at FRC with conventional stimulus parameters (50Hz, 15mA) was 66±8 cmH2O. Moreover, airway pressure generation increased in linear fashion in response to increasing lung volume during high frequency spinal cord stimulation (HF-SCS) and during conventional stimulation, over the vital capacity range.

Summary: These results suggest that HF-SCS produces a comparable level of expiratory muscle activation and positive airway generation to that achieved with conventional stimulus parameters, but with much lower stimulus amplitudes. Conclusion: HF-SCS may be a useful method to restore an effective cough in patient populations with intact sensation and who would benefit from restoration of an effective cough.

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Blood pressure modification following one-month adaptation to oral ingestion of high potassium isotonic fluid (cocos nucifera water) in human subjects

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Potassium adaptation has been well reported in humans and lower animals. Increase in Na⁺/K⁺-ATPase activity has been implicated in the adaptive response mechanism. Slightly elevated plasma potassium concentration has been reported to be vasodilatory. Cocos nucifera water and Colubrina arborescens have been used synergistically to control hypertension. Cocos nucifera water is the liquid found within the endosperm of coconut. It is an isotonic fluid known for its natural high potassium content and health benefits. It is literally claimed to reduce blood pressure among other benefits without much scientific evidence in the literature. This study was designed to highlight the possibility of altering blood pressure responses after a chronic exposure to Cocos nucifera water in humans with normal and high blood pressures and to determine at what point does its efficacy register. A total of twenty human subjects comprising of 10 apparently healthy individuals with mean blood pressure of 122.4±5.5 and 10 hypertensives with mean BP of 165±12.5 were included in the study. They were given 10ml/kg body weight of fresh Cocos nucifera water to drink once a day for 30 days. Their informed consents as well as the ethical approval were obtained from the faculty ethical committee, at the University of Benin. Blood pressure data were collected on a weekly basis throughout the experimental period. Data includes Systolic, Diastolic and mean arterial blood pressures with their pulse rates. We recorded a gradual decrease in systolic and diastolic blood pressures which became significant (P<0.05, respectively) from the 2nd week and sustained for the remaining periods in both controls and hypertensives while the pulse rate and MAP in the control remained stable the values decreased significantly (P<0. in the hypertensives at the third week. We therefore conclude that oral intake of Cocos nucifera water has demonstrable blood pressure lowering effect in hypertensive subject after a two-week adaptation process possibly through its high potassium content. The reduction in the normal subjects was within normal range.


We greatly appreciate our subjects who participated fully in this study

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Hypoxia inducible factor 1α in nucleus tractus solitarii glutamatergic neurons is necessary for ventilatory acclimatization to hypoxia

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Chronic sustained hypoxia (CH) produces ventilatory acclimatization to hypoxia (VAH), which is a time-dependent increase of resting ventilation and hypoxic ventilatory response (HVR). VAH involves increased O₂-sensitivity in carotid bodies and neural plasticity in medullary respiratory centers, including the nucleus tractus solitarii (NTS) at the primary synapse of carotid body chemoreceptors. We hypothesized that VAH requires gene expression induced by hypoxia inducible factor 1-α (HIF-1α) in respiratory neurons in the NTS. To test this, we anesthetized (ketamine/xylazine 40/3 mg/Kg i.p.) transgenic mice (loxP sites flanking HIF-1α gene, 1) and micro-injected adeno-associated virus expressing green fluorescent protein and Cre-recombinase in neurons (Vector Biolabs AAV2-Cre-GFP) in the NTS to delete HIF-1α gene before mice were acclimatized CH (PiO₂=70 Torr). Transgenic mice were micro-injected in the NTS with AAV expressing only GFP for CH controls. After 7 days of CH, we measured hypoxic and hypercapnic ventilatory responses using whole body plethysmography and metabolic rates (V̇O₂ and V̇CO₂) during acute normoxia and hypoxia (21% and 10% O₂). We also tested for HIF-1α deletion (GFP-Cre expression) in glutamatergic and GABAergic neurons in the NTS using immunohistochemistry (Vglut2 and GAD67, respectively) in another group of CH mice. Finally, we used cFos immunoreactivity as a marker for neural activity in the NTS during acute hypoxia (10% O₂ 2-3 hrs).

HIF-1α deletion in the NTS significantly (p<0.05 multivariate ANOVA, Fisher’s test, n=11-14) decreased the HVR in CH mice by decreasing V̇E during 10% O₂ breathing (3320±250 in GFP-Cre vs. 4349±278 ml/(min kg) in GFP mice); there was no significant difference in V̇E breathing 21% O₂ or 7% CO₂. Metabolic rate was not different with HIF-1α deletion. However, we measured ventilation normalized for decreased metabolism in acute hypoxia (V̇E/V̇CO₂) and HIF-1α deletion significantly increased VAH during 10% O₂ breathing (109.9±7.7 in GFP vs. 86.0±5.6 ml/(min kg) in GFP-Cre mice.). Acute hypoxia increased cFos immunoreactivity in GFP-Cre positive neurons in the NTS and GFP-Cre positive neurons in the NTS of CH mice co-localized with Vglut2 but not GAD67-positive neurons. The results support HIF-1α-inducing glutamatergic plasticity that is necessary for VAH (2) but experiments are necessary to determine why adeno-associated virus preferentially infects glutamatergic vs. GABAergic neurons in the NTS during CH.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Phenanthrene is the cardiotoxic polycyclic aromatic hydrocarbon

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The Deepwater Horizon disaster drew global attention to the toxicity of crude oil and the potential for adverse health effects among spill responders and the numerous animals in the northern Gulf of Mexico. Crude oil from the spill released complex mixtures of polycyclic aromatic hydrocarbons (PAHs) into marine areas included pelagic spawning habitats for tunas, billfish, and other ecologically important top predators. PAH exposure of whole fishes during development and exposure to heart cells from adults, reveal the heart is vulnerable to oil-toxicity. However, the precise PAHs that cause cardiotoxicity, as well as the mechanisms underlying contractile dysfunction, are not known. Here we used electrophysiological and confocal microscopy techniques in tunas (Pacific bluefin tuna, Thunnus Orientalis, yellowfin tuna, Thunnus albacares) and Pacific mackerel (Scomber japonicus) to demonstrate that phenanthrene, a PAH with a benzene 3-ring structure, is the key compound disrupting cardiac function. Phenanthrene prolongs the action potential due to potassium channel blockade and decreases the amplitude of the cellular Ca2+ transients that drive force generation. Because there are many important environmental sources of phenanthrene in addition to petroleum based oil spills, including urban air pollution, our findings suggest that phenanthrene may be a major worldwide cause of vertebrate cardiac dysfunction.

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Microbiota and respiratory control: Blunted ventilatory responsiveness to hypercapnia in adult male rats following chronic antibiotic treatment

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Studies of early life stress models and germ free animals, amongst other experimental models, have revealed that the microbiome is vital for physiological homeostasis, including diverse aspects of brain function. We previously demonstrated in rats that early life stress results in disordered breathing and impaired ventilatory responsiveness, which persists into adulthood, effects that correlate with concomitant alterations in gut microbiota. We sought to further explore the putative link between altered microbiota and respiratory control using chronic antibiotic treatment (ABX) to disrupt the adult microbiome.

Forty adult male Sprague Dawley rats were studied. To deplete the microbiota, rats (n=20) were treated with an antibiotic cocktail for 4 weeks beginning at 8 weeks of age, consisting of ampicillin (1g/L), vancomycin (500mg/L), ciprofloxacin HCL (20mg/L), imipenem (250mg/L) and metronidazol (1g/L) prepared in autoclaved deionised water, changed every 2 days. Sham animals (n=20) received autoclaved deionised water. Animal weights were taken daily. Cages were cleaned every second day. Half the animals in each group were studied after 4 weeks; following a washout period of 72 hours, the remaining animals in both groups received transplantation by oral gavage of control faeces and transfer to sham bedding for a period of 4 weeks in an effort to re-colonize with standard microbiota. Ventilation and metabolism during air breathing and in response to hypercapnic (5% CO2) gas challenge were assessed by whole-body plethysmography. Data are reported as mean ± SD and were compared by unpaired t-test.

Baseline ventilation (V̇e) in rats was unaffected by ABX (0.49 ± 0.07 ml/min/g) compared with WT (0.50 ± 0.06); resting metabolism (VCO2 production) was not different between the two groups (0.018 ± 0.002 versus 0.016 ± 0.003 ml/min/g). Ventilatory responsiveness to hypercapnia was blunted in ABX animals. Thus, V̇e/VCO2 was lower (p= 0.038) during 5% CO2; mean V̇e/VCO2 in hypercapnia was (90.6 ± 30.2) versus (66.5 ± 17.4). The blunted response to hypercapnia related to significant depression of the respiratory frequency response to chemostimulation. Baseline ventilation and ventilation during hypercapnic exposure were not different in control re-colonized and ABX re-colonized animals.

ABX has been shown to dramatically alter the gut microbiome, with associated changes in mood and cognition. Here we show that ABX blunts ventilatory responsiveness to hypercapnic chemostimulation, suggestive of altered reflex control of breathing. Blunted ventilatory responses to classical activation of chemoreceptors suggests aberrant plasticity in sensory pathways key to the maintenance of respiratory homeostasis.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
hypertension and progressive heart failure by the age of 20 weeks. We used lean littermates of ZSF rats as controls as they do not develop diabetes or heart failure. All rats were maintained for 23 weeks from 8th week and euthanized and hearts were harvested. Total RNA was isolated from the hearts and processed for transcriptome analysis using Illumina HiSeq 2500. Hearts from obese ZSF1 rats were compared to those from lean ZSF rats. The differential gene list from this analysis was imported into Ingenuity Pathway Analysis (IPA) for global transcriptome analysis and pathway mapping. Results: The top canonical pathways that were differentially affected include cardiac β adrenergic signaling, mitochondrial dysfunction, dopamine receptor signaling, creatinine phosphate biosynthesis, superoxide degradation and CDK5 signaling. Overall about 400 genes demonstrated a significant change of at least 2 fold change on log 2 fluorescence intensity values with majority of these genes down regulated. The important downregulated molecules included PPP2R1A (protein phosphatase Regulatory subunit A alpha), PPP2R14C, AKAP7 (A Kinase anchoring protein 7), SLC8A2 (solute carrier family 8 Na-Ca) SOD 2 (superoxide dismutase 2), CKM (creatine kinase muscle) CITED2 (c (~p300 interacting transactivator) and ALDH8A1 (aldehyde dehydrogenase 8). Conclusions: Our data indicates that Next Gen sequencing of hearts from ZSF1 rats identified several pathways and molecules which are involved in myocardial function and failure. A number of these specific proteins and molecules are incriminated in biochemical and signaling cascades that result in myocardial inflammation, oxidative stress, apoptosis and fibrosis. These observations provide new insights into understanding the pathogenic pathways resulting in heart failure. Diabetic Nephropathy is associated with oxidative stress and decreased renal nitric oxide production. Prabhakar S, Starnes J, Shi S, Lonis B and Tran R. J Am Soc Nephrol 2007 Nov:18 (11) 2945-52 Obese ZSF1 rat as a new model of heart failure with preserved ejection fraction accompanying the metabolic syndrome. Louren A, Falcao-Pires I, Cerquiera R, Fontoura D, Miranda D, Hamdani N, Paulus W and Leite-Moreira A. Circulation 2012;126:A17471 The investigators acknowledge Woirihaye Research Foundation for the support of these studies Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB016

Sympathetic overactivity and hypertension in rats submitted to chronic intermittent hypoxia are not dependent of medullary C1 neurons

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Chronic intermittent hypoxia (CIH) produces expiratory-related sympatho-excitation in rats, which might be an important contributor to the development of neurogenic hypertension. We evaluated the role of sympatho-excitatory catecholaminergic medullary C1 neurons in mediating the expiratory-related sympatho-excitation and hypertension in CIH rats. In awake and in situ perfused preparations of rat, C1 neurons were acutely silenced by application of the insect peptide allatostatin following cell-specific targeting with a lentiviral vector to express the inhibitory Drosophila allatostatin receptor in C1 neurons of control and CIH rats (10 days). In awake rats, inhibition of ~72% of the C1 neurons resulted in a profound fall in arterial pressure and heart rate that was similar in control (n=12) and CIH rats (n=12). However, CIH rats still presented high arterial pressure after C1 inhibition (p<0.05) when compared to control rats. In in situ, C1 neuron inhibition resulted in reversible reductions of firing frequency, perfusion pressure, and amplitude of inspiratory-related bursts of thoracic sympathetic nerve activity in control (n=7) and CIH (n=9) rats. We also documented that CIH- (n=9), acute hypoxia- (n=7) or CO2-induced expiratory-related sympatho-excitation (n=10) were not affected by C1 inhibition. These data confirm an important physiological role of C1 neurons in regulating sympathetic activity and arterial pressure, but apparently they are not involved in the expiratory-related sympatho-excitation that is characteristic of the hypertension evoked by CIH in rats.

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PCB017

Inhibitory glial modulation of neurotransmission is reduced in nucleus tractus solitarius (NTS) after short-term sustained hypoxia in rats

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Sustained hypoxia (SH) produces cardiovascular and respiratory changes in unacclimatized humans ascending to high altitude due to peripheral chemoreflex activation. Moreover, hypoxia also produces changes in structural and functional integrity of glial cells, which are essential for neuronal activity. In this study we evaluated the effect of short-term SH (24 hours, pO210%) on the neuron-glia interaction at the NTS, the first synaptic station of peripheral chemoreflex in the brainstem. Using brainstem slices and whole-cell patch clamp, we observed that passive properties of NTS astrocytes were not affected by SH [input resistance: control: 44.5 ± 14 MΩ (n=8) vs SH: 31.5 ± 10 MΩ (n=4)] [RMP control: -84 ± 1.6 mV (n=10) vs SH: -75 ± 5.2 mV (n=7)]. Since protease-activated receptors 1 (PAR1) are found only in NTS astrocytes and not in neurons, we also evaluated the glial modulation on neurotransmission by activation of astrocytes with SFLRN-NH2 [PAR1 agonist (20 uM)]. SFLRN-NH2 decreased the amplitude of evoked excitatory post-synaptic currents (eEPSCs) in NTS neurons from SH animals to levels similar to that observed in control group [control: -51 ± 5 pA (n=3) vs SH: -56 ± 8 pA (n=5)]. SFLRN-NH2 did not change the RMP of NTS neurons in both groups [control: aCSF: -65.5 ± 1.5 mV vs aCSF + SFLRN-NH2: -61 ± 5 mV, (n=4); SH: aCSF: -63 ± 5 vs aCSF + SFLRN-NH2: -56 ± 8 mV, (n=4)]. In addition, using in vivo labeling and multi-photon microscopy, we observed that SH decreased the cellular density of astrocytes in NTS [control: 0.3 ± 0.004 (n=5) vs SH: 0.1 ± 0.01 cells / µm² (n=5)]. Recording intracellular calcium concentration (calcium waves), we observed that SH reduced the astrocytic response to another hypoxia exposure [Average of fluorescence alterations (∆F/∆F) to 5 min exposure to hypoxic solution - control: 43% vs SH: 3%]. We conclude that SH decreased the number of astrocytes as well their inhibitory activity.
modulation on excitatory transmission on the NTS neurons, which may contribute to the enhancement of baro- and chemoreflex responses observed in this experimental model.

Financial support: FAPESP and CNPq

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PCB018

Accelerated degradation of SCN5A channel L1239P mutant through Nedd4-2-mediated ubiquitination underlies Brugada syndrome

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Brugada syndrome (BrS) is a genetically determined disease which predisposes affected individuals to fatal arrhythmias and sudden cardiac death (1). Although mutations in SCN5A leading to a loss of function of the cardiac sodium channel (Nav1.5) are the most common genotype found among BrS patients, the precise mechanisms are not well understood (2). E3 ubiquitin ligase Nedd4-2 binds to the PxxY motif at the C-terminus of Nav1.5 with its WW domain and downregulates the protein expression and functional current of Nav1.5 channel (3). Sequence alignment showed that BrS-associated L1239P mutant of SCN5A produces a new binding site (from LLEY to LPEY) of Nedd4-2 (4). We hypothesize that this additional Nedd4-2-binding site may enhance Nav1.5 ubiquitination and decrease mutant channel function. To test this, site directed mutagenesis was used to generate mutant DNA, Western blot analysis was used to quantify the protein expression and whole-cell patch clamp was used to record sodium current. Our data revealed that L1239P-SCN5A mutation resulted in loss-of-function properties with decreased protein expression and no detectable sodium current. Treatment of HEK293 cells transiently expressing L1239P-SCN5A with proteasome inhibitor MG-132 robustly increases the expression of the L1239P, which almost equal to wild type (WT), implying that increased degradation of Nav1.5 type may be the possible mechanism. To further investigate whether Nedd4-2 takes part in this process, WT-SCN5A or L1239P-SCN5A together with Nedd4-2 were transfected into HEK293 cells and co-immunoprecipitation was used to detect Nav1.5-Nedd4-2 interaction. Our results demonstrated that the interaction between L1239P and Nedd4-2 is stronger than that between WT and Nedd4-2. Furthermore, a double mutation L1239P/Y1977A was generated which disrupted the basal binding site at Y1977 for Nedd4-2. Western blot result showed that the expression of L1239P/Y1977A increases almost two folds compared with that of L1239P. Co-immunoprecipitation result showed the interaction between L1239P and Nedd4-2 is stronger than that between L1239P/Y1977A and Nedd4-2, which indicate that L1239P is an extra binding site besides Y1977. In order to investigate the importance of L1239P in the degradation of Nav1.5L-1239 was also mutated to R and H. Our data showed that protein expression and sodium current in L1239R-SCN5A and L1239H-SCN5A are almost equal to that of WT-SCN5A, thus supporting the role of L1239P as a significant binding site for Nedd4-2. In summary, these data demonstrated a novel mechanism of BrS-associated L1239P-SCN5A mutation, that additional Nedd4-2-binding site increases the Nedd4-2-Nav1.5 interaction and enhances the ubiquitination and degradation of Nav1.5 channel, which underlying the pathophysiology of BrS.

Figure 1. Proposed scheme illustrating that BrS-associated L1239P mutant of SCN5A produces a new binding site (from LLEY to LPEY) of ubiquitin ligase Nedd4-2. This extra Nedd4-2-binding site enhances Nav1.5 ubiquitination and decreases mutant channel function.


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PCB019

Estimated glomerular filtration rate and c-reactive protein measures enhance the specificity for left ventricular hypertrophy detection using electrocardiographic criteria

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Left ventricular hypertrophy (LVH), the detection of which is recommended for routine risk prediction by all guidelines, is more prevalent in groups of African ancestry. This is in part attributed to higher prevalence rates of obesity. The detection of LVH using standard electrocardiographic (ECG) criteria (ECG-LVH) has poor sensitivity and specificity and therefore needs modification in groups of African ancestry. The usefulness of independent associations between Left Ventricular Mass Index (LVMI) and estimated glomerular filtration rate (eGFR) or serum c-reactive protein (CRP) concentrations to complement ECG criteria for LVH detection in predominantly obese African populations was therefore assessed in patients that had provided informed consent. LVH determined by ECG using at least 12 different criteria (formulae) was compared to LVH determined by echocardiography (LV mass index>51g/m2.7) in a random sample of 358 participants.
from a prospective cohort from an urban, developing community of African ancestry in South Africa (41% obese) and used together with CRP concentrations and eGFR above or below the median for the sample. A combination of CRP concentrations and eGFR above or below the median for the sample respectively showed significant performance (AUC=0.61±0.03, p<0.0005), but a low specificity (ability to report negative results as negative) for LVH detection (77%). When eGFR and CRP concentrations were employed to complement the R wave amplitude of the electrocardiographic lead aVL (RaVL) the specificity increased (93%), although the overall performance did not improve (AUC=0.71±0.03, p<0.0005, RaVL alone: AUC=0.70±0.03). The sensitivity (ability to report positive results as positive) of 25% was however in-line with previously reported sensitivities for LVH detection using ECG criteria in alternative population samples. However, without changing overall performance, eGFR together with RaVL increased the specificity to 88% and CRP concentrations when considered together with RaVL increased the specificity to 87%. Therefore routine measurements of CRP and GFR can be used to enhance the specificity of electrocardiographic tests for LVH (especially the RaVL criterion) in obese African patients since these exhibit poor results from ECG alone. Since in groups of African ancestry, obesity contributes toward a poor validity and performance of all voltage criteria for the detection of LVH, the use of eGFR and/or CRP concentrations to complement ECG criteria increases the specificity without altering the overall performance.

Changes in E-C coupling proteins and transverse and axial tubular structures in guinea pig ventricular muscle during pre- and post-natal development to adulthood

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Transverse tubules (TT), plasmalemma invaginations perpendicular to the long axis of the cardiomyocyte, facilitate rapid action potential transmission to the cell interior and efficient cardiac excitation-contraction coupling (ECC). TTs have also been noted to branch in longitudinal directions, such structures being termed transverse-axial tubules (TATs). Tubule alteration, and changes in expression of proteins important for normal ECC, have been noted in heart disease[1]. We explored the relationship between ECC-related protein expression and TT/TAT structure during pre- and post-natal cardiac development to adulthood as this may be informative for understanding pathological maladaptation that involve reversion to fetal molecular phenotypes.

Hearts were collected from guinea pigs, killed according to Home Office licensed regulations, at developmental stages: fetal (between gestation days (G) 55-68; term=G67-68); neonatal weeks one(NW1), two (NW2) and three (NW3); and adult. Excised hearts were flushed with cold cardiologic solution and (i) left ventricles (LV) frozen for subsequent protein analysis by western blotting or (ii) retrogradely perfused with fixative and LV processed for ultrastructural examination by serial block face-scanning electron microscopy. Values are mean ± SEM, compared by one-way Anova and Bonferroni posthoc test (p<0.05).

Expression of β2 adrenocceptor, a putative TT marker, increased in adults (0.87±0.14-fold relative to positive control) and later neonates NW2(0.90 ±0.04-fold) compared to fetal G55/57 (0.44±0.04-fold) or early neonates NW1 (0.5±0.04-fold). Junctophilin2, a determinant of TT integrity, was expressed in G55/57 and, surprisingly, was invariant among the biological groupings. Expression of Cavin1, a general plasmalemmal marker, increased through fetal and neonatal development to adult. In contrast, flotillin2 expression was unchanged between groupings.

Analysis of digitally reconstructed (Amira 6.0) serial EM images revealed developmental changes in cardiomyocyte structure. Sarcomere length narrowed from G55/57 (2.28±0.01 μm) to NW1 (1.90±0.011μm) and adult (1.92±0.01 μm; assessing 250 sarcomeres from 15 regions of interest). TTs were observed at G58-59 although longitudinal tubular elements predominated. Total tubular surface area (30 serial sections, n=6 for each condition), increased from 0.68±0.08% at G64/68 to 2.54±0.48% in adult. TT diameter (100-150 serial sections, >50 TTs) was 0.27±0.02 μm in G65/68 and 0.40±0.02 μm in adult. Fetal cardiac TT/TATs as early as the mid-third trimester of guinea pig pregnancy. Changes in TT/TAT abundance through pre- and post-natal development to adulthood mirror the changes in expression of some (β2 adrenocceptor, cavin1) but not other (junctophilin2) proteins likely to be important for maturation of ECC

In FDB, simvastatin increased Ca\(^{2+}\) spark frequency (38%; software (NIH) using the Sparkmaster plugin. Data are from 3-6 rats per group, and compared with the Student’s t-test. In FDB, simvastatin increased Ca\(^{2+}\) spark frequency (38%; P<0.05) without significant effects on duration, amplitude or width (n=53-70 cells from 6 animals). By contrast, in cardiac cells a minor reduction in frequency (17%) and amplitude (9%) was observed (P<0.01; n=57-61 cells from 3 animals). These data show that simvastatin has disparate effects on SR Ca\(^{2+}\) release in skeletal and cardiac muscle. In support of a direct interaction of statins with RyR, the SR Ca\(^{2+}\) release channel, we assessed left ventricular mass (LVM) and strain parameters (indexed to body surface area). In four patients, extracellular volume fraction (ECV), myocardial cell volume (MCV) and interstitial volume (IV) were also quantified using T1 mapping. Measurements were performed pre- and 6-12 months post-RDN. Data analysed using Student’s t-test and Pearson’s correlation coefficient, and reported as mean ± SEM. Results: At baseline; age 55 ± 3 years, 53% male, 5.0 ± 0.4 anti-hypertensive drugs, office BP 193 ± 4/107 ± 5 mmHg. 12/19 patients (63%) responded to RDN (±10 mmHg drop in office systolic BP; SBP); change in BP -18.3 ± 8.8/-2.2 ± 5.1 mmHg. 12/19 patients had MSNA of sufficient quality to analyse. Following RDN, there was a reduction in LVM (96 ± 6 vs 88 ± 7 g/m\(^2\), p<0.05) and improvements in peak myocardial strain (radial strain 29.8 ± 1.9 vs 35.3 ± 2.1 %, p<0.01; longitudinal strain -17.4 ± 0.8 vs -19.3 ± 0.8 %, p<0.05; circumferential strain -16.6 ± 0.7 vs -18.6 ± 0.8 %, p<0.01). However MSNA was unchanged (58.4 ± 6.4 vs 61.6 ± 6.4 bursts/100 heartbeats, p=0.67). Whilst there was no correlation between SBP and LVM at baseline (R=0.36, p=0.12), the change in LVM correlated with the reduction in SBP (R=0.56, p<0.01). There was no correlation between the change in MSNA and changes in SBP or LVM. In the four patients assessed with T1 mapping, there were reductions in LVM (-10.6 ± 3.3 g/m\(^2\), p<0.05), and specifically IV and ECV (4.1 ± 1.0 ml/m\(^2\), -0.011 ± 0.002 %, both p<0.05), with a borderline fall in MCV (6.5 ± 2.2 ml/m\(^2\), p=0.06). Conclusions: Similar to previous studies, we have shown improvements in LV mass and myocardial strain following RDN. The novel finding is that these were independent of MSNA. In contrast to previous studies, LV mass reduction correlates with SBP reduction in this cohort, and may therefore be dependent on afterload. Preliminary T1 mapping data also demonstrates a reduction in myocardial interstitial fibrosis post-RDN, giving additional insights into the potential mechanisms that contribute to hypertensive heart disease. Brandt MC, Mahfoud F, Reda S, Schirmer SH, Erdmann E, Bohn M, et al. (2012). Renal sympathetic denervation reduces left ventricular hypertrophy and improves cardiac function in patients with resistant hypertension. J Am Coll Cardiol. 2012;59(10):901-9
Effects of streptozocin-induced type I diabetes mellitus on the cardiac conduction system of the rat heart

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It has been estimated that 5 million people will suffer from diabetes in the UK by 2025.1 Cardiovascular complications are common in type I diabetes mellitus (T1DM). There is increased risk of bradycardias, atrioventricular block and bundle branch block as a result of dysfunction of the cardiac conduction system (CCS).2,3 The CCS is responsible for the generation and transmission of electrical activity in the heart and consists of the sinoatrial and atrioventricular nodes (SAN; AVN), bundle of His (HIS), right and left bundle branches (SAN; AVN; HIS, RBB, LBB, RPFs and LPFs). In the rat streptozotocin (STZ)-induced model of T1DM, in vivo ECG recordings have shown a significant (P<0.05) decrease in heart rate (HR) and prolongation of the QRS complex, evidence of dysfunction of the CCS.4 The aim of this study was to investigate the cellular basis of the effect of T1DM on the CCS using the rat model.

A possible direct action of STZ on the heart was investigated using the Langendorff-perfused rat heart. Protein expression was investigated immunohistochemistry. Antibodies were used to label the cardiomyocyte outer membrane (Caveolin3, Cav3), the funny channel (HCN4), Ca2+-handling proteins (RyR2 and SERCA2a), sympathetic neurones (NF165) and gap junction proteins (Cx40 and Cx43). The research was conducted in accordance with the Guide for the Care and Use of Laboratory Animals in UAE and UK.

We showed that STZ had no direct effect on the functioning of the CCS - there were no changes in RR, PR and QRS parameters after perfusion of hearts with STZ (60 mg/kg for 90 min; n=5, P>0.05). T1DM rats showed decrease in body weight and heart weight but increase in heart to body weight ratio and blood glucose (n=16, P<0.05). Based on Cav3 labelling, significant cellular hypotrophy was observed in the HIS, RBB, RPFs and LPFs (n=5, P<0.05). Surprisingly, HCN4 expression was significantly increased in the SAN, AVN and HIS. However, RyR2 labelling was significantly decreased in the AVN, HIS, RBB, LBB, RPFs and LPFs and SERCA2a labelling was significantly decreased in the AVN, HIS, RPFs and LPFs; downregulation of these Ca2+-handling proteins (involved in the Ca2+ clock pacemaker mechanism) could account for the lower HR. NF165 labelling was significantly decreased in the SAN, HIS, RPFs and LPFs; the assumed downregulation of sympathetic innervation could also help explain the reduced HR in diabetic hearts. The upregulation of HCN4 (involved in the membrane clock pacemaker mechanism) could be a compensatory mechanism. Cx40 expression was significantly decreased in the HIS, LBB, RPFs and LPFs and Cx43 labelling was significantly decreased in the RPFs and LPFs. The downregulation (and redistribution) of the connexins in His-Purkinje system could account for the prolongation of QRS complex.

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Effects of Caveolin-3 knock-down on Ca autoregulation in mouse ventricular myocytes


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In the myocyte, the Ca transient determines the force and speed of contraction and therefore tight control of Ca movement is paramount to normal physiology. The Ca transient is controlled by autoregulation [1]; the balance between Ca influx and Ca efflux which occur mainly via L-type Ca channels (LTCC) and Na/Ca exchanger (NCX) respectively. LTCCs [2] and NCX [3] are predominantly located in t-tubules, and thus autoregulation is hypothesised to occur predominantly within these structures [4]. Caveolin-3 (Cav3) is a scaffolding protein associated with t-tubule formation in myocytes [5]. This study therefore aims to investigate the consequence of Cav3 knockout out (KO) on autoregulation in mouse ventricular myocytes. Myocytes were isolated from 12 week old male Cav3 KO and their littermate controls by enzymatic digestion. Cells were field stimulated at 1Hz and the Ca transients were monitored using Fura-2 AM, and reported as the ratio between the fluorescence excited at 340nm and 380 nm (F340/F380). Autoregulation was investigated during the application of 200 μM caffeine, increasing ryanodine receptor (RyR) opening probabilities, and following the application of 10 mM caffeine, to empty the sarcoplasmic reticulum (SR). Data are presented
Poster Communications

PCBO25

Evolutionary and biochemical characterization of histidine rich calcium binding proteins

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Introduction: Ca\textsuperscript{2+} release from mammalian sarcoplasmic reticulum (SR) via type 2 ryanodine receptor (RyR2) channels is a pivotal step in cardiomyocyte contraction. This Ca\textsuperscript{2+} release is regulated by several factors, including interaction of RyR2 channels with accessory proteins. The SR intraluminal proteins calsequestrin-2 (CASQ2) and histidine-rich Ca\textsuperscript{2+} binding protein (HRC) exert opposing effects on RyR2 gating: CASQ2 inhibits diastolic Ca\textsuperscript{2+} release, whereas HRC promotes RyR2 gating. HRC could act via interactions with the luminal domains of SR proteins, including RyR2, the SERCA2a Ca\textsuperscript{2+} pump and triadin (Zhang, Waddell & Jones (2015)). Despite their importance in cardiac health and disease, little is known about the evolution or roles of HRC proteins. The current study aims to address these deficits.

Methods: HRC homologs were identified by BLASTP searches of protein databases from a wide range of organisms. The evolutionary relationships between these proteins were reconstructed using the Maximum Likelihood method. Conserved patterns shared between homologs were derived using PRATT software. Apparent mutation rates of HRC and other SR proteins were estimated from alignments and from K/K analyses. Candidate Ca\textsuperscript{2+}-binding proteins (CBPs), staining purple with the cationic dye Stains-All, were partially isolated from chicken heart microsomes by stepwise potassium chloride washing, sodium carbonate (pH 11.2) extraction and heat denaturation (as mammalian HRCs are heat stable). Two chicken heart SR proteins, of 122 kDa and 325 kDa apparent molecular weight, were analysed by the Protein Identification Service (University of York, UK).

Results and Discussion: HRC homologs were encoded within many genomes, including those of most vertebrates, sponges, molluscs, bacteria and archaea. These homologs clustered poorly in phylogenetic reconstructions, even between closely related species, suggesting rapid rates of evolution. Analyses of apparent mutation rates support this hypothesis. A conserved pattern of cysteine residues in HRC homologs suggests a relationship with the ferredoxin superfamily and participation in redox events. Birds apparently lack HRCs: homologs were not detected in proteomes of 26 avian species, but all of these encoded homologs of triadin. Several candidate CBPs were detected in microsomal preparations from chicken heart. Of these, mass spectrometry data suggest that a 122 kDa protein is sarcalumenin, an SR CBP. The identity of other bird heart SR intraluminal CBPs, including counterparts of mammalian HRCs, are currently being investigated.

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PCB026

Dual dye optical mapping of P21-activated kinase deficient mouse hearts to assess regional differences in arrhythmogenic potential

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P21-activated kinase 1 (Pak1) is a serine/threonine protein kinase implicated in cardioprotection (1). Mice lacking Pak1 in cardiac tissue (Pak1\textsuperscript{-/-}) respond to chronic isoprenaline (Iso) stress with a more pronounced hypertrophy and higher propensity to arrhythmias than wild type (WT) counterparts (1). The role of Pak1 during acute beta-adrenergic stress is under-studied in these mice. We used a high-speed (1kHz) optical mapping system to assess simultaneously calcium and voltage responses of WT

as mean±SEM and compared by mixed model ANOVA and Sidak post hoc test.

Cav3 KO myocytes showed no significant difference in steady-state Ca transient amplitude compared to littermate controls [F340/380 0.17±0.03 (KO, n=19) vs 0.17±0.01 (WT, n=12)] and the application of 200 μM caffeine produced similar increase in transient amplitude in both cell-types [F340/380 0.26±0.05 (KO) vs 0.27±0.03 (WT)]. During the continued presence of 200 μM caffeine, the transient amplitude recovered to steady-state in both cell-types, however the time taken to achieve this was significantly increased in the Cav3 KO cells [48±2.82 (KO) vs 30±2.12 s (WT), p<0.0001].

The increase in fluorescence during the application of 10 mM caffeine, a measure of SR Ca content, was not significantly different in Cav3 KO cells compared to WT controls [F340/380 0.40±0.03 (KO, n=10) vs 0.41±0.04 (WT, n=10)] and no significant difference was observed in the rate of decay of the caffeine-induced transient [0.35±0.03 (KO) vs 0.38±0.02 s\textsuperscript{-1} (WT)]. This would suggest that NCX activity is similar in both cell-types. Following washout of 10 mM caffeine, stimulation was resumed, and Ca transient amplitudes, which were initially small, recovered to steady state. As with the application of 200 μM caffeine, the time taken to reach steady state was significantly increased in Cav3 KO cells [15±1.44 (KO) vs 10±1.44 s (WT), p<0.01].

These data suggest that autoregulation is slower in Cav3 KO mice, which is unlikely to be due to changes in NCX.


This work was supported by the British Heart Foundation and the Faculty of Biomedical Sciences, University of Bristol

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and Pak1<sup>cko</sup> tissue to acute Iso exposure (10nM). Hearts were loaded with calcium (rhod-2-AM) and voltage (rh237) dyes via Langendorff perfusion. Iso caused a significant reduction in refractory period of WT left atrium (LA) (15±6% reduction, n=8) whereas Pak1<sup>cko</sup> LA exhibited no change (2±3% increase, n=10). Pak1<sup>cko</sup> LA also exhibited significantly shorter action potential durations (APD) whilst calcium transients (CaTD) remained unaltered. At 135msec pacing interval during control CaTD was 31.73±2.5 ms in WT but 36.01±1.6 ms in Pak1<sup>cko</sup> (n=15 regions from 8 atria, both genotypes) whilst APD was 25.47±3.3 ms in WT and 20.22±2.3 ms in KO (n=16 regions from 8 atria and n=18 regions from 9 atria respectively). Pak1<sup>cko</sup> APDs were also significantly shorter in right ventricular preparations (p<0.05, n=17 regions from 9 RV for WT and n=14 regions from 7 RV for Pak1<sup>cko</sup>). Our data are consistent with a role of Pak1 in both calcium handling and electrophysiology. Lack of APD response to isoprenaline in Pak1<sup>cko</sup> LA suggests this protein is important in beta-adrenergic signalling. Further work will be required to pinpoint underlying mechanism(s).

1. Wang et al. (2014) Circ Arrhythm Electrophysiol. 7:938-48

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**PCB027**

**Effects of intracellular acidosis on the hERG K<sup>+</sup> channel**

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Potassium channels encoded by human Ether-a-go-go-Related Gene (hERG) underlie the cardiac rapid delayed rectifier K<sup>+</sup> channel current (I<sub>Kr</sub>), which plays an important role in ventricular action potential repolarisation (Sanguinetti and Tristani-Firouzi, 2006). Acidosis occurs in a number of pathological situations and there is consensus that extracellular acidosis can attenuate the amplitude and accelerate the deactivation of I<sub>hERG</sub> (Berube et al. 1999, Du et al. 2010). However, comparable data are lacking for intracellular acidosis. Replacement of extracellular sodium chloride (NaCl) with sodium acetate (NaAc) is established experimentally to produce intracellular acidosis, as uncharged protonated acetate can cross the cell membrane and then release protons with sodium acetate (NaAc) is established experimentally to produce intracellular acidosis, as uncharged protonated acetate can cross the cell membrane and then release protons. We previously demonstrated that increasing the intracellular proton concentration (-7.9±1.3 mV; n=6 cells, p<0.01) respectively by NaAc and NaBu. The V<sub>0.5</sub> of inactivation was negatively shifted by 80 mM NaAc (-7.9±1.3 mV; n=6 cells, p<0.01). These findings provide evidence that increasing the intracellular proton concentration produces multiple effects on I<sub>hERG</sub>, with modulation of both current amplitude and kinetics.

Berube J et al. (1999). Pflugers Arch 438, 419-422.


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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

**PCB028**

**Autonomic imbalance and cardiac function deterioration is associated with increased expression of AT1R and NF-κB in the RVLM of rats with HFpEF**

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Heart failure with preserved ejection fraction (HFpEF) is characterized by a progressive decline in diastolic cardiac function with a normal ejection fraction (EF). Furthermore, sympathoexcitation in HFpEF has been linked to cardiac arrhythmias. We recently described that activation of the central chemoreflex pathway (CC) induced cardiac autonomic imbalance. Therefore, we aimed to determine whether the retrotrapezoid (RTN) nuclei, a main chemoreceptive area, and the rostral ventrolateral medulla (RVLM), a major region involved in the regulation of sympathetic outflow, displayed chronic neuronal activation in the setting of HFpEF. Furthermore, we explored if both inflammation and angiotensin II signalling pathways could be associated with the changes in neuronal activity in the RTN and RVLM from rats with HFpEF. Male Sprague-Dawley rats were anesthetized (isoflurane 2% in O2) and subjected to aorto-caval shunt to induce HFpEF. Ventilatory response to acute hypercapnia (FICO2 7%) was assessed to determine CC sensitivity. Finally, rats were anesthetized (α-chloralose 40 mg/kg and urethane 800 mg/kg i.p.) and cardiac function was determined by pressure-volume loops. Neuronal activation was assessed in RTN and RVLM micropatches by measuring FosB expression by immunoblot. Compared to Sham rats, HFpEF rats display (HFpEF vs. Sham): normal EF (51±3 vs. 50±7 %), cardiac hypertrophy (heart to body weight ratio, 6.1±0.3 vs. 4.0±0.5 mg/g; P<0.05), increased arrhythmia incidence (196±84 vs. 19±7 events/h; P<0.05) and increased end diastolic pressure-volume relationship (p<0.0039±0.001 1/ul; Pc<0.05). In addition, we found that HFpEF rats display enhanced CC sensitivity compared to Sham animals (165.3±9.1 vs. 127.3±10.3 ml/min/100g, HFpEF vs. sham, respectively; P<0.05). Despite the significant increase in CC gain, HFpEF rats showed no changes in normoxic minute ventilation compared to Sham. RVLM FosB expression was increased by ~2.5 fold in HFpEF compared to sham rats. On the contrary, no change in FosB in the RTN was found between groups. In order to determine the plausible mechanisms that
 could mediate the RVLM hyper activation we screened for changes in angiotensin II type 1 receptor (AT1R) and for p65 subunit of the NF-kB pathway. We found an augmented expression of AT1R in the RVLM from HfPEF rats compared to sham rats (276±48% vs. 100±20%, HfPEF vs. sham, respectively; P<0.05). NF-kB p65 expression was also increased in the RVLM of HfPEF. Our results show that autonomic imbalance in HfPEF is associated with chronic neuronal activation of the RVLM but not with the activation of the RTN. Furthermore, AT1R and p65 are highly expressed in the RVLM of HfPEF rats. Our results suggest that central angiotensin II and inflammation may partially play a role in RVLM activation and sympathoexcitation in HfPEF.

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PCB029

A short QT phenotype secondary to PGC-1β ablation in mice

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Energetic dysfunction is important in the pathogenesis of multiple cardiac pathologies. Increasing evidence implicates causal links between energetic dysfunction and potentially arrhythmogenic, primary cardiac electrophysiological abnormalities (Adabag et al. 2015). The precise physiological mechanisms linking metabolic disturbance to arrhythmic tendency remain uncertain. These relationships were investigated in PGC-1β-/- mice known to display abnormal mitochondrial bioenergetics.

Wild type (WT) and PGC-1β-/- C57/B6, mice aged ≥12 weeks (Bar Harbour Laboratories, Maine), matched for baseline characteristics, were weighed and anaesthetised with tribromoethanol (240mg/kg i.p.) for lead I and lead II electrocardiogram (ECG) recordings. Three hundred seconds of intrinsic ECG recordings were followed by dobutamine (0.3mg/kg i.p.) challenge, and a further 300s of ECG recorded. ECG signals were analysed using a bespoke program in the open-source R programming language. Multivariate ANOVA examined for significant differences in ECG features between groups. Demonstration of significant differences between groups then prompted further, post hoc MANOVA decomposition and post hoc Tukey HSD tests for significance (P<0.05).

WT (n=13) and PGC-1β-/- (n=15) mice showed indistinguishable pre-treatment baseline heart rates (WT 6.78 ± 0.16 Hz vs. PGC-1β-/- 6.52 ± 0.32 Hz, P > 0.05). However, PGC-1β-/- mice displayed chronotropic incompetence with dobutamine challenge (8.30 ± 0.10 Hz vs. 9.11 ± 0.28 Hz, P = 0.025) (Fig. 1). Dobutamine-treated PGC-1β-/- mice also showed shorter corrected QT (QTc) intervals than the corresponding WT mice (126.06 ± 1.15ms vs. 119.94 ± 2.23ms, p<0.05). The late component of the murine R wave has previously been correlated with ventricular repolarisation (Boukens et al. 2014). QT waveforms were accordingly analysed in greater detail to clarify aspects of electrical activity affected. Mean QT' durations were indistinguishable between groups whether before or after dobutamine challenge. However, R'Tc intervals were shorter in PGC-1β-/- mice following such challenge (25.91 ± 0.37ms vs. 24.24 ± 0.44, p < 0.05).

The present study is the first report of a short QT phenotype associated with energetic dysfunction, secondary to PGC-1β-/- ablation in mice. In humans short QT has been correlated with increased arrhythmic risk (Gollob et al. 2011). Short QT syndromes are poorly characterised disorders for which there is limited understanding of arrhythmic mechanisms. The PGC-1β-/- mouse model will enable exploration to its underlying mechanistic pathways.

Figure 1: Mean heart rate at baseline and after dobutamine administration. * denotes p < 0.05


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PCB030

Spontaneously hypertensive rats express increased levels of TNF and TNFR1 in cardiovascular centres of the brain

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Introduction: Tumour necrosis factor (TNF) is an archetypal proinflammatory cytokine implicated in cardiovascular
diseases (1). A growing body of evidence shows that brain TNF is involved in blood pressure regulation and sympathoexcitation (2), (3). Neuroinflammation of the brain nuclei involved in the regulation of the cardiovascular system has been recognized as an important contributing factor to the pathogenesis of hypertension (4). The proinflammatory effects of TNF in the central nervous system are mediated by TNF type 1 receptors (TNFR1) (5).

Aim: In the present study we checked if spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats differ in concentrations of TNF and its receptor TNFR1 in the key cardiovascular centres of the brain.

Methods: We measured systolic blood pressure in adult male SHR (n=6) and WKY (n=6) rats with tail-cuff method. Then under anesthesia with urethane (1.5 g/kg b.w., i.p.), we collected blood and brains, which were snap frozen in liquid nitrogen immediately after euthanasia. Brains and serum were stored at -80°C for further analysis. We sectioned coronal slices of the brain in the brain matrix and isolated the hypothalamus (HTH), the rostral ventrolateral medulla (RVLM) and the nucleus of the solitary tract (NTS). After homogenization and centrifugation of the tissues, we used the enzyme-linked immunosorbent assays to determine concentration of TNF in the obtained supernatants of HTH, RVLM and NTS and concentration of TNFR1 in the precipitates of the respective areas. We also measured norepinephrine (NE), TNF and TNFR1 in serum. Student’s t-test was used for statistical analysis. Values are expressed as means ± SD.

Results: SHR rats had significantly higher systolic blood pressure than WKY rats, 182±13 vs 142±19 mmHg (p<0.001). Protein expression of TNF in RVLM and NTS of SHR rats was significantly higher than in WKY rats, 2243±193 vs 1523±154 pg/g of tissue (p=0.001) and 2290±219 vs 1643±105 pg/g of tissue (p=0.002), respectively. Concentration of the cytokine in HTH and serum did not differ significantly between SHR and WKY rats. TNFR1 expression was significantly higher in NTS of SHR than in WKY rats, 5494±311 vs 4577±565 pg/g of tissue (p=0.029). There were no significant differences in concentration of TNFR1 in HTH, RVLM and serum between normotensive and hypertensive rats. Serum NE was 12.6 ± 2.0 ng/ml in SHR vs 3.8 ± 1.4 ng/ml in WKY rats (p=0.036).

Conclusions: Our results show that expression of TNF is increased in the brainstem of SHR rats and this increase is accompanied by augmented expression of TNF type 1 receptor. Increased expression of both, the cytokine and its receptor, may provide a setting for inflammatory response contributing to the hypertensive milieu.


Probert L. TNF and its receptors in the CNS: The essential, the desirable and the deleterious effects. Neuroscience. 2015; 302:2-22.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Mechanoreflex sensitivity is elevated in patients with hypertension

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Background: Exaggerated increases in systolic blood pressure (SBP) during exercise are associated with adverse cardiovascular events [1]. The SBP and muscle sympathetic nerve activity (MSNA) responses to mechanoreflex stimulation are exaggerated in untreated hypertensives [2]. However, it is unclear whether patients with treated-controlled vs. never treated hypertension (HTN) have different cardiovascular responses to mechanoreflex stimulation. We aimed to assess the change in SBP during the first 30 seconds of isometric handgrip (IHG) exercise.

Methods: In 20 normotensive (NTN) (50±3 years, body mass index (BMI) 24±0.2 kg/m²), 9 treated-controlled HTN (56±3 years, BMI 30±0.5 kg/m²) and 7 untreated HTN (58±4 years, BMI 27±0.7 kg/m²) participants, beat-to-beat blood pressure (BP; Finapres) was measured during baseline (supine rest, 10 mins) and 30s of IHG (30% of maximal voluntary contraction). Peroneal microneurography was used to measure MSNA at baseline. Data were analysed using one-way analysis of variance (ANOVA) with Tukey test for multiple comparisons or Pearson’s correlation coefficient. Data are presented as mean ± SEM.

Results: Age was similar among groups (P=0.18). BMI was higher in treated HTN (P=0.004). Office SBP was greater in untreated HTN vs treated HTN vs NTN (175±11, 139±5 vs 124±2; P<0.0001). Baseline MSNA (bursts/min) was higher in untreated HTN and treated HTN vs NTN (39±4, 38±5 vs 25±2 bursts/min P=0.009). Resting HR was similar in untreated HTN, treated HTN and NTN (61±2, 64±2 vs 62±2 beats/min; P=0.28). Delta SBP and diastolic BP (DBP) during IHG was higher in combined HTN participants (SBP: 14±3 vs 6±2 mmHg; P=0.03; DBP: 7±1 vs 2±1 mmHg; P=0.005). Delta HR was not different between combined HTN vs NTN during IHG (4±1 vs 6±1 beat/min; P=0.11). Delta SBP during IHG was not different among untreated HTN, treated HTN vs NTN (10±5, 15±5 vs 6±2 mmHg; P=0.1680). However, delta DBP was higher in treated HTN vs untreated HTN and NTN (7±2, 6±1 vs 2±1 mmHg; P=0.02). Delta HR during IHG did not differ among untreated HTN, treated HTN vs NTN (6±1, 7±2 vs 4±1 beats/min; P=0.22). There was no correlation between baseline MSNA (bursts/min) and delta SBP during IHG in NTN (R=0.17, P=0.54). In untreated HTN there was an inverse correlation of baseline MSNA (bursts/min) to delta SBP during IHG (R=0.78, P=0.04). Conversely, treated HTN had a positive correlation of baseline MSNA (bursts/min) to delta SBP during IHG (R=0.73, P=0.04).

Conclusion: The SBP response to IHG is exaggerated in HTN participants. Interestingly, untreated HTN with higher baseline MSNA had a lower SBP response to IHG, but this relationship was the opposite in treated-controlled HTN. Potentially, anti-hypertensive medication may result in re-sensitisation or upregulation of vascular adrenergic receptors, augmenting the response to increased SNA during IHG.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Ventilatory pattern of lung function in patients with metabolic syndrome and its association to components of metabolic syndrome, systemic inflammation and insulin resistance

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The restrictive lung functions have been associated with an increased risk for cardiovascular disease. Therefore we investigate the ventilatory pattern of lung functions in patients with metabolic syndrome and its association to components of metabolic syndrome, high sensitivity C-reactive protein and insulin resistance. This cross-sectional study included 200 subjects with metabolic syndrome (MetS) after written consented, in the metabolic group and further divided into normal, restrictive, obstructive and mixed ventilatory pattern subgroups according to a modified classification of the Global Initiative for Chronic Obstructive Lung Disease, and 100 healthy volunteers’ without metabolic syndrome in the non-metabolic group. They were examined at Saurashtra centre, India between 2011 and 2014. Metabolic syndrome defined, National cholesterol Education Program’s-Adult Treatment Panel III Criteria. Lung function test was performed by automated flow-sensing spirometer (Helios401’RMS India) based on American Thoracic Society/European Respiratory Society. Fasting glucose and lipid profile levels determined by enzymatic, serum high sensitivity C-reactive proteins (hs-CRP) by latex turbidimetry method, fasting insulin by ELISA and homeostatic model assessment was used to assess insulin resistance. Statistical analysis was made by ANOVA and multiple leaner regression models using SPSS window version 20.0. The overall prevalence of lung functions impairment in patients with MetS was 50% with high prevalence of restrictive ventilatory patterns (33%) followed by obstructive (13%) and mixed (4%). The mean ± SD of insulin resistance (MetS, 8.87 ± 6.95 Vs 3.12 ± 1.91, Non-MetS) and hs-CRP (MetS, 3.71 ± 5.095 Vs 0.86 ± 0.682, Non-MetS) values were statistically very significantly (P<0.001) higher while mean of % predicted FVC (77.48 ± 14.06 Vs 94.15 ± 6.19, FEV1 (81.71 ± 15.10 Vs 103.29 ± 7.14), FEV1/FVC (104.91 ± 13.79 Vs 109.84 ± 4.53) and FEF25-75% (75.37 ± 23.32 Vs 101.52 ± 16.04) values were significantly (P<0.001) lower in MetS group as compared to Non-MetS group. There were significant differences in the body mass index (P<0.05), waist circumference (P<0.01), fasting glucose (P<0.01) and hs-CRP (P<0.05) between ventilatory pattern subgroups. Abdominal obesity (β=-0.330P=0.017), tri- glycerides (β=-0.274P=0.043) and hs-CRP(β=-0.534P=0.002) levels were sturdily negatively associated with restrictive pattern. Eosinophils (β=-0.417P=0.028) counts were significant negative and hs-CRP (β=0.545; P=0.009) was strongly positive associated with obstructive pattern. It is concluded that increased abdominal obesity and systemic inflammation are risk factors for decline lung functions in metabolic. Keywords: Metabolic syndrome, Lung functions, Obstructive pattern, Restrictive pattern, Insulin resistance, Systemic inflammation

Renal oxygen homeostasis is perturbed following exposure to long-term but not short-term intermittent hypoxia in the rat

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Renal tissue hypoxia is a key contributor to the progression of chronic kidney disease (CKD), which occurs in more than 50% of patients with obstructive sleep apnoea (OSA). OSA is characterized by repetitive cycles of intermittent hypoxia (IH) causing oxidative stress. However, the impact of acute and chronic IH on renal function and O2 homeostasis is unclear. Male Sprague Dawley rats (8-10 weeks old) were exposed to IH (270 sec @ 21% O2; 90 sec @ 6.5% O2 at nadir) for 4 hrs in plethysmography chambers (AIH) (n=7) and for 8hrs/day for 2wks in an oxyccylert™ environmental chamber (n=7, CIH). Sham animals were exposed to normoxia under similar conditions (n=6 for AIH study and n=7 for CIH study). Animals were anesthetized (Euthatal:60mg/kg IP) and surgically prepared.
for the measurement of mean arterial pressure (MAP), left renal excretory function, renal blood flow (RBF) (transonic flow probe), and oxygen tension (PO2) in the renal cortex (C) and medulla (M) (fluorescence quenching oximetry). Animals were euthanized by anesthetic overdose at the end. Data: 2X2 (gas x duration) ANOVA with P<0.05 taken as significant. AIH had no effect on MAP (123±6 (mean±SEM) versus (v) 129±5mmHg), whereas animals exposed to CIH were hypertensive (122±3 v 144±6mmHg (P<0.05)). AIH heightened glo- merular filtration rate (GFR) (0.92±0.11 v 1.33±0.13ml/min), (RBF) (3.8±0.6 v 7.2±0.9ml/min) and transported sodium (TNa) (132±16 v 201±18µmol/min) (all P<0.05). Conversely, CIH had the opposite effect, reducing GFR (1.15±0.61 v 1.33±0.57ml/min (P<0.05)) and TNa (160±13 v 120±15µmol/min (P<0.05)) (Interaction: P<0.05). Oxygen consumption (QO2) was elevated by AIH (15±0.6 v 160±7µmol/min (P<0.05)) (Interaction: 13 v 120±15µmol/min (P=0.06)), RBF (4.13±0.61 v 3.08±0.57ml/min (P<0.05)) and medulla (M) (fluorescence quenching oximetry). Animals where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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PCB036

Baroreflex function in congestive heart failure assessed by the sequence method

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The baroreflex is a sympatho inhibitory reflex being a major determinant of sympatho-vagal balance. Despite the impaired baroreflex control of heart rate (HR) and increased renal sympathetic activity (RSNA), observed in patients and animals with heart failure (HF), the gain of the arterial baroreceptor reflex control of RSNA can be found preserved in this cardiovascular disease (1,2,3). The sequence method is an approach that assesses spontaneous beat-to-beat arterial pressure (AP) fluctuations and their related HR changes. The sequence method has never been applied to assess baroreflex control of RSNA. We propose the use of the sequence method for analysis of spontaneous baroreflex function, using AP and RSNA signals, as an alternative approach to spectral methods. We analyzed the baroreflex gain by the barocurve, cross spectral analysis and sequence method in control (n=7) and rats with HF induced by myocardial infarction (n=6). The experimental protocol was approved by the Committee of Ethics in Animal Research of the School of Medicine of Ribeirão Preto, Brazil (Protocol n. 1477/2007). Rats were anesthetized with ketamine (50mg/kg,ip) and xylazine (10mg/kg,ip) and underwent surgery to implant stainless steel electrodes around the renal nerve and catheters into the femoral artery and vein for AP recording and phenylephrine and sodium nitroprusside administration. Barocurves were calculated fitting systolic AP vs RSNA curves by sigmoidal regression. The spectral method applied estimates the gain of the transfer function in the high frequency band, considering AP as the input and RSNA as the output of a linear time-invariant system. The sequence method identifies successive spontaneous increases or decreases (ramps) in AP that are actually producing a reflex response by baroreflex. All methods employed to assess the baroreflex gain (%RSNA/mmHg), barocurves (1.78±0.4 vs 1.63±0.2 HF rats), transfer function (14.6±4.7 vs 15.2±1.5 HF rats) and sequence method (17.1±6.9 vs 22.1±2.4 HF rats) did not detect any difference between control and HF group. However, HF rats showed lower BEI (0.6±0.02 vs 0.3±0.04 HF rats, p<0.05). It means that, when the baroreflex is acting, its sensibility is similar in control and HF animals. Nevertheless, its effectiveness to buffer changes in the efferent pathway is markedly affected in HF. In conclusion, these data demonstrated that while the gain of the baroreflex could not reveal any difference between control and HF rats, BEI is markedly decreased in HF rats, indicating attenuated baroreflex effectiveness to buffer changes in RSNA in HF rats.


CNPq, FAPESP and CAPES

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB037

Testing the interaction between central and peripheral chemoreceptors in humans using a transient hypoxic chemoreflex test

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Arterial blood gas levels are maintained through respiratory chemoreflexes mediated by central (CCR; brainstem) and peripheral (PCR; carotid body) chemoreceptors. The CCRs are stimulated slowly (25-30 sec) by accumulating brainstem hypercapnia (central chemoreflex). The PCRs are stimulated quickly (10-15 sec) by rapid changes in arterial hypercapnia and hypoxia in a synergistic fashion (peripheral chemoreflex). There is currently controversy regarding the potential interaction between central and peripheral chemoreceptors, and few studies have investigated this interaction in humans. Exper-
imental data using reduced animal models suggest one of three possibilities: (a) simple addition (i.e., no interaction), (b) hypo-additive (one reflex is inhibited with stimulation of the other) or (c) hyper-additive (one reflex is augmented with stimulation of the other). We aimed to investigate the interaction between the CCRs and PCRs in informed human volunteers using a transient hypoxic test of the PCR. Sixteen healthy human participants (23.3±2.9 yrs; BMI 24.3±8.4 kg/m²; 9 females) underwent a series of transient hypoxia tests (TT-N2; three consecutive breaths of 100% N2), which exploits the temporal and stimulus specificity of the PCRs. These TT-N2 were superimposed upon three background levels of steady-state inspired normoxic CO2 (FICO2: 0, 2 and 4%; randomized). Respiratory variables and end-tidal gases were assessed via a pneumotachometer and dual O2-CO2 gas analyzer. Following an eight-minute baseline, participants were exposed to three consecutive TT-N2 with a minimum two-minute recovery period between each test. Respiratory responses were averaged from all three TT-N2 trials to represent an individual’s response at each FICO2 level. Using TF RM ANOVAs, we assessed the hypoxic frequency response (HFR), hypoxic tidal volume response (HVTIR) and the hypoxic ventilatory response (HVR) at each FICO2 to elucidate the interaction between PCRs and CCRs on various components of ventilation. The HFR (Δ/min/Δ%ScO2) was not different between FICO2 levels (P=0.07), whereas the HVTIR (Δ%ScO2) significantly decreased with increasing FICO2 levels (P<0.05). However, the overall HVR (Δ/min/Δ%ScO2) was not different between FICO2 levels (P=0.50). Although HVTIR significantly decreased with incrementally larger FICO2, the HFR and HVR were not different across FICO2 levels. Thus, respiratory chemoreceptors in humans interact via simple addition between the CCRs and PCRs, mediated through additive and hypo-additive regulation of frequency and tidal volume, respectively.

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PCB038

Increased right cardiac sympathetic and parasympathetic nerve activity in type 2 diabetes

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Imbalance between sympathetic and parasympathetic inputs to the heart is strongly linked to cardiac dysfunction in type 2 diabetes. We recently showed via in vivo recordings that left cardiac sympathetic nerve activity (SNA) was increased in Zucker type 2 Diabetic Fatty (ZDF) rats. Importantly, in diabetes disturbances in heart rate regulation are a common dysfunction, however right cardiac SNA and parasympathetic nerve activity (PSNA) are unknown. Therefore, we aimed to directly measure right cardiac SNA and vagal PSNA in type 2 diabetes. 20-week old male diabetic ZDF rats (DM, n=6-9) and their non-diabetic (ND, n=6-7) littersmates were anaesthetised with sodium pentobarbital intraperitoneally (80mg/kg), and maintained by variable femoral vein infusion (0.6mg/kg/min). The right cardiac sympathetic nerve and right parasympathetic vagal nerve were placed uncut over bipolar platinum recording electrodes. SNA and PSNA were recorded under baseline conditions and following intravenous injection of β-agonist isoproterenol (ISO; 1µg/kg). Animals were euthanised by overdose, and differences between groups were assessed via t-test. Right integrated SNA was increased in DM (ND 1.7 ± 0.4 vs DM 6.0 ± 2.1 µV/s, p<0.05), in agreement with our measures of left cSNA, but despite reduced basal HR in diabetes. However, basal vagal PSNA firing rate was significantly increased in DM (ND 2.3 ± 1.1 vs DM 15.7 ± 5.6 Hz, p<0.05). Integrated cSNA was increased following ISO in ND animals, whereas the HVTIR (Δ%ScO2) was not different between FICO2 levels (P=0.50). Although HVTIR significantly decreased in DM at basal conditions, with most likely the PSNA changes being dominant because basal HR is lower in diabetes. Interestingly, the reduced reponsiveness of both SNA and PSNA to β-adrenergic stimulation suggests that severe impairment of autonomic control of the heart is likely a key contributor to the vast burden of cardiac dysfunction in type 2 diabetes.

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PCB039

Exogenous insulin-like growth factor 1 preserves cardiac function after myocardial infarction independent of insulin-like growth factor 1 receptor in cardiomyocytes

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Motivation: Multiple cardiac and non-cardiac cell types are involved in the remodelling process after myocardial infarction. Insulin-like growth factor 1 (IGF1) preserves cardiac function after myocardial infarction. However, it is unknown whether the beneficial effect of IGF1 is mediated by IGF1 receptor (IGF1R) signalling in cardiomyocytes.

Methods: All experiments were performed after approval of the local animal ethics committee. Two independent experimental series were conducted: In series 1, C57BL/6 mice were investigated, and in series 2, IGF1R flox/flox mice, which were crossedbreed with alpha-MHC Cre-deleter mice to achieve a tamoxifen inducible cardiomyocyte specific knock out (iCMIGF1RKO; KO and WT, respectively) were investigated. All mice of both series underwent 45 minutes regional myocardial ischemia followed by four weeks of reperfusion. Regional myocardial ischemia was initiated in anesthetized mice (2% isoflurane; 0.1 mg/kg buprenorphine, s.c.) after ligation of the right coronary artery. At the onset of reperfusion, mice received either vehicle (Con) or IGF1 as bolus (40 ng/g, i.p.) followed by continuous infusion over three days using osmotic mini pumps (1 µg/g/d, s.c.). Left ventricular function (end diastolic (EDV), end systolic volume (ESV), and ejection fraction (EF)) was analyzed by echocardiography at baseline and week 1 and 4 after I/R. In addition, regional wall motion was analyzed using strain analysis in series 2.

Results: No differences in left ventricular function between groups in both series were observed at baseline (EF: series 1; 64±4% (Con), 66±3% (IGF1); series 2; 60±4% (WT-Con), 60±2% (WT-IGF1), 60±5% (KO-Con), 58±2% (KO-IGF1)). In C57BL/6 mice (series 1), myocardial infarction caused a moderate left ventricular dilatation shown by an increase in EDV of about 33% (92±17 µl at week 4 vs. 69±10 µl at baseline). IGF1-treatment reduced left ventricular dilatation compared
Effects of ischemic postconditioning and physiological and pharmacological concentrations of melatonin on ischemia reperfusion induced change of irisin

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Myocardial ischemia-reperfusion (I/R) represents a clinically relevant problem associated with thrombolysis, angioplasty and coronary bypass surgery. Myocardial ischemic postconditioning (PostC) is a strong endogenous cardioprotective phenomenon, which targets the increased tolerance of the myocardium against I/R. It has been reported that protective effects of PostC decrease/disappear with age and chronic heart disease. Similarly low serum melatonin levels have been reported in the same risk groups. Irisin is a thermogenic protein and it is thought to play regulatory role in energy metabolism in the pathogenesis of myocardial infarction.

The aim of this study was to investigate the effects of PostC and physiological and pharmacological concentrations of melatonin on I/R induced change of irisin using an in vivo model of myocardial I/R injury.

Rats were pinealectomized (Px) or sham-operated (non-Px) (control) 2 months before the I/R studies. In order to produce cardiac damage, the left main coronary artery was occluded for 30 min, followed by 120 min reperfusion, in anesthetized rats. Melatonin was administrated by intraperitoneal injection last 10 days (10 mg/kg). PostC was induced by 3 cycles of R/I (10 s each) after the ischemia. Irisin level was detected by qRT-PCR.

The levels of the irisin increased with I/R (20%) and Px (92%), decreased with PostC and melatonin both Px and non-Px groups. Additionally, it decreased with applications of melatonin and PostC together in Px group. These results suggest that irisin level may increase with Px and cardiac I/R injury and decrease with melatonin and PostC. Irisin may play an important role in cardioprotection of ischemic PostC and physiological and pharmacological concentration of melatonin.

This study is supported by TUBITAK (115S323)

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Sarcoclemmal distribution of Na-Ca exchange and Ca autoregulation in mouse ventricular myocytes

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Intracellular Ca in cardiac myocytes is regulated by the balance between Ca influx and Ca efflux (1). In ventricular myocytes, the main Ca influx pathway, L-type Ca current (I_{L},Ca), is predominantly located in the t-tubules (TT) (2), and data from rat indicate that the main Ca efflux pathway, Na-Ca exchange current (I_{Na,Ca}), is also greater at the TT than at the surface sarcolemma (SS) (3). However the distribution of I_{Na,Ca} in mouse myocytes is not known.

In the current study, the effects of melatonin and atorvastatin on aorta and liver vaspin, visfatin and serum L-arginin levels in hypercholesterolemic rats

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Hypercholesterolaemia, characterized by nitric oxide bioavailability, lipid profile and oxidative stress changes is one of the important risk factors for atherosclerosis. Melatonin secreted by the pineal gland is protective against oxidative stress and endothelial dysfunction. Atorvastatin is a HMG-CoA reductase inhibitor, used in the treatment of hypercholesterolemia. This study was aimed to investigate effects of melatonin and atorvastatin on vaspin, visfatin levels in liver and aorta and serum L-arginin levels in high-cholesterol diet-induced hypercholesterolemia.

Rats were divided into 5 groups (n:7). While control group was fed with normal diet, other groups were fed with % 2 cholesterol and % 0.5 cholic acid diet to develop hypercholesterolemia for 8 weeks. Melatonin was administrated by intraperitoneal injection both concurrently with cholesterol and during last 2 weeks. Atorvastatin (10 mg/kg) was administered by gavage during last 2 weeks. The tissue vaspin and visfatin levels were detected by Western-Blot and serum L-arginin level was measured by HPLC.

The changes of vaspin, visfatin levels in liver and aorta tissues and serum L-arginin level were decreased by melatonin and atorvastatin treatments in high-cholesterol diet-induced hypercholesterolemia.

These results suggest that melatonin may have protective and therapeutic effect on hypercholesterolemia and create similar effects with atorvastatin.

This study is supported by TUBITAK (115S323)

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myocytes, and the role of t-tubular I\textsubscript{Ca} and I\textsubscript{NCX} in Ca autoregulation, are unknown.

Animal procedures were performed in accordance with UK legislation. Mouse ventricular myocytes were isolated by enzymatic digestion, and detubulated (DT) by osmotic shock (3). Membrane current recordings were made from intact and DT myocytes at room temperature using the whole-cell voltage-clamp technique. Intracellular Ca concentration was monitored simultaneously using fluo-4. Sarcoplasmic reticulum (SR) Ca release was triggered using caffeine (10 mM) and the associated I\textsubscript{NCX} recorded at -80 mV. Following washout of caffeine, pulses to 0 mV were applied at 1 Hz to activate I\textsubscript{Ca} and trigger a Ca transient (CaT); Ca influx during each pulse was measured as the integral of I\textsubscript{Ca} at 0 mV and Ca efflux as the integral of I\textsubscript{NCX} upon repolarisation to -80 mV (1). Data are presented as mean±SEM of n cells and statistical significance determined by Student’s t-test (p<0.05 was the limit of confidence).

There was no significant difference in caffeine-induced Ca transient amplitude between intact and DT myocytes [F/F\textsubscript{0}: 3.16±0.26 (intact; n=13) vs 3.06±0.54 (DT; n=10)] or in the associated peak I\textsubscript{NCX} density [1.03±0.09 vs -1.18±0.13 pA/pF respectively], suggesting equal I\textsubscript{NCX} density in TT and SS.

Recovery of CaT amplitude following washout of caffeine reflects restoration of SR Ca content to steady state (autoregulation). Recovery was slower [half-time 8.7±1.0 (intact) vs 12.6±2.0 beats (DT), P<0.001] and steady-state CaT amplitude was reduced [F/F\textsubscript{0} 2.80±0.28 (intact) vs 1.57±0.16 (DT), P<0.01] in DT compared to intact cells. Ca influx and efflux were both smaller in DT than in intact cells, but recovery of CaT amplitude to steady-state was associated with a reduction in Ca influx of approximately 15% in both cell types, from -108.8±10.5 to -91.3±8.0 pA/s/pF (intact) and from -65.3±4.4 to -56.6±3.5 pA/s/pF (DT). However, Ca efflux increased 4-fold during recovery of the CaT in intact cells from -15.1±1.5 to -59.2±6.7 pA/s/pF, but only 2-fold in DT cells from -14.9±2.9 to -26.0±3.5 pA/s/pF.

These data suggest that I\textsubscript{NCX} density is similar in the SS and TT in mouse myocytes, and is the main mechanism for autoregulation (4), and that loss of Ca fluxes across the TT membrane slows recovery following DT.


This work was supported by the British Heart Foundation

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Fatty acid metabolism, TCA cycle intermediates and electron transport (45 proteins). There was also an increase in the expression of two pro-apoptotic proteins (diablo Homolog & Apoptosis-inducing factor 1) with ageing. On the other hand, mitochondrial aldehyde dehydrogenase expression. Further work is being performed to compare protein expression between adult and aged groups.

Results: Proteomic analysis yielded 6077 cardiac proteins in total. There were 435 (7%) mitochondrial proteins and only 127 of these proteins displayed a statistically significant (p<0.05) change (increase or decrease) with ageing. The majority (95%) of the mitochondrial proteins that changed with age were significantly upregulated with ageing. In particular there was an upregulation of proteins associated with fatty acid metabolism, TCA cycle intermediates and electron transport (45 proteins). There was also an increase in the expression of two pro-apoptotic proteins (diablo Homolog & Apoptosis-inducing factor 1) with ageing. On the other hand, ageing was associated with a marked downregulation in mitochondrial aldehyde dehydrogenase.

Conclusion: This work reports a significant remodelling of mitochondrial proteome as a result of ageing. The changes in mitochondrial proteins are consistent with increased stress and vulnerability to cardiac insults as shown by increased mitochondrial proteins are consistent with increased stress and to cardiac vulnerability to I/R. Whether the mitochondrial proteome of the ageing heart reflects this is not presently known. The aim of this study was to investigate the effect of ageing on cardiac mitochondrial proteins and to establish whether the changes are related to stress and to cardiac vulnerability to I/R.

Methods: Cardiac protein extracts from adults (2 months old) (n=4) and from aged (18 months old) (n=5) male C57BL/6 mice were processed using isobaric tandem mass tagging and analyzed by reverse phase nano-LC-MS/MS as described previously. Mice were killed by a lethal dose of anesthetic (intraperitoneal (IP) injection of 20mg of pentobarbital sodium), and cardiac ventricles were used for protein extraction and quantification as described previously. Mitochondrial proteins were identified, and statistical analysis (unpaired t-test) was performed to compare protein expression between adult and aged groups.

Hypoxia-inducible factor (HIF) is a transcription factor which plays a pivotal role in the cellular response to reduced oxygen availability. Manipulation of the HIF system could have therapeutic potential in the treatment of ischaemic cardiac disease. HIF activity is regulated by two families of oxygen sensitive enzymes; the prolyl hydroxylase domain (PHD) family, and factor-inhibiting HIF (FIH1). The role of FIH1 in the heart is unknown.

We compared cardiac function and metabolism in hearts from mice with a null mutation in the FIH1 gene (FIH1-/-, n=5 hearts) and wild type littermate controls (WT, n=6 hearts). In vivo cardiac function was investigated using cine MRI in anesthetized mice (2% isoflurane maintenance). Individual ventricular myocytes were isolated by collagenase digestion and contractility measured using an inverted fluorescence microscope (IonOptix sarcomere length detection, 32°C, 1 Hz stimulation, n=50 cells per group). Intracellular calcium (Ca2+) transients were recorded using the fluorescent Ca2+ probe, fura-2. Glycolytic flux was investigated in Langendorff perfused beating hearts using 3H labelling techniques. Data are expressed as mean ± standard error and compared using unpaired t-tests.

In vivo cardiac function was impaired in FIH1-/- mice, with stroke volume (23.4 ± 1.5 μl) reduced by 15% in FIH1-/- hearts compared to WT (27.4 ± 2.3 μl, p<0.05). Contractility was reduced in myocytes isolated from FIH1-/- hearts, with percentage sarcomere shortening significantly lower in FIH1-/- cells (3.01 ± 0.20 %) than in WT cells (3.92 ± 0.17 %). This was accompanied by reduced Ca2+ transient amplitude (fura-2 ratio 0.21 ± 0.02 in FIH1-/-, compared to 0.29 ± 0.02 in WT, p<0.05). Furthermore, the time from peak Ca2+ transient to 50% decline (RT50) was significantly slower in FIH1-/- (198 ± 6 ms) compared to WT (150 ± 6 ms, p<0.05). Reduced Ca2+ transient amplitude and impaired Ca2+ transient decline in FIH1-/- myocytes persisted during β-adrenergic stimulation (10 nM isoproterenol). Hence, Ca2+ transient amplitude and RT50 were 0.39 ± 0.03 and 96 ± 5 ms respectively in FIH1-/- compared to 0.48 ± 0.02 and 78 ± 3 ms respectively in WT (p<0.05).

Cardiac metabolism was also altered in FIH1-/- mice. Glycolytic flux was significantly higher in FIH1-/- hearts (1.17 ± 0.04 μmol/min/g) than WT (0.79 ± 0.12 μmol/min/g, p<0.05). Furthermore, FIH1-/- hearts demonstrated increased pyruvate kinase and hexokinase activity.

In conclusion, our data suggest a novel role for FIH1 in modulating cardiac E-C coupling and metabolism. Genetic ablation of FIH1 produced cardiac effects comparable to those observed following chronic hypoxic exposure, notably decreased contractility, impaired relaxation and increased glycolysis. Potential mechanisms for these observed changes are currently being explored.

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Poster Communications

PCB046

Alveolar macrophages express the bone morphogenetic protein antagonist gremlin-1

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Background

Pulmonary hypoxia secondary to lung disease results in inflammation and the subsequent development of hypoxia-induced pulmonary hypertension (HPH). We have previously shown that the bone morphogenetic protein antagonist, gremlin-1, is lung-selectively upregulated at levels of hypoxia equivalent to those found in hypoxic lung diseases. Additionally, we have shown that gremlin-1 plays a key role in the development of HPH by increasing pulmonary vascular resistance. Macrophages play a critical role in the development of HPH although their exact mechanism of action remains to be elucidated. Preliminary data suggests that the macrophage may be an important source of gremlin-1 during the development of HPH. We hypothesize that macrophages are sources of gremlin-1 in the hypoxic lung and that macrophage-derived gremlin-1 plays a role in the development of HPH. In this study we sought to: confirm gremlin-1 upregulation in the hypoxic lung, investigate whether gremlin-1 is produced by macrophages and assess whether hypoxia regulates expression of gremlin-1 by macrophages.

Methods

Adult male C57Bl6/J mice were used for all experiments. All experiments were carried out under licence from the animal research ethics committee of University College Dublin. Mice were maintained in normoxia (21% O2) or hypoxia (10% O2) for indicated time points then sedated by inhalation of isoflurane and subsequently killed by intra-peritoneal injection of sodium pentobarbitone (200 mg/kg). Bronchoalveolar lavage was performed on mice to isolate alveolar macrophages. Lungs from mice were removed and rapidly flash frozen in liquid nitrogen. Bone marrow-derived macrophages (BMDMs) were generated using standard protocols with L-929 cell conditioned medium.

Results

Expression of gremlin-1 mRNA was significantly increased in lungs isolated from hypoxic mice (n=6) compared to normoxic controls (n=6). Immunostaining demonstrated that alveolar macrophages isolated by bronchoalveolar lavage are sources of gremlin-1. In addition, alveolar macrophages isolated from hypoxic lungs (n=6) display increased gremlin-1 immunostaining compared to normoxic controls (n=6). Furthermore, gremlin-1 mRNA expression is increased in alveolar macrophages isolated from hypoxic lungs (n=6) compared to normoxic controls (n=6). BMDMs display gremlin-1 immunostaining and mRNA expression but unlike alveolar macrophages, BMDMs do not increase gremlin-1 levels in response to hypoxia.

Conclusions

Our findings establish alveolar macrophages as sources of gremlin-1 in the lung. Furthermore, alveolar macrophages increase gremlin-1 expression in the lung in response to hypoxia. Absence of a hypoxia-induced increase in gremlin-1 levels in BMDMs suggests that this response may be tissue specific. Future work will investigate the role of macrophage-derived gremlin-1 in the development of HPH.


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Poster Communications

PCB047

Effects of maternal high fat diet and metformin treatment on adult mouse offspring cardiac ventricle wall thickness

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Maternal obesity and high-fat (HF) diet is associated with fetal myocardial thickening and contractile dysfunction, and with adult offspring hypertension and insulin resistance (1-3). These early life effects of HF may influence the risk of disease in a subsequently obeseigenic adult environment. The insulin-sensitising drug metformin is linked to improved cardiac function (4), and it is prescribed for use in diabetic pregnancies. In mature adult mouse offspring, we determined the interactive effect of maternal and post-weaning HF diet on left ventricular wall thickness (LVWT), a risk factor for cardiovascular disease, and the effect of maternal metformin treatment. C57Bl6/J female mice received a control (C, 7% kcal fat) or HF (45% kcal fat) diet 6 weeks pre-mating, and throughout pregnancy and lactation. Half of the dams were given metformin (m) in drinking water (250mg/kg bodyweight/day) throughout pregnancy and lactation. Male and female offspring were weaned onto the C or HF diet until they were killed at 30 weeks old, creating 8 diet groups: C/C, C/HF, HF/C, HF/HF, Cm/C, Cm/HF, Hfm/C, Hfm/HF. LVWT was determined in three-dimensional images by microcomputed tomography of parafin-embedded ventricles (SkyScan, Bruker). Data were analysed by 2-way ANOVA. In females and males, LVWT per gram heart weight was decreased in HF/C vs. C/C offspring (female, 8.08±0.60 vs. 10.70±0.70, p<0.001; male, 8.14±0.39 vs. 9.44±0.37, p<0.01). With maternal metformin treatment, these effects were no longer apparent.

In contrast to immature offspring (1,2), a thinner left ventricular wall relative to heart size was observed in older offspring of HF fed mothers. Amelioration of this effect by maternal metformin treatment could be due to its insulin sensitizing actions, or via cardiac-specific AMPK activation (4). These novel findings have potential implications for minimizing cardiovascular risk in offspring of obese pregnancies.


Sexual dimorphism in cardiac mitochondria following intrauterine hypoxia

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A suboptimal prenatal environment can affect organogenesis and the natural development of an individual by epigenetic modifications of the genome. While these changes are permanent, it is common not to see any pathological effects until adulthood. The impact of nutritional insults during development has been well-studied in a wide variation of physiological systems. Less studied however, are the effects of hypoxic developmental insults. To this end, our aim is to investigate the long-term effects of prenatal hypoxia on cardiovascular metabolism of adult offspring. We have utilised spectrophotometry to investigate mitochondrial enzyme activity combined with high resolution respirometry to investigate in vivo mitochondrial efficiency and production of reactive oxygen species. With these methods we aim to identify changes in myocardial mitochondrial energy production, taking a step towards understanding the effect of intrauterine hypoxia on cardiac energetics.

Pregnant mice were placed in hypoxic chambers with 14% O₂ from gestational day 3-19 and reared in normoxia until six months of age. Heart tissue was harvested and enzymatic activity of citrate synthase and mitochondrial Electron Transport Chain Complexes I-IV was measured using spectrophotometry. High resolution respirometry lets us further investigate the status of the mitochondria, with emphasis on oxygen consumption and ROS production.

Preliminary data show promising differences between treatment and control groups, as well as sexual dimorphism regarding response and effect. We hope to be able to identify possible mechanistic changes, on a cellular level, that underlie the pathological cardiovascular phenotype associated with intrauterine hypoxia.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

The effect of inflammatory cytokines on the baroreflex control of heart rate in cisplatin-induced renal failure rats

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Kidney failure is associated with renal inflammation and there is evidence that pro-inflammatory cytokines can activate the renal sensory innervation causing a dysregulation of baroreflex regulation of sympathetic outflow. This study investigated the effect of tumour necrosis factor alpha, an anti-inflammatory agent, on the baroreflex-mediated control of heart rate (HR) in cisplatin-induced renal failure rats. Wistar rats (275-350g, n=30) were randomly assigned into renal failure (RF) and control (C) groups and received either I.P. cisplatin (5mg/kg) or saline (5ml/kg) respectively, 7 days prior to the acute study. RF and C rats received either I.P. tacrolimus (0.25mg/kg/day) or saline (0.75ml/kg/day) for 7 days after the injection of cisplatin or saline. In another group of rats, during the acute experiment TNF-alpha was infused intra-renal (2µg/kg/h) and the HR baroreflex gain curve (DHR vs. DMAP) was examined. Anaesthesia was induced with chloralose/urethane (1ml 16.5:250 mg/ml, I.P.) and the right femoral artery and vein were canulated to allow measurement of mean arterial pressure (MAP) and HR, and infusion of sustaining saline and supplemental anaesthetic. The right kidney was exposed via a flank incision and a cannula was inserted 4.5mm into renal cortex to allow infusion of saline or TNF-alpha at 17µl/min. Animals were allowed to stabilise for 60-90min. High-pressure baroreflex gain curves for HR were generated using I.V. injections of phenylphrine and sodium nitroprusside (50µg/kg/min) to increase and decrease blood pressure, respectively. Data are expressed as means ± s.e.m. and compared using student’s t-test or ANOVA where relevant. P<0.05 indicated significance. In the RF group (MAP: 98±6 mmHg; HR: 39±10 beats/min), the HR baroreflex gain curve sensitivity was 61% (P<0.05) lower than C group (MAP: 91±3 mmHg; HR: 382±12 beats/min) but in rats receiving tacrolimus the baroreflex sensitivity was restored to normal values. The HR baroreflex sensitivity in the group of C rats which received intrarenal TNF-alpha was similar to the vehicle (saline). However, the mid-point of the baroreflex curve was shifted by 15% (P=0.05) to a higher MAP value. In the renal failure model, blockade of the inflammatory response with tacrolimus restored the high pressure baroreflex control of HR to normal levels while TNF-alpha depressed the gain sensitivity in control rats. Previous studies have shown that in this renal failure model that renal denervation restores HR baroreflex to normal levels. The present findings suggest that pro-inflammatory mediators could be important in increasing renal afferent nerve activity to the central nervous system causing a dysregulation of the high pressure baroreflex.

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Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NOS, is formed by proteolytic breakdown of methylated core proteins. In our research we examined the correlation of airway resistance (normalized to standard value (Raw%)) - as an indicator of airway inflammation in BA- and serum ADMA levels in asthmatic patients.

After obtaining informed consent we enrolled every patient who visited the outpatient unit of Department of Pulmonology at the University of Debrecen between 15.08.2012 and 15.10.2013 (167 patients, 91 female, 76 male, average age 46.64±14.89 years) and had the diagnosis of BA. The respiratory function was measured by whole-body plethysmography while the quality of life was measured using the St. George’s Respiratory Questionnaire (SGRQ). The laboratory parameters were determined by routine measurements and the ADMA levels were measured using HPLC. The data was analyzed by simple and multiple linear regression. At first we identified the parameters showing significant correlation with Raw% and ADMA. Based on this we compiled the initial model for multiple linear regression that involved the parameters showing significant correlation with Raw% and ADMA, and other a priori identified parameters. We set out to define the least parsimonious model that didn’t differ significantly from the initial model.

In the final model we observed a significant correlation between Raw% and serum ADMA levels (p=0.003, β=-1.08). The strong positive correlation of Raw% and ADMA flow 25-75% normalized to the standard values (p<0.001, β=57.099), SGRQ activity score (p=0.003, β=0.715) and forced expiratory flow 25-75% normalized to the standard values (p<0.001, β=1.08)). The strong positive correlation of Raw% and ADMA in the multiple regression model may indicate that the ADMA contributes to the development of bronchoconstriction in BA thus endogenous NO causes bronchodilation even under the asthmatic inflammatory condition of airways. Based on the correlation between ADMA and Raw%, the surrogate parameter of inflammation quantification we shed light on the potential importance of ADMA in the pathomechanism (and therapy) of BA. Our results further suggest that the Raw% is possibly a valuable parameter in the assessment of airway inflammation in BA patients.

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PCB051

Mass spectrometry based proteomic analysis of adult and fetal guinea pig heart muscle reveals differences in key proteins involved in branched-chain amino acid metabolism

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Fetal and adult heart muscles differ markedly at molecular, structural and physiological levels. There is a great interest in identifying those differences, their underlying mechanisms and downstream effects, particularly in light of reports showing that in certain pathological conditions, leading to heart failure, the adult heart reverts toward a “fetal” gene expression program(1, 2). In the era of high throughput technologies it is possible to undertake wide-ranging cardiac protein expression profiling. Therefore, using LC-MS-based approaches, we have performed a proteome scale comparison of fetal and adult guinea pig heart tissue (left ventricular), to identify proteins and pathways involved in heart development/maturation.

Adult female (pregnant and non-pregnant) guinea pigs were killed according to Home Office guidelines and excised fetal (gestation day 65-67, term 7 day 67) and adult hearts flushed clear of blood (glucose 227.5 mM, mannitol 34.5 mM, KCl 30 mM, NaHCO3 25 mM, pH 7.4) and stored frozen for subsequent analysis. Two separate protein extraction methods were applied in an attempt to combat issues of a massive protein dynamic range typical for muscle tissues (100mM Tris base pH7.6, 4% SDS, 0.1M DTT, 20µl/ml protease inhibitor (Sigma P8340) for total lysate and 4mM EDTA, 2mM EGTA, 5mM DTT, 150mM sucrose, 20µl/ml protease inhibitor for myofibrils depletion). Lysates were tryspinized and analyzed using two separate instrument platforms (Thermo Q-Exactive Plus in Data Dependent Acquisition mode and AB-Sciex TripleTOF-6600 with SWATH acquisition). The raw data was searched against a customized guinea pig proteome database and quantified using MaxQuant (Thermo) or Peak View (AB-Sciex). Statistical analysis of a combined dataset was performed in R.

Under our confidence criteria (2 unique peptides per protein with FDR <0.01, quantifiable for each of 3 biological replicates in at least one experimental condition) we have quantified 2158 unique proteins. Expression of 678 proteins was significantly different (Student t-test, FDR <0.05, fold change >1.5) with 489 more abundant in fetal and 189 in adult heart muscle. Subsequent pathway analysis in String and PathVisio revealed reduced fetal cardiac expression of proteins essential to branched-chain amino acid (BCAA - leucine, isoleucine, valine) degradation e.g. Branched Chain Amino-Acid Transaminase (BCAT2), Branched Chain Keto Acid Dehydrogenase (BCKDHA, BCKDHb), Dihydrolipoamide Branched Chain Transacylase (DBT). This suggests that regulating the expression of proteins key to BCAA catabolism is important for physiological cardiac remodeling and identifies these proteins as interesting candidates to study in the progression of hypertrophic cardiopathologies.

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PCB052

Is the blunted β-adrenergic response in right ventricular heart failure linked with changes in the caveolar microdomain?

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Desensitisation of the β-adrenergic (βAR) response is typically seen in heart failure (HF), caused by decreased β1AR expres-
the importance of linking protein distribution/organisation

to RV HF by 21-28 days post-injection. MCT+BB and CON ani-
mals were given metoprolol (10 mg/kg; MCT+BB group) or
saline (CON group) or 60 mg/kg of monocrotaline (MCT). MCT
animals induced RV hypertrophy which progresses
progressively and after 30-40 min, IF approached the extracel-
ular solution, the intracellular fluorescence (IF) increased
µM vs 6 µM) and

post injection. MCT+BB and CON ani-
mals were given metoprolol (10 mg/kg; MCT+BB group) or

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requirements.

Poster Communications

Flecainide accumulates within intact ventricular cardiomycocytes and its intracellular effect on RyR2 is
potentiated by sarcoplasmic reticulum counter-current block

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Flecainide can be an effective treatment for catecholaminergic polymorphic tachycardia (CPVT) in both animal models and
humans [1]. While flecainide is an inhibitor of Na,1.5,
initial studies on adult ventricular myocytes (AVMs) from wild
type (WT) rat or CPVT (calsequestrin-2 knockout) mouse con-
cluded that flecainide acts primarily on RyR2 to reduce the
frequency of spontaneous Ca2+ waves (SCWs) [1, 2]. However,
in subsequent studies it was suggested (i) that the antiarrhyth-
mic effect of flecainide was due to an action on Na,1.5, not
RyR2 in WT rat AVMs [3, 4] and (ii) that flecainide does not
affect gating of isolated RyR2 under physiologically relevant
conditions [4].

Here, the effects of flecainide were further investigated in
permeabilised Wistar rat AVMs using confocal imaging [2].
Data were analysed using one or two way repeated mea-
sures ANOVA where appropriate, and are presented as mean ± SEM. Flecainide (25 µM) decreased SCW frequency by 21.2 ± 5.43 % (n=12; p<0.01). This compares with previous find-

ings on permeabilized AVMs from CPVT mice, where a lower
level of flecainide (6 µM) caused a 50 % greater decrease in
wave frequency [5]. Interestingly however, when the SR counter-current was inhibited by substitution of K+ with Cs+, flecainide (25 µM) reduced SCW frequency by 42.8 ± 13.6 %
(n=13; p<0.001) in WT rat AVMs. This might be explained if a
transient polarisation of the SR membrane during SR Ca2+
release (inside negative) facilitates the action of flecainide on
RyR2.

In further experiments, flecainide entry into intact WT rat
AVMs was detected using a fluorescent (FITC) labelled form
(flecainide-F). When 6 µM flecainide-F was added to the extra-
cellular solution, the intracellular fluorescence (IF) increased
progressively and after 30-40 min, IF approached the extracel-

ular fluorescence (EF). After 3 hours in 6 µM flecainide-F, the IF
was 2.32 ± 0.17 times the EF (n=11-12; p<0.0001), suggesting
accumulation of flecainide-F. These data show that flecainide has qualitatively similar
effects on SCWs in WT rat to those reported in CPVT mouse
AVMs [2], although a higher intracellular flecainide concen-
tration was required in WT rat AVMs (25 µM vs 6 µM) and the
decrease in wave frequency was less pronounced. The
flecainide-F experiments suggest that it may take several hours
incubation to achieve the intracellular drug level needed to
affect RyR2 in WT rat AVMs. The greater effect of flecainide
following counter-current inhibition may relate to recent find-
ings on isolated RyRs within lipid bilayers, where flecainide
preferentially blocked the channel when there was a negative
charge across the bilayer.


Where applicable, the authors confirm that the experiments
described here conform with the Physiological Society ethical
requirements.

PCB053

Flecainide accumulates within intact ventricular
cardiomyocytes and its intracellular effect on RyR2 is
potentiated by sarcoplasmic reticulum counter-current block

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Flecainide can be an effective treatment for catecholaminergic polymorphic tachycardia (CPVT) in both animal models and
humans [1]. While flecainide is an inhibitor of Na,1.5,
Cellular electrophysiology of cardiomyocytes from rat pulmonary vein: Responses to noradrenaline

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Atrial fibrillation (AF), the most common sustained arrhythmia, usually arises at the junctions of the left atrium (LA) with the pulmonary veins (PV) (1). The autonomic nervous system is important in AF initiation (2). In rat LA cells, noradrenaline (NA) increases L-type Ca current (I_{Ca}), inhibits a steady state K current (I_{Kss}), and prolongs action potential duration at 30% repolarization (APD30) (3). It has been suggested that NA may trigger arrhythmic activity in PV cells (4). The aim of this study was to examine the electrophysiology of rat PV cardiomyocytes and their responses to NA in comparison with LA cells. Procedures were approved by local ethics committee and performed in accordance with UK legislation. LA and PV cardiomyocytes were isolated from adult male Wistar rat hearts, superfused with a Tyrode’s solution (37 °C) and subject to whole-cell recording using a ruptured patch-clamp technique. Action potentials were recorded during stimulation at 1 Hz using a nominally Ca-free pipette solution. Whole-cell currents were recorded from a holding potential of -80 mV using a Ca2+-buffered pipette solution. I_{Ca} was activated by depolarisation to +10 mV (300 ms) following a pre-pulse to -40 mV to inactivate Na current, and measured as the difference between the peak inward current and the steady-state current at the end of the pulse. I_{Kss} was recorded as the outward current on depolarisation to +50 mV. Inward rectifier current (I_{K1}) was measured as the K-dependent inward current following a voltage ramp to -120 mV. NA was applied at 1 µM. Data are presented as mean ± standard error of the mean and were compared by either unpaired or paired t-test. P<0.05 was used as the limit of statistical confidence. Membrane capacitance, an index of cell size, was not different between LA (57.5±1.8 pF, n=125) and PV (58.2±2.2 pF, n=125) cardiomyocytes. In control conditions, there were differences between LA and PV cardiomyocytes in I_{Ca} (LA: 9.13±0.51 pA/pF, n=29; PV: 7.19±0.70 pA/pF, n=26; P=0.0330) and I_{Kss} (LA: 9.98±0.87 pA/pF; PV: 14.28±1.18 pA/pF; P=0.0052). I_{K1} density was also slightly smaller in PV (-13.17±0.62 pA/pF, n=26) compared with LA (-15.78±0.81 pA/pF, n=50; P=0.0339) cardiomyocytes. However, there were no differences between the cell types in APD30 (LA 7.2±0.5 ms, n=24; PV 6.1±0.6 ms, n=24), APD90 (LA 19.7±1.3 ms; PV 15.5±1.6 ms) or APD95 (LA 51.5±3.0 ms; PV 43.8±3.9 ms). In PV cells, NA had no effect on I_{K1} or on currents during the ramp at diastolic potentials. NA inhibited PV I_{Kss} by 41.9±4.1% (n=23, P<0.0001), similar to LA cells (3). In contrast, NA did not increase I_{Ca} in all PV cells. Heterogeneity between PV cells in APD95 prolongation by NA was also observed: of 12 cells, 6 showed no response. PV cardiomyocytes show differences to LA cells and respond heterogeneously to NA.


Disease modelling on new scales: a 3D in vitro cardiac model using fluorescent Zebrafish (Danio rerio)

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Zebrafish (ZF) are an excellent platform for cardiac disease modelling, owing to their electrophysiological similarity to humans and the availability of numerous mutants that mimic human cardiac disease phenotypes. Homogenised ZF larval tissue forms spontaneously contracting ZF Heart Aggregates (ZFHAs) in vitro which represent a simple, high-throughput cardiac model. Here, we demonstrate at a cellular level the generation of a 3D in vitro cardiac cell culture model using transgenic ZF with enhanced green fluorescent protein expressed in myocardial cells, specific to the cardiac myosin light chain 2 gene.

We created fluorescent ZFHAs from homogenised ZF larval tissue; live cell microscopy was used to assess migration of the fluorescent myocardial cells during formation. ZFHAs formed from wild type and fluorescent ZF were characterised using immunohistochemistry and electron microscopy, whilst contraction frequency, size and electrophysiology were analysed at multiple time points in development. Angiotensin II was utilised to induce hypertrophy of the ZFHAs, with further electrocardiogram analysis performed to assess subsequent changes in electrophysiology. This work is ongoing but results so far demonstrate that ZFHAs are an exciting complementary in vitro model for human cardiac disease phenotypes, safety pharmacology and tissue regeneration.

I would like to thank Holly Shiels, Lisa Mohamet and Bianka Grunow for their incredible support and enthusiasm throughout this project, Jake Ireland and my fellow students at Shiels lab for their valuable advice and the Bioimaging facility for their training and assistance.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Glutathionylation dynamically regulates G protein alpha subunit and Caveolin 3 interaction

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Acute regulation of cardiac function requires cellular mechanisms to elicit changes in protein function through different post-translational modifications. One such modification is palmitoylation, the addition of the 16-carbon fatty acid palmitate to a cysteine thiol side chain, which alters protein function by regulating intracellular sorting, stability, membrane interaction, enzyme activity and targeting to lipid rafts. Glutathione acts as a redox buffer and antioxidant within cells in order to protect proteins from irreversible oxidative damage. Protein glutathionylation (the reversible conjugation of glutathione to protein cysteines in a mixed disulfide) is emerging as a critical signaling event in the cardiovascular system due to its ability to regulate many physiological processes involved in cardiac homeostasis. Both palmitoylation and glutathionylation can change the function and location of a protein in a reversible manner; we investigated competition between these modifications in both freshly isolated rat ventricular myocytes and the rat cardiomyoblast cell line H9c2. The heterotrimeric G-protein alpha subunits Gα and Gβ are palmitoylated (measured using resin-assisted capture) and glutathionylated (measured using biotinylated glutathione ethyl ester labelling) in unstimulated cells at rest. Oligomers of caveolin 3 form caveolae within the cardiac sarcolemma. These lipid rafts concentrate many signalling molecules, including G proteins, facilitating cellular signal transduction. Treatment of H9c2 cells or ventricular myocytes with the selective thiol oxidizing agent diamide increases glutathionylation on Gα and Gβ and caveolin 3 (2.47 ± 0.77, n=7). Concurrently co-immunoprecipitation experiments indicate the physical interaction between both Gα and Gβ and caveolin 3 is lost, however sucrose gradient fractionation indicates both Gα-protein subunits still reside within caveolar membranes. This implies that G protein or caveolin 3 glutathionylation dynamically regulates G protein a subunit interaction with caveolin 3 and thus G-protein coupled generation of intracellular cAMP. Control of intracellular cAMP is functionally crucial in the regulation of cardiac function. Determining the role of glutathionylation on Gα, Gβ and caveolin 3 will therefore provide considerable insight into dynamic regulation of cardiac function both at rest and under conditions of redox stress such as ischaemia reperfusion.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Cell-specific mathematical models of cardiac electrophysiology

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Drug compounds can have off-target effects on cardiac ion channels that can alter heart rhythm and sometimes lead to sudden cardiac death. Automated ion channel screening can now detect to what degree a novel compound blocks particular ion currents. Data from screening with mathematical models of cardiac action potentials can help us to understand what combined effect a drug will have on overall cellular electrophysiology [1]. A recent proposal [2] is to use this approach with a confirmatory experimental whole cell measurement, and a promising cell type to help us explore these effects is the induced human stem cell-derived cardiomyocyte (iSCM) [3]. These cells are more readily available than mature human ventricular cells and so can allow a higher-throughput testing of compounds.

We present a methodology for tailoring the ion channel densities in an existing mathematical iSCM model to individual cells from action potential voltage recordings, and quantify associated uncertainties due to beat-to-beat variation. We provide a proof of principle in silico study using simulated datasets. We use a hierarchical Bayesian approach to construct parameter distributions for how we expect an ‘average’ cell to behave, allowing us to make predictions of drug action for an individual cell with an associated probability. For most ion channel densities, we find that we can successfully infer the ‘correct’ distributions for each dataset. We also infer a higher-level distribution which describes how these ion channel densities were selected. We then detail how this might be extended to look at the case of drug effects on iSCMs and how they compare with what we expect from ion channel screening. Once the combined effects of a drug on these cells is understood in terms of its action on ion channels, we hope to extrapolate this information into predictions in the adult human situation using mathematical models again.

EPSRC, Roche

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Identification of caveolar subpopulations in ventricular muscle

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The lipid raft concept proposes that membrane environments enriched in cholesterol and sphingolipids cluster certain proteins and form platforms to integrate cell signalling. In cardiac muscle, caveolae concentrate signalling molecules and ion transporters, and play a vital role in adrenergic regulation of excitation-contraction coupling and consequently cardiac contractility. Evidence from non-cardiac cells has shown that there are multiple caveolar subpopulations, defined by different content of cholesterol, caveolin isoforms, signal effectors (e.g. kinases) and targets (e.g. ion channels).

The aim of this investigation was to identify caveolar proteins in cardiac muscle, investigate dynamic regulation of caveolar content, and define co-localised subpopulations of caveolar proteins.
We defined the cardiac caveolar proteome using quantitative proteomics to identify proteins depleted from caveolar membranes prepared from rat ventricular myocytes using a standard discontinuous sucrose gradient after treatment of these myocytes with methyl-β-cyclodextrin (MβCD) to deplete cholesterol and disrupt caveolae. We defined 249 proteins as high-confidence caveolar residents. Functional annotation clustering indicates cardiac caveolae are enriched in integrin signalling, guanine nucleotide binding, ion transport, and insulin signalling clusters.

In order to investigate dynamic changes in caveolar protein constituents following adrenoceptor (AR) stimulation we selectively activated α-, β1- and β2-AR by applying agonist/antagonist pairs for 10 min to field-stimulated myocytes prior to preparation of caveolae. Quantitative proteomic analysis indicates that with the notable exception of cavins 1, 2 and 4, very few proteins show altered abundance in caveolae following AR activation, suggesting signalling complexes are pre-formed to ensure a rapid and high fidelity response to adrenergic stimulation in cardiac muscle.

To define subpopulations of cardiomyocyte caveolae we enriched caveolar membranes using a sucrose gradient. Membranes were fractionated by size exclusion chromatography using Sephacryl S500 and S1000 columns, and fractions blotted for caveolin1, caveolin3, Na pump α1 subunit and cavin1. Preliminary results show that caveolin1 and caveolin 3 are not enriched in the same fractions, suggesting a subset of cardiomyocyte caveolae is caveolin 1-free. We find cavin1 is associated preferentially with Caveolin 3 over Caveolin 1/3-enriched membranes. This confirms that myocyte caveolae exist in different subpopulations. Hence physical and functional co-localisation of subpopulations of caveolar residents may contribute to the complexity of signalling through these microdomains in cardiac muscle.

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**PCB059**

**Chronic Tempol supplementation restores diaphragm muscle force-generating capacity in the dystrophin-deficient mdx mouse**

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Duchenne muscular dystrophy (DMD) is a neuromuscular disease characterised by skeletal muscle weakness (limb & respiratory muscle). DMD is caused by disruption to the dystrophin gene, leading to the absence of the structural protein – dystrophin. Patients die due to respiratory and cardiac failure. Reactive oxygen species (ROS) are important modulators of respiratory muscle function in health and disease. There is evidence of aberrant redox signalling in DMD and a pre-clinical model of DMD, the mdx mouse. We sought to examine the effects of acute and chronic antioxidant (Tempol; superoxide scavenger) treatment on diaphragm muscle dysfunction in the mdx mouse.

Fourteen week old mdx (C57BL/10ScSn-Dmd<sup>mdx</sup>; n=22) and wild-type (WT; C57BL/10ScSn; n=7) mice were studied. Ex vivo diaphragm muscle preparations were examined in four groups: WT (n=7), mdx (n=7), mdx & Tempol <i>in vitro</i> (n=7) and mdx & Tempol <i>in vivo</i> (n=8). Diaphragm muscle mechanical properties were examined using a dual-mode lever transducer system. For mdx & Tempol <i>in vitro</i>, diaphragm muscle was incubated in Tempol (10μM). Mdx & Tempol <i>in vivo</i> received Tempol (1μM) supplementation in the drinking water for 2 weeks (12-14 weeks of age). The enzymatic activity of citrate synthase (CS), lactate dehydrogenase (LDH) and phosphofructokinase (PFK) was examined in diaphragm muscle from the WT, mdx and mdx & Tempol <i>in vivo</i> groups by use of commercial spectrophotometric assays. Data are reported as mean±SD and were statistically compared by unpaired Student t-test.

Peak specific force (Fmax) was significantly reduced in mdx (12.0±1.8 N/cm²; p<0.0001) compared with WT (22.4±4.1). Application of 10μM Tempol to mdx diaphragm had no effect on mechanical function (12.1±1.4). Chronic Tempol supplementation significantly increased mdx diaphragm force (20.1±5.8; p=0.006; mdx Tempol <i>in vivo</i> vs mdx). CS activity was significantly reduced in mdx (0.5±0.1 μmol/min/mg protein; p=0.0003) compared with WT (0.8±0.1; in vivo Tempol significantly increased CS activity in mdx (0.7±0.1; p=0.005). LDH activity was significantly reduced in mdx (0.5±0.2; p=0.001) compared with WT (1.0±0.3; in vivo Tempol significantly increased LDH activity in mdx (0.9±0.2; p=0.002). PFK activity was equivalent in all groups. Pronounced muscle weakness and redox stress is evident in the mdx mouse diaphragm at 14 weeks of age. Acute treatment with Tempol did not rescue force in mdx diaphragm suggestive of structural modelling due to chronic oxidative stress. Chronic treatment with Tempol <i>in vivo</i> restored mdx diaphragm muscle force and metabolic enzyme activity indicating that antioxidant intervention prevents aberrant muscle plasticity secondary to oxidative stress. Antioxidants could serve as useful adjunctive therapies in the treatment of DMD.

Supported by Muscular Dystrophy Ireland and the Department of Physiology, UCC

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**PCB060**

**Tracking the evolution from compensation to decompensation in aortic-banded hearts: a computational modeling approach**

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The heart is a dynamic system that adapts to external constraints to fulfill its physiological role. Systemic hypertension hinders blood ejection into the aorta and occurs for example in aortic-valve stenosis, a common heart disease in which the aortic-valve opening is narrowed, impeding blood flow from the left ventricle (LV). This results in an enhanced pressure gradient across the aortic valve during LV ejection to maintain the required blood-flow rate. Increased afterload tends to reduce the ventricular stroke volume, and leads to ventricular hypertrophy, a thickening of the muscular wall that compensates the increased wall stress and allows generation of higher pressures. Over time, however, the short-term benefits of cardiac remodeling give way to decompensatory behaviour and ultimately heart failure. A better understanding of the
nature of the transition from compensation to decompensation is essential for developing diagnosis and treatment of heart failure. Aortic banding (AB), a common experimental model, involves artificially constricting the aorta to impede blood flow. After several weeks, AB rats typically display an increased heart mass and reduced LV diameter at diastole and systole, but while demonstrating ejection fractions remarkably similar to control rats, indicative of compensatory tendencies in these early stages. To better understanding the nature of the transition from compensation to decompensation, we sought to identify changes in the balance of compensatory contributions using a computational-modeling approach supported by heart-specific measurements. We investigated the interplay of mechanistic contributions to LV contraction to identify how these might evolve at different stages of compensation. We fitted a finite-element computational model to geometrical, fibre-configuration, electrophysiological, and hemodynamic measurements derived from individual aortic-banded or control rats. The model was simplified so as to allow an objective parameterisation of essential functional parameters based on the experimental data. These heart-specific models provided a testbed for simulating the contraction and comparing the sensitivities of the LV ejection fraction to the different mechanistic parameters and physiological conditions. Our simulation results suggest a significant enhancement of the sensitivity of the ejection fraction to muscle activation in aortic-banded hearts, whereas geometrical features and passive mechanical properties play a more significant role in a control heart. Despite the near-identical ejection fraction displayed in both heart types, this difference in the underlying compensatory balance may reflect a fundamental evolution in the heart’s response to the aortic banding that will eventually lead to heart failure.

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PCB062

Carotid body dysfunction and hypoventilation in the dystrophin deficient mdx mouse

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Duchenne muscular dystrophy (DMD) is a fatal X-linked neuromuscular disease. Patients have severe respiratory muscle wasting and chronic respiratory insufficiency. Hypoventilation and sleep disordered breathing are both common features of the disease and are known to culminate in periods of hypoxemia and hypercapnia. There is a paucity of information regarding the control of breathing in pre-clinical models of DMD. We measured ventilation, oxygen consumption (VO2) and carbon dioxide production (VCO2) in freely behaving mdx (C57BL/10ScSn-Dmd-mdx/J; n=12) and wild-type (C57BL/10ScSn; n=9) mice at 8 weeks of age during normoxia (FiO2=0.21) and in response to a graded hypoxic challenge (FiO2=0.15, 0.12, 0.1 & 0.08). Carotid sinus nerve activity was measured ex vivo in an artificially perfused preparation in WT (n=6) and mdx (n=6) mice during normoxia (PO2=100 Torr) and graded hypoxia (PO2=80, 60, 40 Torr). Diaphragm muscle force-frequency relationship was examined ex vivo. qRT-PCR was used to examine gene expression in diaphragm muscle from WT (n=8) and mdx (n=8) mice. Data were expressed as mean±SD and were statistically compared by unpaired Student t-test unless otherwise stated.

Minute ventilation (V̇e) was significantly reduced in mdx (0.7±0.2 ml/min/g; p<0.001) mice compared with WT (1.2±0.3) controls. There was no significant difference in VO2 and VCO2 between WT and mdx when expressed in absolute terms. Inspiratory drive (VI/TI) was significantly reduced in mdx (0.06±0.02 ml/sec/g; p<0.001) compared with WT (0.1±0.02). Carotid sinus nerve unitary discharge was significantly depressed during normoxia in mdx (1.6±0.4 Hz; p<0.01) compared with WT (2.4±0.4) ex vivo. Diaphragm force was
Species-specific comparison of the cardiacsodium/potassium pump based on a minimal biophysical model

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The Na+/K+ ATPase (NKA) plays a critical role in maintaining the concentration gradients, across the plasma membrane, of potassium (which determines the cell’s membrane potential) and sodium, the driving force behind crucial ion-exchange processes, including calcium extraction via the sodium/calcium exchanger. This function has been extensively studied, experimentally and by computational simulations, within the context of the excitation/contraction coupling in cardiac myocytes. An important source of complexity in these strongly couple systems is the significant species-dependent variability of physiological conditions under which NKA operates, particularly the intracellular sodium concentration [Na+]i. For example, [Na+]i ~ 11 mM in rat ventricular myocytes, and ~5 mM in guinea pig. An important question is whether (1) NKA is maintained across species and operates in different species-specific regimes; or (2) NKA shows significant species-dependent variations and hence participates directly in defining physiological conditions. Most existing models neglect this fundamental question by assuming a generic NKA formulation derived from disparate experimental sources.

To address this problem, we propose a biophysical framework for characterizing NKA function, specifically designed for species-specific parameterization, and produce separate models for rat and guinea pig NKA, each parameterized from fully consistent data sets. We find that the apparent binding affinity for sodium in the rat is lower by a factor of approximately three, whereas the overall pump current magnitude is roughly doubled, relative to guinea pig. These trends mirror those for the [Na+]i differences, suggesting that NKA kinetics compensates or has adapted to its physiological conditions. Such comparisons allow an analysis of the relative influence of cellular components, ionic conditions, and the action potential on ion transport in cardiac contraction, and ultimately enable the quantification of variations in physiological function of NKA across biological contexts.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB064

Age-related cardiac conduction slowing in PGC-1β−/− hearts

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Sudden cardiac death accounts for 70,000 deaths in the United Kingdom and 250,000 in the United States annually. In a significant proportion the aetiology remains obscure, however recent reports implicate metabolic dysfunction as a causative factor. Metabolic dysfunction secondary to PGC-1β−/− ablation in mice has been shown to increase the incidence of lethal ventricular arrhythmias (Gurung et al. 2011). As metabolic dysfunction progresses with advancing age, we investigated interactions between ageing and mitochondrial dysfunction, upon action potential conduction characteristics as a contributor to their arrhythmic phenotype.

Wild type (WT) and PGC-1β−/−, C57/B6 mice (Bar Harbour Laboratories, Maine) were divided into young WT (n=5), young PGC-1β−/− (n=9), old WT (n=8) and old PGC-1β−/− (n=6) groups. Mice were weighed, anaesthetised with tribromoethanol (240mg/kg i.p.) and electrocardiogram (ECG) signals were recorded at baseline and following dobutamine challenge (0.3mg/kg i.p.). Components of the ECG waveform and relevant intervals were analysed for statistically significant differences between groups pre and post dobutamine challenge using multivariate ANOVA. Significant differences between groups then prompted further, post hoc MANOVA decomposition and Tukey HSD tests respectively.

P wave duration and atrio-ventricular node (AVN) conduction appeared normal in all animals, with no significant differences between groups at baseline. All WT mice demonstrated a normal positively dromotropic response to dobutamine. However 44% of young PGC-1β−/− and 83% of old PGC-1β−/− mice exhibited a paradoxically negatively dromotropic effect with dobutamine administration (Fig. 1). A reduction in conduction velocity is a recognised mechanism of arrhythmogenesis (King et al. 2013). We therefore next assessed ECG surrogates of ventricular conduction velocity. MANOVA analysis demonstrated significant interactive effects of genotype and age on QR and QS duration, both prior to, and following dobutamine administration (F(2,23) = 4.0031, p = 0.03223 and F(2,23) = 3.7015, p = 0.04039 respectively). Post hoc ANOVA and Tukey HSD tests showed that this difference lay in the aged PGC-1β−/− mice (Table 1).

The present findings implicate age-related abnormalities in AVN function and slowed conduction through the ventricular system in the PGC-1β−/− arrhythmogenic phenotype.
Table 1: Depolarisation intervals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dobutamine PR Interval</th>
<th>Post Dobutamine PR Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild Type</td>
<td>6.05±0.07 ms</td>
<td>7.14±0.75 ms</td>
</tr>
<tr>
<td>Wild Type</td>
<td>6.52±0.49 ms</td>
<td>7.48±0.45 ms</td>
</tr>
<tr>
<td>PGC 1β -/-</td>
<td>6.52±0.50 ms</td>
<td>7.47±0.40 ms</td>
</tr>
</tbody>
</table>

Mean +/- SEM, * p < 0.05 after post hoc Tukey HSD.

Impact of maternal and postnatal obesity on gene expression on the mouse lung

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Obesity is a major global health concern and associated with a range of cardiometabolic disease. More recently, it has been linked to respiratory diseases such as asthma and COPD (Reilly et al., 2003). Beyond direct reproductive consequences, maternal obesity has proven associations with increased prevalence of respiratory disease in the offspring. Specially, children born to obese mothers are more likely to suffer respiratory infections and childhood wheeze (Haberg et al., 2009). The aim of this project was to investigate the impact of maternal obesity and early postnatal diet upon the expression of genes associated with lung: development, architecture and function, in the lungs of offspring.

Female C57/BL6J mice (n=29) were fed either standard chow (C; 7% kcal fat, 18% kcal protein, 75% carbohydrates) or an obesogenic high-fat diet (HFD; 45% kcal fat, 17% kcal protein, 35% kcal carbohydrates) for 4-6 weeks prior mating and throughout gestation and lactation. At weaning, pups were transferred to either C or HFD to give four dietary phenotypes, CC, HFC, CHF & HFHF (n=4-6 males and females per group). Offspring were killed by cervical dislocation at 30 weeks of age and the lungs were snap frozen. Expression of genes involved in inflammation (IL6, IL33 & TNFa), remodelling (TGF-β2 & ADAM33) and obesity (FTO) were measured by quantitative real-time RT-PCR. Data was analysed by 2-way-ANOVA for associations between prenatal and postnatal diet; significance was accepted at p<0.05.

Increased ADAM33 expression was seen in lungs of male mice born to obese dams (prenatal HFD mice) compared with prenatal C mice (p<0.012, F=2.299). Increased FTO expression was seen in lungs of male postnatal HFD mice compared with postnatal C mice (p<0.013, F=0.544). Decreased expression of IL6 was seen in the lungs of male postnatal HFD mice compared with postnatal C mice (p<0.023, F=0.824). No changes were seen in levels of TNF-a, TGF-b2 and IL-33.

These data suggest that both maternal and postnatal obesity differentially alter gene expression in the offspring lung. That similar changes in these genes have previously been associated with impaired lung function suggests that maternal obesity alone can lead to long term respiratory changes.


This work was supported by the Gerald Kerkut Trust and Diabetes UK.

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Figure 1: Representative PR intervals for (A) WT and (B) PGC-1β⁻/⁻ mice


This work was supported by the Medical Research Council (MR/M001288/1), Wellcome Trust (105727/Z/14/Z) and Fundamental Research Grant Scheme (FRGS/2/2014/SKK01/PERDANA/02/1) from the Ministry of Education, Malaysia.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Predicting drug-induced arrhythmic risk using simulated afterdepolarisations

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New drug candidates must be shown not to cause life-threatening Torsades de Pointes arrhythmia. The present metrics used to predict this are block of the hERG potassium current, and prolongation of QT interval. However, some drugs which are strong hERG blockers are not torsadogenic (Kramer, 2013) and not all drugs which prolong QT interval cause Torsades (Segers, 2008). In an effort to find a more specific marker of drug-induced pro-arrhythmic risk, we used multi-ion channel block in combination with mathematical electrophysiological modelling to investigate the link between ion channel effects and susceptibility to afterdepolarisations.

Physiological causes of afterdepolarisations include increase in L-type calcium current conductance; decrease in rapid delayed rectifier potassium current conductance; increasing late sodium current (Noble & Noble, 2006); and shifting the voltage inactivation curve of the fast sodium current. We implemented these effects in mathematical models of cardiac cells by altering conductances, concentrations, and ion channel kinetics. The level of these interventions necessary to cause afterdepolarisations were measured (see figure). Using data for drug effects on multiple ion channels, ion channel conductances were reduced to mimic the effects of drug block in the cells, and the intervention thresholds required to provoke afterdepolarisations were measured for each drug.

The change in threshold was used to classify drugs into risk categories using linear discriminant analysis, based on training data of clinical drug-induced torsades incidence. The errors in classification were used as a metric for the predictivity of the intervention. All but five of the interventions were more predictive than hERG-only risk markers, which had a mean error of 1.5, with a standard deviation of 1.2. The lowest error was the L-type calcium current increase protocol in the Ten Tusscher 2006 M cell model, with mean error 0.48 and standard deviation 0.62, the same mean error as the APD90 measure developed by Mirams et al. (2011). These results indicate that simulating afterdepolarisation tendency has potential for use in the early stages of drug development as an improved marker for CiPA and drug companies to use for predicting drug-induced arrhythmic risk.


GPR30 resisted the stress cardiomyopathy via down-regulating G/p-Akt signaling pathway

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Stress cardiomyopathy(SCM), is an increasingly recognized clinical syndrome of acute but reversible apical ventricular dysfunction. High levels of epinephrine (Epi) trigger a switch of G protein signaling pathway, from Gs to Gi signaling, producing SCM (Lyon AR, 2008). SCM usually occurs in postmenopausal women, suggesting that the incidence of SCM is associated with the drop in estrogen levels (Kida K et al. 2010). G protein-coupled receptor (GPR30), as a novel estrogen receptor, confers rapid cardioprotection in rat heart. Female Sprague-Dawley (SD) rats (n=6) were anaesthetised with 10% chloral hydrate intraperitoneal injection (3.5ml/kg). Epi was injected via tail vein to establish the SCM model. G1 and G15, as GPR30 agonist and antagonist respectively, were also injected via tail vein. Left ventricular internal diameter at end-systolic dimension (LVIDd), left ventricular internal diameter at end-systolic dimension (LVIDs), ejection fraction (EF) were detected by echocardiography during 4 mins. The expression of p-Akt in hearts was measured. In vitro, the contraction of myocytes (n=6), LDH of supernatant above groups were detected (n=6), Gs protein and activity of Gi protein of cardiomyocytes were detected. Values are means ± S.E.M., compared by one-way or two-way ANOVA. Compared with Normal group, Epi group showed an increasing LVIDs(0.386±0.005 VS 0.139±0.006,P<0.001), and decreasing EF(73.22±1.630 VS. 94.36±0.6778, P<0.001). Compared with Epi group, G15+Epi group had a higher LVIDs (0.466±0.031 VS 0.386±0.005, P<0.01) and a lower EF(52.20±2.345 VS.73.22±1.630, P<0.001) at the beginning 2 mins, and the EF of two groups recovered to normal level at 4 min. In G1+Epi group, except LVIDd, the LVIDs and EF were kept at the normal level during the 4 mins. Epi and G15 pretreatment increased the p-Akt, which is more obvious in the latter group. G1 pretreatment decreased the p-Akt(n=3). On the cell level, E2 or G1 pretreatment decreased contraction inhibition induced by Epi(E2: 9.318±0.273 VS. 7.541±0.559,G1:10.080±0.444VS. 7.541±0.559, P<0.05 respectively). However, G15 pretreatment abolished the effect of E2(9.318±0.273 VS. 7.207±0.444,P<0.05) Compared with Normal, Epi increased the LDH concentrations (937.6±27.79 VS. 511.5±48.26, P<0.001). LDH concentrations were declined in E2+Epi, which was abolished by G15 pretreatment(725.7±40.48VS.936.2±27.04, P<0.05). G1 pretreatment decreased LDH concentrations (724.5±55.7 VS. 937.6±27.79, P<0.05). There was no difference in the expressions of Gs protein. The activity of Gi protein increased after stimulated with Epi. Gi protein partly declined when pretreated with E2 or G1 but still higher than Normal group. G15 pretreatment abolished this effect of E2(n=3). These data suggested that activation of GPR30 resisted SCM via down-regulating the activity of G/p-Akt signaling pathway.


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PCB068

Cigarette smoke disrupts the functions of SPLUNC1 in regulating airway surface liquid volume and anti-microbial activity

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Chronic obstructive pulmonary disease is the third leading cause of death worldwide with tobacco smoke being the most common causative factor. Airway surface liquid (ASL) is a thin layer that lines the luminal side of airways which plays a major role in innate defense facilitating the clearance of inhaled pathogens as well. SPLUNC1 is a secreted protein in the upper airway that regulates ASL height and has anti-microbial activity against respiratory pathogens. Exposure of cigarette smoke (CS) to airway epithelia is known to induce inflammation, mucus hypersecretion, and depletion of the ASL volume which together result in in bacterial infection and mucus stasis. However whether CS affects SPLUNC1 activity directly is unknown.

We investigated the role of CS on SPLUNC1 activity using an in vitro CS exposure model, ASL height measurements, mass spectrometry and antimicrobial assays. After exposing recombinant SPLUNC1 to CS we observed a reduction in the ability of SPLUNC1 to regulate ASL height (P<0.05) (N=6). Overnight dialysis of SPLUNC1 did not reverse this effect (N=6). Furthermore antimicrobial activity against Gram Negative bacteria such as Haemophilus influenzae and Pseudomonas aeruginosa was attenuated following CS exposure (P<0.05) (N=3). Further, analysis of smoked SPLUNC1 using mass spectrometry revealed oxidative modifications including tri-oxidation and crotonaldehyde, to the cysteine residues at positions 180 and 347. Smoking disrupted the function of SPLUNC1 in vitro with a reduction of ~90% in PLUNC1 activity and ~60% in antimicrobial activity.

We conclude that cigarette smoking modifies SPLUNC1 resulting in its loss of ASL height regulation and attenuation of anti-microbial activity. This data expands on the severe impact of cigarette smoking on lung physiology through inactivating SPLUNC1 function. The identification of modified secreted proteins in the airway following smoke exposure may serve as biomarkers that can be used to assess the toxicity of cigarette compounds.

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PCB069

Ageing alters Ca²⁺ spark characteristics in the mouse sinus node

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In the pacemaker of the heart, the sinus node (SN), localised Ca²⁺ release events (Ca²⁺ sparks) occur from the sarcoplasmic reticulum (SR) during diastolic depolarization. The resulting elevation of intracellular Ca²⁺ activates the forward mode of the Na⁺-Ca²⁺ exchanger (NCX), generating an inward current and accelerating pacemaking. This is referred to as the Ca²⁺ clock.1 Sick sinus syndrome (including sinus bradycardia) is primarily a disease of ageing. We have previously shown that mRNA and protein expression of RYR2 (SR Ca²⁺ release channel) is decreased in the sinus node of the aged rat.2 The aim of this study was to determine if the Ca²⁺ sparks in SN myocytes are altered as a consequence of ageing as this may partially explain the sinus bradycardia associated with age.

Single myocytes were isolated by enzymatic digestion of the SN from either 12-14 week or 19–20 month old male C57BL/6J mice (young, n=6 cells from 3 mice; aged, n=4 cells from 3 mice). All procedures were in accordance with the Animals (Scientific Procedures) Act 1986. Changes in intracellular Ca²⁺ concentration, [Ca²⁺], were detected by loading cells with 2.5 µM Fluo-8 AM. Cells were bathed in Tyrode’s solution (containing in mM: 140 NaCl, 5.4 KCl, 1 MgCl₂, 5 HEPES, 5.5 glucose, 1.8 CaCl₂). Confocal Ca²⁺ imaging was performed using an Andor Revolution XD confocal system with a Yokogawa CSU spinning disk. Ca²⁺ sparks were analysed offline from x-y image stacks using xYSpark software.3 Data is expressed as mean ± S.E.M. Statistical analysis was performed using a Mann-Whitney test and statistical significance was accepted when P<0.05.

In aged SN myocytes, mean Ca²⁺ spark frequency (measured as sparks/1000 µm²/s) was reduced to 35% of the frequency in young SN myocytes (29.0±7.7 in aged vs 94.3±21.6 in young SN myocytes, P<0.05). Mean spark amplitude (ΔF/F₀) was 0.06±0.02 in aged and 0.67±0.36 in young SN myocytes (P<0.05), a reduction of ~90%. Average spark spatial width (FWHM) was ~40% greater in aged SN myocytes (4.15±0.21 in aged vs 2.9±0.1 in young SN myocytes; P<0.01). Spark mass was lower in aged SN myocytes (4.11±2.2 in aged vs 14.3±4.1 in young myocytes; P<0.05). Ca²⁺ spark duration was unchanged between aged and young SN myocytes. These preliminary results show that ageing alters Ca²⁺ spark frequency and amplitude in the mouse SN. The altered Ca²⁺ spark characteristics in aged mice could be related to a reduction in RYR2. The changes may at least partially explain the sinus bradycardia observed during ageing and the development of sick sinus syndrome.


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Atrial fibrillation (AF) is the most common cardiac arrhythmia which leads to stroke. It is mediated by the activation of persistent Na channels (NaP) which in turn raises intracellular Ca2+ concentration ([Ca2+]i), a factor known to be associated with AF. Episodes of atrial arrhythmias, exhibit diurnal patterns suggesting a correlation between circadian rhythms and atrial electrophysiology. Circadian rhythms are mediated by cellular circadian clocks, made up of transcriptional-translational feedback loops consisting of several genes, in particular Bmal1. So far the correlation between the activated NaP channels and Bmal1 rhythmicity is unknown.

The aim of this study is to assess the effects of ATXII (Anemone Sulcata toxin II, NaP channels opener) in the absence and presence of ranolazine (NaP channels blocker) on Bmal1 gene, driven by bioluminescence in cultured mouse atrial (HL-1-6) and embryonic fibroblast (MEF) cells. HL-1-6 were grown in Claycomb medium (10% FBS, 0.1 mM norepinephrine and 2 mM L-glutamine), whereas, MEFs were maintained in DMEM with 10% FBS. The cells were transfected with Bmal1::luciferase (BMAL1::LUC) probe to determine bioluminescence rhythmicity through long-term bioluminescence recording over several circadian cycles. Cells were serum shocked for 2 hrs in 50% FBS containing media to synchronise all cellular clocks. Cells were then incubated in control media alone or with 1nM ATXII in the absence/ presence of 10µM ranolazine throughout the duration of the experimental protocol. All data was expressed as mean ± S.E.M. Differences were deemed significant at P<0.05. Two-Way ANOVA was used to test significance between groups followed by Bonferroni post hoc comparison.

Both HL-1-6 cells and MEFs exhibited 24 hrs circadian patterns of Bmal1 driven bioluminescence, under control conditions, with HL-1-6 cells exhibiting a period of 26.32 ± 1.76 hrs (n=8) and the later a period of 25.59±1.47 hrs (n=8). The circadian patterns in bioluminescence were highly disrupted in presence of ATXII in HL-1-6, with a 9 hrs shortening (17.28 ± 1.06 hrs: n=8; P<0.05) in their period, but not in MEFs which remained to show stable periods of 25.89 ± 2.1 hrs (n=8). The effect of ATXII on HL-1-6 cells was reversed in presence of both ATXII and ranolazine (26.54 ± 1.67; n=8; P<0.05 vs ATXII).

This study showed that in cultured atrial myocytes ATXII treatment leads to a significant shortening of Bmal1 driven bioluminescence by almost 9 hours, and such effect is fully reversed by ranolazine. Unlike MEFs where ATXII failed to affect Bmal1. In conclusion, this study suggests a possible correlation between ATXII induced atrial arrhythmias and Bmal1 gene and hence proposing a role for NaP channels in the disruption of atrial circadian rhythm.

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Smooth muscle cells-generated methylglyoxal is responsible for cardiac and myocyte dysfunctions in diabetes mellitus

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Heart failure is a common pathophysiology in individuals with diabetes mellitus (DM). This defect has been attributed in part to disruption of sarcoplasmic reticulum (SR) Ca²⁺ cycling in cardiac myocytes arising from post-translational modification and dysregulation of ryanodine receptor Ca²⁺ release channels (RyR2) and sarco(endo)plasmic reticulum Ca²⁺ ATPase (SERCA2a) by the di-carbonyl species (RCS). The most potent SR-dysregulating RCS is the diffusible methylglyoxal (MG), but its source remains elusive. This study which received ethical clearance from UCLan and the University of Nebraska Medical Center, Omaha, tests the hypothesis that smooth muscle cells in the microvasculature (cSMCs) of the heart are responsible for generating the MG that perturbs SR Ca²⁺ cycling in myocytes. DM was induced in young adult male Sprague-Dawley rats via a single intravenous injection of streptozotocin (STZ) (45 mg/kg in 0.1 ml citrate buffer, n = 36). Control animals received citrate buffer injection only (Con, n = 24). One week after STZ injection, DM animals were divided into three groups. One group was injected with an adenov-associated virus (AAV2/9) to selectively increase expression of the MG-degrading enzyme glyoxalase-I in cSMCs (1.7×10¹² viron particles/kg), another was injected with AAV2/9 to increase expression of the non-selective green fluorescent protein (GFP), and the third group remain untreated. After 7-8 weeks of DM, body mass, heart rate, ejection fraction and fractional shortening were significantly lower than in Con [415 ± 10g vs 275 ± 15g; 367.1 ± 10.1 bpm vs 289.1 ± 8.1 bpm; 79.1 ± 1.9% vs 70.9 ± 1.9% and 49.1 ± 1.7% vs 41.9 ± 1.9%, respectively]. Contraction and relaxation kinetics were also significantly lower [cell length, 115.8 ± 3.0 µm vs 110.7 ± 4.2 µm; contractile velocity, 75.0 ± 6.3 µm/s vs 132 ± 10.2 µm/s; relengthening velocity 60.9 ± 5.8 vs 117.9 ± 9.1, p<0.05], as was mean Ca²⁺ transient amplitude [1.1 ± 0.1 vs 3.0 ± 0.2 FU, p<0.05].

Fig. 1: Expression of several hundred micro-RNAs as determined NGS and qPCR

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Tumour necrosis factor and neuronal nitric oxide synthase in the central control of arterial blood pressure in Sprague-Dawley rats

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Introduction: A growing body of evidence shows that brain TNF has prohypertensive effects on the regulation of arterial blood pressure [1,2]. Neuronal nitric oxide synthase (nNOS) participates in the central control of the arterial blood pressure and baroreflex, exerting antihypertensive effects in hypertensive animals [3]. TNF stimulates expression of hypothalamic nNOS and induces synthesis of nitric oxide [3].

Aim: We checked how TNF infused into the cerebral ventricles affects arterial blood pressure, heart rate and spontaneous baroreflex sensitivity, and whether TNF actions are dependent on nNOS in normotensive rats.

Methods: We instrumented 28 adult male Sprague-Dawley rats with arterial catheter and intracerebroventricular (ICV) canula under anesthesia with intraperitoneal ketamine (100 mg/kg b.w.) and xylazine (10 mg/kg b.w.). We did hemodynamic measurements in freely moving conscious rats during hourly ICV infusion of either: 1) saline (5 microl/hr); 2) TNF (200 ng/5 microl/hr); 3) nNOS inhibitor – 7-nitroindazole sodium salt (7-NI) (20 microg/10 microl/hr); 4) TNF together with 7-NI (200 ng and 20 microg/10 microl/hr, respectively). We analysed mean arterial blood pressure (MABP), heart rate (HR) and spontaneous baroreflex sensitivity (sBRS) evaluated by the sequence method. The data were analysed with repeated measures one-way and two-way ANOVAs.

Results: ICV infusion of TNF resulted in a significant increase in MABP, a transient increase in HR and a decrease in sBRS in comparison to pre-infusion values (one-way ANOVA, p<0.05) and to control and 7-NI infused rats (two-way ANOVA, p<0.05). ICV infusion of 7-NI had no effect on MABP, HR, or sBRS. ICV infusion of TNF with 7-NI significantly increased MABP without changes in HR in comparison to pre-infusion values (one-way ANOVA, p<0.05) and to control and 7-NI infused animals (two-way ANOVA, p<0.05). Coadministration of TNF and 7-NI restored sBRS (two-way ANOVA, p<0.05).
Conclusions: Intracerebroventriculally infused TNF increases MABP and blunts sBRS. The pressor effect of TNF appears to be independent of nNOS activity in the brain. However, our results suggest that neuronal NOS participates in the depressor effect of TNF on sBRS.


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Decreased carotid body KLF2 contributes to cardiac autonomic imbalance and arrhythmia incidence in heart failure

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Cardiac autonomic imbalance is associated with increased arrhythmia incidence and may contribute to mortality in chronic heart failure (CHF). Increased carotid chemoreflex (CC) sensitivity is also associated with higher mortality risk and is thought to play a role in autonomic imbalance. Chronic blood flow reduction plays an important role in enhanced CC sensitivity. We hypothesize that downregulation of the shear-sensitive transcription factor Kruppel-like Factor 2 (KLF2) mediates increased CC sensitivity in CHF and contributes to cardiac autonomic imbalance and increased arrhythmia incidence. Rabbits (male, 3-3.5 kg, n=8-9 per group) were anaesthetised with isoflurane (2%) in oxygen and radiotelemetry transmitters and pacing wires were implanted. Rabbits were paced into CHF over the course of 3-4 weeks (320-380 bpm). Once in CHF animals were anaesthetised as previously described and an adenovirus (KLF2) solution was delivered to the carotid sinus (CHF-KLF2). Ventilation, arterial pressure, and ECG were measured at rest and in response to CC activation with isopropnic hypoxia (IsoH) in pre-pace, CHF, and CHF KLF2 conditions. Heart rate variability (HRV) in the time domain was analyzed for the SD of interpulse intervals (SDNN). Power spectral analysis in the frequency domain was performed using the following frequency cutoffs: 0.0625–0.1875 Hz low-frequency (LF) band, 0.1875–0.5625 Hz high-frequency (HF) band. Cardiac function (CF) was quantified with echocardiography. Values are means ± S.E.M., compared by ANOVA or paired t-test. HRV in the time domain (pre-pace SDNN 15±1 ms, CHF SDNN 10±5 ms) and HF power (pre-pace 39±3 n.u., CHF 27±2 n.u.) were decreased in CHF. LF/HF ratio (pre-pace 0.5±0.06, CHF 0.8±0.1) and arrhythmia incidence (pre-pace 50±10/h, CHF 300±100/h) were increased in CHF (all p<0.05 vs. pre-pace), as were CC responses to IsoH. Transfection of KLF2 to the carotid bodies (CB) in CHF animals resulted in increased HRV (SDNN 15±2, HF 43±4 n.u.) and attenuation of arrhythmia incidence (46±13/h) (all p<0.05 vs. CHF). CC responses to IsoH were attenuated as well. CF was not different between CHF and CHF KLF2 conditions. Protein expression of KLF2 in the CB were decreased in CHF animals (0.5±0.1 sham vs. 0.3±0.1 CHF, n=8), and was restored with adenovalenic transfection (0.6±0.1, n=8). Conversely, KLF2 knockdown with lentiviral KLF2 siRNA resulted in decreased CB KLF2 expression (0.4±0.1, n=8) and decreases in SDNN (12±1 ms) and HF power (30±6 n.u.) and increased LF/HF (1±3). Our findings indicate that increased CC sensitivity, cardiac autonomic imbalance, and arrhythmia incidence during CHF may be caused by down-regulation of KLF2 in the CB.

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Hyperoxia promotes ventilator-induced lung injury through apoptosis signal-regulating kinase-1 (ASK1)

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Patients with acute respiratory distress syndrome require mechanical ventilation and supplemental oxygen, but animal models demonstrate that the combination of these two factors can contribute to lung injury. We previously showed that pre-exposure of mice to hyperoxia before mechanical ventilation exacerbated ventilator-induced lung injury (VILI), and that deletion of apoptosis signal-regulating kinase-1 (ASK1) ameliorated this effect. We hypothesize that ASK1 promotes VILI through activation of apoptotic pathways and inhibition of cell survival pathways. Using mice pre-treated with hyperoxia (95% oxygen for 12 hr) and then ventilated with an injurious tidal volume (25 ml/kg for 4 hr), we found an increase in apoptotic cells that was reduced in ASK1-/- mice and in wild type mice treated with a p38 inhibitor (SB202190). P38, a downstream effector of ASK1, was activated in untreated wild type mice, but was not activated in ASK1-/- mice. Survivin, a member of the inhibitors of apoptosis protein family, was up-regulated in ASK1-/-, but not wild type mice, in response to hyperoxia and VILI. These data suggest that injury caused by the combined effects of hyperoxia and mechanical ventilation are in part mediated by ASK1, which promotes p38-mediated apoptosis and inhibits survival pathways.

This work was funded by NIH grants HL123540, HL094366, and HL081297.

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Leg cuff deflation on International Space Station: a model of “standing up” in space
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Human spaceflight removes the daily gravitational stress placed on the cardiovascular system and greatly reduces the normal levels of physical activity. Both of these factors could lead to cardiovascular deconditioning and increased risk of orthostatic hypotension on return to Earth. The spaceflight experiment “BP Reg” was designed to investigate the primary hypothesis that an inflight challenge to BP regulation by rapid deflation of thigh cuffs could be used as a test of inflight arterial baroreflex response to a sudden reduction in arterial blood pressure. Eight male astronauts provided written informed consent on a project approved by five independent Ethics Review Boards (including NASA MPA 7116301606HR). They then completed three repetitions in the pre-flight (supine position) and in the inflight condition of rapid deflation of two cuffs (Leg-Arm Cuff System) placed on the upper thighs for 3-min to pressures above systolic BP. Finger arterial pressure was recorded throughout the leg cuff tests (inflight by the Continuous Blood Pressure Device) and Modelflow cardiac output (QMF) was recorded. In addition, a rebreathing measurement of cardiac output was conducted to correct the QMF estimates. The change in QMF underestimated the change in cardiac output by rebreathing: no change was seen for QMF while rebreathing increased 47%. With release of the leg cuffs, there were no significant differences in the drops in systolic BP (16.4 ± 3.7 and 16.4 ± 5.6 mmHg, pre-flight to inflight) and in diastolic BP (15.7 ± 3.4 and 14.4 ± 3.2 mmHg), the increases in heart rate (18.6 ± 7.2 and 16.4 ± 3.3 bpm) and QMF corrected (2.4 ± 1.0 and 2.5 ± 0.9 L/min). However, there was a greater drop in calculated total peripheral resistance pre-flight (8.9 ± 2.3 vs. 5.7 ± 0.9 units). The results showed that the leg cuffs were effective in spaceflight in causing a reduction in arterial blood pressure that activated the arterial baroreflex response, and that the heart rate component of the arterial baroreflex was unchanged from pre-flight. However, the responses were biased by the large increase in cardiac output with spaceflight. It is currently unknown where the 47% increase in cardiac output is directed during spaceflight but an hypothesis is that it goes to the splanchnic circulation and acts as a buffer for blood pressure regulation during a challenge such as the rapid change in total peripheral resistance with leg cuff release. The results suggest that the arterial baroreflex is not changed during spaceflight. The blood pressure response to other challenges during spaceflight such as lower body negative pressure should be assessed.

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Biophysical, molecular, and pharmacological characterization of Na channel from induced pluripotent stem cell-derived cardiomyocytes
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Background: The ability to differentiate patient-specific human induced pluripotent stem cells (hiPSC) into cardiac myocytes (hiPSC-CM) offers exciting perspectives for cardiovascular research. A number of studies have used hiPSC-CM to model voltage-gated Na channel (Na) dysfunctions such as long QT and Brugada syndromes. These studies mainly reported current-voltage curves but the expression patterns and precise biophysical and pharmacological properties of Na channels from hiPSC-CM remain unknown.

Objective: We propose to study Na channels characteristics from hiPSC-CM to assess the appropriateness of this novel cell model.

Methods and results: We generated hiPSC-CM using the recently described monolayer-based differentiation protocol. hiPSC-CM expressed cardiac-specific markers, exhibited spontaneous electrical and contractile activities, and expressed several Na channels. Electrophysiological, pharmacological, and molecular characterizations revealed that, in addition to the main Na channel, the neuronal TTX-sensitive Na channel was also significantly expressed in hiPSC-CM. Most of the Na currents were resistant to blocking by tetrodotoxin (TTX) a specific Na channel blocker, and therapeutic concentrations of lidocaine, a class I antiarrhythmic, also blocked Na currents in a use-dependent manner. The Na expression and maturation patterns of hiPSC-CM and native human cardiac tissues appeared to be similar. The four Na regulatory subunits were expressed, with β3 as the preponderant subtype in hiPSC-CM.

Conclusions: Based on our results, we propose that Na channels from hiPSC-CM recapitulate most of characteristics reported for native human cardiomyocytes. hiPSC-CM can also be adapted to model cardiac diseases, perform drug screening, and assess the safety of novel compounds. As with any experimental model, hiPSC-CM have limitations that should be kept in mind in order to benefit from their full potential.

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The effects of carbon monoxide on ionic currents in isolated murine cardiomyocytes
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Endogenous carbon monoxide (CO) is a by-product of heme breakdown. Recently some physiological functions of CO were revealed. However, mechanisms of its action on cardiac func-
tion remain unclear. Our group has shown that CO causes decrease of electrical and mechanical activity in mammalian myocardium (1), however, the ionic mechanisms of this effect was questionable. The aim of the present study was to investigate the effects of CO on ionic currents in isolated murine cardiomyocytes.

Standard microelectrode technique was used to investigate the effects of CO (3×10^{-4}M) on action potential duration (APD) in isolated electrically paced (6 Hz) ventricular preparations. The preparations were dissected from male outbred mice (20 g). Animals were euthanized by cervical dislocation before heart excision. The effects of CO on ionic currents in isolated ventricular cardiomyocytes were investigated using whole-cell patch clamp. Ventricular cardiomyocytes were enzymatically isolated from freshly excised hearts of male outbred mice. CO stock solution (10^{-3}M) was prepared by bubbling Tyrode solution with 99% gaseous CO and used instantly before application to the experimental chamber. CO working solution (3×10^{-4}M) was made by diluting the stock solution in standard Tyrode solution.

CO (3×10^{-4}M) caused significant (p(T)<0.05, n=6 for all groups) decrease of APD in murine ventricular preparation at 50 and 90% repolarization level (51.55±3.94% and 57.75±5.54%, respectively; values are means±S.E.M., compared by Wilcoxon test). Whole-cell patch clamp data indicated that 3×10^{-4}M CO does not activate potassium delayed rectifier currents (I_{K1}). On the contrary, it inhibited steady-state potassium current (10.98±2.24%). Also, CO significantly reduced peak calcium L-type current (I_{CaL}) by 26.78±2.58% of control peak amplitude measured at +10 mV. These results were confirmed by microelectrode experiments: in the presence of I_{CaL} blocker nifedipine (10^{-6}M), CO failed to induce any significant changes in electrical activity of murine ventricular myocardium preparations.

It was previously shown that CO electrophysiological effects in myocardium can be mediated by cGMP-dependent signaling pathway (2). In accordance with this finding, in our whole-cell patch clamp experiments with GTP-free pipette solution CO did not affect I_{CaL}.

Thus, our data suggest that AP shortening in mammalian myocardium caused by CO is mediated mainly by its inhibitory effect on I_{CaL}, which is putatively mediated by cGMP intracellular signaling pathway.


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PCB079

Investigation of the role of irisin on physiopathology in the experimental myoglobinuric acute kidney injury

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Myoglobinuric acute kidney injury (MAKI) is a uremic syndrome that develops after a damage in the skeletal muscle cells and passing into the intracellular components of the cells to the circulation, rhabdomyolysis, with traumatic or non-traumatic reasons. In this study, we aimed to investigate of the role of irisin that produced by the skeletal muscle, on physiopathology of experimental MAKI. In the study, 42 Sprague Dawley male rats which were 170-200 g weight were used. Rats were deprived of water for 24 hours. The control groups (groups 1, 3 and 5, n=6) were received saline solution (8 ml/kg) and the MAKI groups (group 2, 4 and 6, n=8) were received intramuscular 50% glycerol solution (8 ml/kg). The rats in group 1 and 2 after 6 hours, rats in groups 3 and 4 after 24 hours, rats in groups 5 and 6 after 48 hours of intramuscular injection were sacrificed under anaesthesia. The blood samples were taken and kidney tissues of the animals were removed. Urine samples were collected for 24 hours. We examined kidney irisin expression immunohistochemically and plasma/urine irisin concentrations using an ELISA system. Values are means ± SD., compared by Mann-Whitney U test. There was a significant increase in plasma irisin levels between 5th and 6th groups (205.38±38.36 vs 289.05±39.16, p<0.05) and between 4th and 6th groups (230.73±37.14 and 289.05±39.16, p<0.05). There was a significant decrease in the urine irisin levels between 3rd and 4th groups (213.40±17.39 vs 42.93±17.00, p<0.05) and between 5th and 6th groups (161.75±42.07 vs 29.61±11.57, p<0.05). There was not a significant increase in the kidney tissue irisin expression immunohistochemically between 1st and 2nd groups (275.00±12.25 and 255.00±22.68, p>0.05). On the other hand, there was a significant decrease between 3rd and 4th groups (270.00±18.98 and 101.25±20.31, p<0.05). For the renal injury score, there was a significant increase between 1st and 2nd groups (0.17±0.41 vs 1.18±0.35, p<0.05), between 3rd and 4th groups (0.17±0.41 vs 2.63±0.52, p<0.05), between 5th and 6th groups (0.17±0.41 vs 3.75±0.46, p<0.05). There was a significant increase in serum urea, creatinine, potassium levels, alanine transaminase, aspartate transaminase, lactate dehydrogenase, creatine kinase activities between control and MAKI groups (p<0.05). We observed a time-dependent degradation in the kidney function and increase in the histopathological damage. While there was a decrease in the urinary irisin levels and kidney tissue irisin expression, serum irisin levels were increased depending on the time basis. These findings suggest that irisin might be used as a diagnostic marker in the pathogenesis of MAKI.

This study was supported by Trakya University Scientific Research Projects (TUBAP 2014/124).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB080

Effects of Shisha (Water Pipe) smoking on the lung function indices of the youth of Karachi, Pakistan

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Introduction/ Objectives: Shisha smoking (also known as Water pipe, Hubble bubble, Argilhe) is becoming a global phenomenon in the past few years and inhalation of shisha expose our body to hazardous substances like charcoal, nicotine, arsenic, cobalt, chromium and lead etc which may lead to many life-threatening diseases including lung cancer, mouth cancer and urinary bladder Cancer. The aim of this study is to assess
whether there is any difference in the lung function indices of those who smoke shisha as compared to non-shisha smokers. Methodology: 152 shisha smokers and 153 age matched non-shisha smokers (between 18-35 years of age), who were all non-cigarette smokers and had no apparent lung diseases were recruited for this study. All subjects were volunteers and underwent screening with detailed history, anthropometry and spirometric measurements. The study was approved and supported by department of health management, Institute of Business Management, Karachi, Pakistan.

Results: There was a significant reduction in the force vital capacity (FVC) [mean difference (95% CI) 0.54L (0.45, 0.64) P < 0.001], force expiratory volume in first second (FEV1) [mean difference (95% CI) 0.52L (0.43, 0.61) P < 0.001], and peak expiratory flow (PEF) [mean difference (95% CI) 78.6L/min (65.2, 92.04) P = 0.001] in the shisha smokers as compared to the control individuals. The study also indicated that most of the shisha smokers these days are educated young adults who smoke shisha for pleasure (79.5%) or for a social status or for friends. Most of the subjects heard about the shisha smoking either from their friends gathering (85.4%) or from their educational institute (13.2%) indicating that shisha smoking is getting common among educational institutes as well. It has been found that most of the shisha smokers smoked it occasionally (54.3%), and some did it either monthly (14.6%) or weekly (13.9%).

Conclusion: It can be concluded from this study that shisha smokers had statistically significant lower FVC, FEV1 and PEF as compared to the non-shisha smokers. Therefore, it is suggested that government and regulatory bodies should take necessary steps to implement the law regarding selling and use of shisha smoking in close cafes and in public places as this would help in reducing the morbidity and mortality associated with respiratory complications secondary to shisha smoking in younger age group.

Lung function indices of shisha smokers and non-smokers

<table>
<thead>
<tr>
<th>Indices</th>
<th>Shisha Smokers (Mean±SD)</th>
<th>Non Shisha Smokers (Mean±SD)</th>
<th>Mean Differences</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC (L)</td>
<td>2.80±0.43</td>
<td>2.34±0.41</td>
<td>0.54</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FEV1 (L)</td>
<td>2.00±0.30</td>
<td>2.00±0.30</td>
<td>0.52</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PEF (L/min)</td>
<td>51.7±12.8</td>
<td>54.10±13.2</td>
<td>0.50</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>


Ziad M El-Zaatari, Health effects associated with waterpipe smoking, Tob Control doi:10.1136/tobaccocontrol-2014-051908.


Institute of Business Management and MBQMDC for their support

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PCB081

Purinergic P2X3 receptor signalling in the carotid body accounts for its aberrant discharge in the spontaneously hypertensive rat

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Carotid body chemoreceptor afferent firing is normally of low intensity at sea level but exhibits aberrant discharge in conditions of hypertension. This pathological signalling contributes to the sustained levels of sympathetic activity and hypertension (McBryde et al., 2013). The mechanism(s) for the elevated afferent activity remains unknown but its elucidation might reveal a potential new target for the treatment of hypertension. Given the presence of P2X3 receptors within both glomus cells of the carotid body and petrosal neurones of Wistar rats (Prasad et al., 2001), as well as their role in sensitisation of other respiratory afferent modalities (Smith et al., 2015), we hypothesised that this receptor was upregulated and became tonically stimulated in the carotid body of spontaneously hypertensive (SH) rats.

Patch clamp recordings from physiologically characterised chemoreceptive and baroreceptive petrosal neurons were made in an in situ preparation. Single cell RT-PCR was performed on cytosol extracted from petrosal neurons.

We found a selective upregulation of P2X3 receptor mRNA (~4.5 fold) in chemoreceptive petrosal neurons in SH versus normotensive (Wistar) rats (P<0.01). In contrast, there was no change in P2X3 receptor expression in baroreceptive petrosal neurones between rat strains. Current clamp recordings from petrosal chemoreceptive neurones revealed ongoing firing in the SH rat (1.5 ± 0.8 Hz) never seen in the Wistar rat. SH vs Wistar rat neurones were more depolarised (-51.5 ± 0.9 mV vs. -55.7 ± 1 mV; P<0.001) and showed profound reflex sensitisation following stimulation of the carotid body with sodium cyanide (22mcg i.a.; 123 vs. 52 evoked spikes, P<0.001). Local instillation of a highly selective and potent P2X3 receptor antagonist (AF-353, 20µM) into the carotid body of SH rats abolished spontaneous afferent firing and hyperpolarised the membrane potential to levels not significantly different to those in Wistar rats. Additionally, chemoreflex evoked action potential barrages were not different to those in Wistar rats. We conclude that carotid body aberrant signalling in hypertension is caused in part by an upregulation and stimulation of P2X3 receptors. Furthermore, antagonising P2X3 receptors within the carotid body has the distinct advantage of preserving normal reflex function while nulling aberrant spontaneous discharge and the hyperreflexia. We propose that P2X3 receptors are a potential novel therapeutic target for controlling carotid body activity in diseases in which aberrant carotid body behaviour is prevalent.


Restoration of pharyngeal dilator muscle force in dystrophin-deficient (mdx) mice following co-treatment with neutralizing IL-6R antibody and Urocortin-2

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Duchenne muscular dystrophy (DMD) is a genetic disease caused by a mutation in the dystrophin gene, leading to the absence of the structural protein - dystrophin. Patients have severe muscle weakness and die prematurely due to respiratory and cardiac failure. The mdx mouse, a model of DMD, shows evidence of reduced nornoxic ventilation and impaired respiratory muscle function at 8 weeks of age. 6 week old mdx (C57BL/10ScN-Dmdmdx/J; n=24) and wild-type (WT; C57BL/10ScN; n=23) mice received either saline (0.9% w/v) or a co-treatment of neutralizing IL-6 receptor antibodies (xIL-6R; 0.2 mg/kg) and corticotrophin releasing factor (0.9% w/v) or a co-treatment of neutralizing IL-6R antibody and Urocortin-2 receptor agonist (Urocortin-2; 30 µg/kg). Animals received 2 subcutaneous injections over 2 weeks. Following this, sternohyoid muscle (pharyngeal dilator) contractile function was examined ex vivo. Muscle fibre number and inflammatory cell infiltration were examined by Haematoxylin and Eosin staining. Fibre type analysis of cross-sectional area (CSA) and areal density was determined by myosin heavy chain (MHC) staining. Fibre type transitions were ameliorated by co-treatment. Improved mechanical force and power in dystrophic sternohyoid muscle. The percentage of centrally-nucleated muscle fibres was significantly increased in mdx (25±1%) compared with WT (0.5±0.1%). The areal density of inflammatory cell infiltrates was significantly increased (5±1% vs. 0.9±0.1% mdx vs. WT). Both indices were unaffected by drug co-treatment. Fibre type transitions were apparent in the mdx sternohyoid muscle. The areal density of MHCIIx fibres was significantly increased (31±2% vs. 20±2%; mdx vs. WT), whereas MHCIIb fibres was significantly decreased compared with WT (52±2% vs. 62±2%; mdx vs. WT). Fibre transitions were ameliorated by co-treatment. Improved force in mdx co-treated sternohyoid was not related to fibre hypertrophy. Co-treatment had a positive inotropic effect, restoring mechanical force and power in dystrophic sternohyoid muscle. Co-treatment reversed fibre transitions in mdx but did not affect the infiltration of inflammatory cells or the proportion of fibres with evidence of central nucleation. Preservation of MHCIIb fibres may underpin, at least in part, recovery of force production in the mdx co-treated mice. These data may have implications for the development of pharmacotherapies for DMD with relevance to respiratory muscle performance.

Supported by Muscular Dystrophy Ireland and the Depts. of Physiology, UCC and TCD. The IL-6R antibodies were gifted by Chugai Pharmaceuticals, Japan.

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PCB082

Endothelial cell activation in vascular disease mediated by hydrogen peroxide in vitro

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The development of cardiovascular disease (CVD) is the main cause of death among chronic kidney disease (CKD) patients (1). Endothelial injury and dysfunction are critical steps in atherosclerosis, a major CVD (2). Increased production of reactive oxygen species (ROS) has been associated with the pathogenesis of cardiovascular diseases such as atherosclerosis, hypertension and heart failure (3). However, hydrogen peroxide ($H_2O_2$) modulates endothelial cell function by intricate mechanisms. Ambient production of O2.- and subsequently $H_2O_2$ at low levels, maintained via basal activity of pre-assembled endothelial NAD (P) H oxidases (4). Endothelial cells play an important regulatory role in the circulation as a physical barrier and as a source of a variety of regulatory substances. Dysfunction of the vascular endothelium is thus leading to atherosclerosis which is characterised by overexpression of adhesion molecule expression, comprising vascular cell adhesion molecule 1 (VCAM1). This adhesion molecule has been found to be up-regulation in human atherosclerotic lesions.

The aim of this study is to evaluate the effect of $H_2O_2$ on the endothelial cells adhesion molecules expression. Primary cultures of Human Umbilical Vascular Endothelial Cells (HUVECs) will be maintained in endothelial growth medium supplemented with penicillin-streptomycin and supplement mix of fetal calf serum in a 37°C humidified incubator in an atmosphere of 5% v/v CO2. HUVECs will be treated with in the presence and absence of 50 µM of $H_2O_2$ for 2, 6, 12 and 24 h. Intracellular superoxide anion production in HUVECs will be detected by using p-Nitro Blue Tetrozolium (NBT) assay to demonstrate whether $H_2O_2$ induce the generation of superoxide anions intracellularly in HUVECs. The formation of blue formazan will be measured spectrophotometrically at 570 nm. Total RNA will be extracted from non-treated and treated cells and RNA quantity and quality will be checked by OD260/280 measurements. VCAM-1 mRNA expression will be assessed using RT-PCR. Our results show that $H_2O_2$ could potentially significantly induce EC activation through increased mRNA expression of ICAM-1 adhesion molecules in cultured HUVECs.

Treatment with N-acetyl cysteine (NAC) (bulk/nano form) could significantly attenuate the effect of $H_2O_2$, administration on adhesion molecule protein expression. This strongly suggests the role of ROS in the endothelial cell damage sustained. Future work is to find reliable methods to test endothelial function. Non-invasive studies such as brachial ultrasound
testing are also needed to determine its predictive value as a potential predictor for cardiovascular disease.


Cai H. Circulation research, 2005, 96(8):818-822


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increasing the number and activity of EPCs in stented CAD patients. A follow-up at 6 and 9 months is scheduled for assessing the risk of restenosis, if further experiments will provide relevant data.

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PCB086

A statistically averaged model of the lungs to predict physiology from imaging

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Background: The human lung is complex system, comprising branching networks of airways and blood vessels embedded in a highly deformable tissue. The contributors to normal pulmonary function and disease progression are difficult to tease apart, and vary between individuals. Personalised computational models of lung structure and function have contributed to improved understanding of the contributors to pulmonary disease and are becoming useful tools to stratify patient severity [1]. However, personalised models are time-consuming to develop and so predicting how population variability contributes to pathological response is not possible, except in very small samples.

Methods: Here we propose a statistical model of the lung which can be used to predict function in both the population average, and for lungs that represent the normal range of variability from this mean. This model is based on a novel framework called SF-eal (Statistical Finite element analysis of Lung) and an Active Shape Model (ASM) concept. The model is parameterised using a training set of 15 healthy non-smoking subjects aged 20-30 years old from a standardised Human Lung Atlas database.

Results: The model is able to capture 90% of inter-subject variability in 7 principal components, which represent 3D shape changes. When lung volume is accounted for, the largest variability in lung shape is diaphragmatic, and there are not significant differences in male and female average lung shape (a maximum of ~30 mm surface distance from the average lung). To demonstrate a use of the SF-eal model to predict lung function we simulate tissue deformation under gravity and elastic recoil pressure in individuals, and in the statistically averaged lung. On an individual basis, model predictions of total lung density lie within 3% of CT measured values, and gradients in tissue density in the gravitational direction are 0.010-0.023 g/cm³ compared with measured values of 0.009-0.014 g/cm³. With the statistically averaged model we are able to predict the normal variability in distribution of lung tissue and elastic recoil pressures under a gravitational load.

Implications: Prediction of a normal range of lung tissue deformation provides the first key steps to assessment of whether an individual’s lung tissue, which can be observed in computed tomography (CT) imaging, lies within a normal range. This improves upon current methodologies which do not capture individual variability. Local elastic recoil influences the patency of pulmonary airways and blood vessels and so the gas exchange ability of the lung so is critical to gas exchange function. Long term we aim to provide statistically averaged representations of pulmonary airways and blood vessels, so predictions of population response to pulmonary disease can be conducted in conditions representative of normal (or pathological) cohorts quickly and accurately.


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PCB087

Transcriptomic and histological analysis of right ventricular myocardium in patients with tetralogy of Fallot repair undergoing pulmonary valve replacement

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Tetralogy of Fallot (TOF) is a congenital heart defect which presents soon after birth and is the most common cause of ‘blue baby syndrome’. It is characterised by subpulmonary stenosis, overriding aorta, a ventricular septal defect and right ventricle (RV) hypertrophy. Early TOF repair now provides affected infants with near-certain post-operative survival and relatively normal development into early adulthood. However, TOF patients approaching adulthood develop pulmonary valve regurgitation, leading to RV volume overload and subsequent complications such as RV dilatation and possible RV failure. For treatment, pulmonary valve replacement (PVR) is required. With infants undergoing TOF repair, gene expression arrays have shown a failure of cardiac development pathways, suppressed expression of genes involved in cardiac contraction, and upregulation of apoptotic regulators [1, 2]. To date there has been no study of the gene expression profile of TOF adults undergoing PVR. Our aim was to utilise a transcriptomic approach to compare RV tissue samples from TOF patients undergoing PVR (n=7) compared with healthy RV from unused donor hearts (n=6), with the goal of understanding potential mechanisms underpinning pathologic changes in the dilated RV myocardium of TOF adults at the time of PVR. All patients gave informed consent for inclusion to the study prior to undergoing PVR and parental consent was gained in the cases where the subject was under 18 years of age. Tissue was obtained during surgery of PVR patients or from the explanted donor hearts and was flash-frozen. mRNA was extracted from the frozen samples and used to perform affymetrix genechip arrays (HTA 2.0 array). Principle component analysis was performed with p-value threshold of <0.004 and false discovery rate (q) of 0.47, which showed segregation of TOF from RV donor clusters, suggesting distinct gene expression profiles. Differential expression of genes revealed ECM remodelling, angiogenesis, cell cycle regulation, apoptosis and development as dominant functional themes. Upregulation of the genes for ECM modifiers lumican (2.16 fold change, p<0.001) and osteoglycin (1.99 fold change, p<0.004), both members of the class II small leucine-rich proteoglycans (SLRPs), suggested a strong ECM component to RV myocardial dysfunction. To investigate the role of the ECM further, Collagen I and III mRNA levels were measured by qPCR and showed
no difference between cohorts. Additionally, histological assessment showed no obvious myocardial disorganisation, whilst image quantitation of picrosirius red staining showed no difference in the collagen deposition of PVR myocardium compared to RV donor. We conclude that gene expression changes in patients undergoing PVR indicate a program of tissue repair characterised by subtle ECM modifications. We speculate that qualitative rather than quantitative changes to ECM composition contribute to pathophysiology of RV myocardium in TOF patients undergoing PVR.


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biology (phosphorylated protein 38/total protein 38; pp38/p38 ratio) WB; histology (muscle layer in small pulmonary arteries) and immunohistochemistry (Ki67) studies. All procedures were approved by the Local Bioethical Committee (CBA4063 FMUCH). Cinaciguat did not change the mPAP, but did change the PVR in the last day of treatment (p<0.05). Cinaciguat induced a decrease response in mPAP during the episode of acute hypoxia (p<0.05) relative to controls lambs. The treatment reduced the RV/LV + Septum ratio (p<0.05), decreased the muscle layer in small pulmonary arteries (p<0.05), showing diminished cardiopulmonary remodelling than control lambs. However, the lung ratio of pp38/p38 and positive Ki67 (proliferative markers) were similar between groups. We conclude that cinaciguat was able to decrease pulmonary artery responsiveness in the last day of treatment and in acute hypoxia in pulmonary hypertensive neonatal lambs. This was associated with a marked decrease in pathologic cardiopulmonary remodelling, presumable due to an increase in apoptosis and/or autophagy in the pulmonary arteries. FONDECYT 1140647, 1120605, 1130424 & 1151119. Herrera EA, Reyes RV, Giussani DA, Riquelme RA, Sanhueza EM, Ebensperger G, Casanello P, Méndez N, Ebensperger R, Sepúlveda-Kattan E, Pulgar VM, Gabello G, Blanco CE, Hanson MA, Parer JT, Llanos AJ, Carbon monoxide and pulmonary vasodilatation in neonatal llamas (Lama glama) at the Andean altiplano. Cardiovasc Res. 77(1):197-201, 2008.

Supported by FONDECYT 1140647, 1120605, 1130424 & 1151119.

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pulmonary hypertension persists despite oxygen therapy, were treated with a daily single dose of 2-APB (10 mg·kg\(^{-1}\)·i.v.) or its vehicle (DMSO:saline 1:10) during 10 days. At the end of the treatment, lung samples were collected for \textit{ex vivo} and \textit{in vitro} experiments. We determined the responses of small pulmonary arteries to the vasoconstrictors endothelin-1 (ET-1) and a thromboxane A2 mimetic (U46619), and the vasodilators PKG activator (8Br-cGMP) and fasudil (Rho-kinase inhibitor), by wire myography. Further, we determined the pulmonary expression of TP receptor, PKG1, ROCK 1, 2 and PKG1 by immunoblot, the expression of calcium-regulated mitogens ppET1, PDGF and VEGF-A by RT-PCR, and the proliferation marker Ki67 by immunohistochemistry. All procedures were approved by the Bioethical Committee of the Faculty of Medicine, University of Chile.

2-APB treatment produced: a) a reduction of the maximal constriction to ET-1 and U46619 without modification of TP receptor expression; b) an increase of the maximal relaxation to 8Br-cGMP, and the maximal relaxation and sensitivity to fasudil, without effect on PKG-1 expression but with an increase in both ROCK1 and ROCK2 proteins; c) a decrease of VEGF-A without modification of PDGF and ppET1 transcripts; and d) a decrease of Ki67 positive nuclei in the medial layer of pulmonary arteries.

These results indicate that the reduction of mPAP and remodeling after 2-APB treatment is the result of a new balance between vasodilator and vasoconstritor function and the reduction of mitogen expression and proliferation of PASMC, despite an increase of ROCK function, suggesting a potential role of SOC inhibitors for neonatal pulmonary hypertension treatment.


Supported by FONDECYT 1120605, 1140647, 1130424 & 1151119.

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PCB092

Opiate exposure during early postnatal life has long term effects on breathing patterns

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The mammalian respiratory system is immature at birth. In mice, this immaturity is characterized by a fragile and highly variable breathing pattern interspersed with apneas and sigh-like breaths during postnatal days 1-3 (P1-3). Around P3-P4, the respiratory system undergoes a step in maturity, after which breathing pattern is less variable and frequency increases. The neural mechanisms underlying this maturity step are unclear. Evidence from \textit{in vitro} studies suggests that two distinct medullary neuronal clusters, the opiate-sensitive preBötzinger Complex (preBötC) and the opiate-insensitive Retrotrapezoid nucleus/parafacial respiratory group (RTN/pFRG)\(^1,2,3\), play a critical role in generating respiratory rhythm but little is known of their interaction during development and early post natal life when the respiratory system is fragile. To pharmacologically tease apart the function of these neuronal clusters during early postnatal maturation of breathing and to investigate the long term effects of opiates on breathing, neonatal mice were exposed to the \(\mu\)-opioid receptor agonist fentanyl (0.08mg/kg·i.p. daily.), or saline as a control, from P1-5 (n=16) or P9-13 (n=16). All procedures were carried out in accordance with current UK legislation. Mice were continuously monitored post-injection and breathing recorded by closed plethysmography at regular intervals from 5 minutes to 2 hours post-injection. Fentanyl had a modest effect on breathing at all postnatal days by increasing variability, decreasing frequency (250±20 vs 150±30 breaths per minute) and increasing the number of apnoeas compared to saline-exposed mice (2±1 vs 5±2 per minute respectively). At 6 weeks of age, all saline and fentanyl exposed mice were exposed to a further dose of fentanyl (0.04 – 1.0mg/kg·i.p.) and monitored as above. Respiratory frequency was significantly decreased (190±10 vs 120±15 breaths per minute, p<0.05 two-way anova) in all mice previously exposed to saline as neonates (P1-5 and P9-13); however, in mice previously exposed to fentanyl as neonates (P1-5 and P9-13) fentanyl exposure in adulthood had no effect on respiratory frequency (180±8 vs 150±10 bpm, p>0.05 two way anova). Tidal volume increased slightly in all mice post fentanyl regardless of whether they had previous exposure of fentanyl or saline. These data suggest that the respiratory system in younger animals is less susceptible to fentanyl compared to adults, and that pre-exposure to fentanyl during early postnatal maturation results in a long term desensitization to further fentanyl insults.


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PCB093

Pulmonary artery reconstruction using cord blood-derived multipotent stem cells \textit{in vitro} and \textit{in vivo} study

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Lack of growing and remodelling potential of available grafts is the bottleneck of current congenital heart defect (CHD) treatment in paediatric surgery. We tested the possibility of solving this problem by engineering a clinically available decellularised graft with multipotent stem cells (SCs) derived from human and swine perinatal tissues, and the feasibility of transplanting swine SC-engineered grafts in a large animal model. Human umbilical cord blood stem cells (HUCB-MSCs) and newborn swine peripheral blood stem cells (SPB-SCs) were isolated, expanded, characterised and tested for their capacity to differentiate into smooth muscle cells (SMCs) \textit{in vitro}.
Cells were then seeded onto the decellularised porcine small intestinal sub-mucosa (Cormatrix) at the density of 0.5x10^6 cells/cm² and incubated in a bioreactor for 10-14 days. Non cell seeded or swine cell-seeded scaffolds were shaped into a conduit with seeded-cells located at the outer side of the tube, and implanted into the left pulmonary artery of 12-15 kg piglets. Six months after surgery, echocardiography was carried out and grafts were harvested and analysed by histology, scanning electron microscopy, and immunohistochemistry. Both HUCB-SCs and SPB-SCs displayed mesenchymal stem cell-like phenotype and were capable of differentiating into adipocytes, osteocytes, chondrocyte and SMCs under inductive conditions. Graft-implanted piglets recovered well and grew at normal rate. All grafts appeared patent at echocardiography 6 months after implantation. However, while the acellular-graft implanted animals showed light stenosis and higher blood flow velocity through the pulmonary artery, no abnormal feature was observed in the cellularised-graft implanted group. At histology, the explanted cellularised-grafts revealed luminal endothelialisation and a multi-layer of smooth muscle-like cells within the vessel wall. The acellular graft exhibited a relative patchy luminal cell layer and a thinner SMC layer. Results indicate that umbilical cord blood is a valuable stem cell source for tissue engineering of grafts to correct cardiac defects, especially in the paediatric surgery.

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**Poster Communications**

**PCB094**

**SR K⁺ channels from TRIC-A knockout mice in a cooperative manner**

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Sarcoplasmic reticulum (SR) K⁺ channels are voltage-sensitive, monovalent cation channels present in high levels throughout the functional and longitudinal SR of cardiac and skeletal muscle. They are suggested to equilibrate K⁺ concentrations and balance charge movements across the SR membrane during Ca²⁺-release and reuptake (1, 2). The novel proteins, TRIC-A and TRIC-B, are thought to represent two subtypes of SR K⁺ channel (3, 4). To study the gating of a homogeneous population of SR K⁺ channels, we incorporated SR membrane vesicles from TRIC-A KO mice into artificial membranes under voltage-clamp conditions in symmetrical 210 mM K⁺ PIPES solutions as previously described (4, 5). SR vesicles incorporated in a fixed orientation and the cytosolic side of the channels was voltage-clamped at potentials relative to the luminal side, which was held at ground. When multiple channels were observed, we added decamethonium to the cytosolic chamber at the end of the experiment to enable us to count the channels in the bilayer and determine the zero current level. Mean values ± SEM were compared by Student’s t test. When only one SR K⁺ channel was gating in the bilayer, the gating was obviously voltage-dependent; Po was 0.099±0.014 (n=35) at -30 mV and 0.007±0.004 (n=19) at -30 mV (p<0.001). At +30 mV, the average SR K⁺ channel Po increased to 0.251±0.046 (n=12) when there were two channels, 0.493±0.067 (n=6) when there were three channels, and 0.591±0.06 (n=3) when there were four channels gating in the bilayer (p<0.001). A similar trend was observed at -30 mV, thus indicating that the channels are behaving in a positively cooperative manner at both holding potentials. The experimental data deviated from the binomial statistical prediction that would be expected if identical, independent SR K⁺ channels were gating simultaneously, further supporting the idea of cooperative gating. We also investigated whether the presence of ryanodine receptor (RyR) channels gating in the bilayer would influence the activity of the SR K⁺ channels. Most SR vesicle fusions led to incorporation of only SR K⁺ channels into the bilayers, however, when an RyR channel incorporated also, this did not appear to influence the gating of the SR K⁺ channels. With an RyR also present in the bilayer, SR K⁺ channel Po was 0.130±0.041 at +30 mV (n=10) and 0.030±0.024 at -30 mV (n=5), similar to SR K⁺ channel Po without an accompanying RyR.

The cooperative gating feature of SR K⁺ channels that we observe may be central to their ability to rapidly respond to signals such as small voltage changes across the SR, thus enabling large SR K⁺ fluxes to rapidly compensate K⁺ gradients or charge differences across the SR. Future work is necessary to identify the mechanisms underlying the cooperativity and to determine whether any SR K⁺ channel modulators could directly affect this behaviour.


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**PCB095**

**Angiotensin II controls theautomaticity of embryonic stem cell-derived cardiomyocytes by autocrine signaling**

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Early differentiating cardiomyocytes, distinct from adult cardiomyocytes, generate spontaneous calcium transients and action potentials. This automaticity resembles what is observed in sinoatrial nodal cells. The underlying mechanism of automaticity is not fully understood. In this study, early differentiating mouse embryonic stem cell-derived cardiomyocytes (mESC-CMs) are used to study automaticity. The objectives of this study were: 1) to investigate if mESC-CMs express the components of the renin-angiotensin system (RAS); 2) to investigate if blocking the angiotensin II (Ang II) receptor would affect the spontaneous calcium transients and
action potentials of mESC-CMs. By immunocytochemistry, our results showed that mESC-CMs express several components of the RAS, including the angiotensin converting enzyme, chymase, Ang II type 1 receptors (AT1R) and Ang II type 2 receptors (AT2R). Interestingly, by calcium imaging and current patch clamp, we found that addition of AT1R blocker losartan alone decreased both the calcium transients and action potentials of mESC-CMs. Our results suggest that mESC-CMs may synthesize Ang II intracellularly; the Ang II in turn controls the automaticity positively in an autocrine manner. Further study would be required to dissect out the role of both AT1R and AT2R in controlling the automaticity of mESC-CMs. This study will yield important information on the mechanism underlying the automaticity of cardiomycocytes. 

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PCB096

Bicarbonate permeability of CFTR, ANO1 and glycine receptor is associated with pore dilation

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Anions are essential for keeping cells alive and mediating diverse functions. Chloride (Cl⁻) and bicarbonate (HCO₃⁻) are two major anions. However we have little information how the anion channel regulates anion permeability. Diverse cellular stimuli can dynamically modulate the bicarbonate permeability (Pbic/Cl⁻) of anion channels. Activation of WNK1/SPAK kinases can increase the bicarbonate permeability of CFTR, and Ca²⁺-calmodulin can increase that of anoctamin-1 (ANO1). To figure out the mechanism of anions selection by anion channels, we measured halide ion permeability using patch clamp experiments. And we also measured the permeability of polyatomic anions in same manners. WNK1/SPAK activation altered the permeability of halide ions of CFTR, and increased dielectric constant (ε) and pore size. ANO1 is highly permeable to HCO₃⁻ at a high intracellular Ca²⁺ concentrations ([Ca²⁺]i > 1μM). The dynamic increase in Pbic/Cl⁻ of ANO1 was also associated with increases in ε and the diameter of the channel pore. The glycine receptor (GlyR) Cl⁻ channel mediates synaptic inhibition in nervous system. Deletion of proline at the -2 position (P-2') makes pore size larger. Pore size of wild type (WT) GlyR was 5.3 Å, whereas that of P-2'Δ mutation was 7.1 Å. Also the bicarbonate permeability and dielectric constant of P-2'Δ increased than WT. Here, we provide evidence that pore dilatation increases the bicarbonate permeability of anion channels by reducing energy barriers of size-exclusion and ion dehydration of HCO₃⁻ permeation. Significantly, changing the pore size by cellular stimuli dynamically modulates the anion selectivity of CFTR and ANO1. Additionally, the anion selectivity of GlyR was altered by pore dilation in a mutation in the pore-lining region. The dynamic regulation in Pbic/Cl⁻ by pore size change may have many physiological and pathophysiological implications ranging from epithelial HCO₃⁻ secretion to neuronal excitation.

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PCB097

Sex-related differences in the effects of nicotine on some indices of renal function of Wistar rats

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Nicotine (NC), the major alkaloid found in cigarettes and tobacco, is also found in some plants which are consumed daily by humans. The adverse and beneficial health effects of NC inhalation or ingestion are still controversial. Most studies on NC have focused on its effects on cardiovascular and nervous functions. However, literature is scanty on its dose- and sex-specific effects on renal function, the gap this study aims at bridging. A study of these effects in animals can point towards its sex-specific benefits or hazards in humans. Forty Wistar rats [20 males (M) and 20 females (F)], 120–150g, were housed in separate metabolic cages. Each sex was divided into 4 groups (n=5) thus: 1 [control (CN)] received 0.2mL/100g/day distilled water i.p. for 28 days, while 2, 3 and 4 received NC at 1, 2 and 4mg/kg/day i.p respectively for the same period. Twenty four hour urine samples were collected thereafter. The rats were sacrificed under ketamine anesthesia (10mg/kg i.m.). Their plasma and urine were assayed for creatinine (Jaffe, 1886), urea and uric acid (Randox kits), Na⁺ and K⁺ (Flame Photometry) and HCO₃⁻ (titrimetry). Creatinine clearance (CCR) was calculated using standard formula. Their kidney sections were stained with H&E for histopathological examination. Values are Mean±S.E.M., compared by ANOVA and t-test. P<0.05 was considered significant. Urine Na⁺ (mMol/L) was significantly (SG) higher in M groups (GP) 2 and 3 [126.40±4.60 and 174.40±3.67 respectively (RS)] and F GP 3 (72.80±2.08) when compared with their respective CN (M=100.60±4.43; F=70.40±2.36). Urine Na⁺ in F GP 1, 2, 3 and 4 (70.40±2.36, 72.80±2.08, 92.80±5.16 and 76.60±6.17 RS) was SG lower than their M counterparts (CP) (100.60±4.43, 126.40±4.60, 174.40±3.67 and 180.20±7.52 RS). Plasma K⁺ (mMol/L) of GP 2, 3 and 4 (M=3.54±0.10, 3.72±0.05 and 3.32±0.08; F=3.26±0.07, 3.16±0.06 and 3.22±0.10 RS) was SG lower than their CN (4.20±0.06 and 3.68±0.12 RS). Plasma K⁺ in F groups 1, 2 and 3 (3.68±0.12, 3.26±0.07 and 3.16±0.06 RS) was SG lower than their M CP (4.20±0.06, 3.54±0.10 and 3.72±0.05 RS). CCR [×10⁻³mL/min] was SG higher in GP 2 and 3 (M=7.38±0.53 and 7.65±0.40; F=9.45±0.49 and 14.29±1.82 RS) than their CN (4.66±0.66 and 6.28±0.37 RS). CCR in F GP 2, 3 and 4 (9.45±0.49, 14.29±0.62 and 4.61±0.34 RS) was SG higher than their M CP (7.38±0.53, 7.66±0.40 and 3.41±0.38 RS). Photomicrographs revealed no appreciable distortion in the kidney histoarchitecture of all the rats. It was concluded that sub-chronic administration of low doses of NC alter the plasma and urine electrolyte balance and increase CCR of Wistar rats presumably by increasing their renal blood flow, an effect that was more pronounced in the females than the males.


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Loss of luminal carbonic anhydrase IVX results in decreased biliary bicarbonate output and a late-stage sclerosing cholangiopathy in mice

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Carbonic anhydrase XIV (Car14) is highly expressed in the hepatocyte canalicular membrane, supposedly with its active site in the extracellular (luminal) milieu. To determine its function in biliary fluid and acid/base output, the common bile duct of anesthetized Car14-knockout (KO) and wild type (WT) mice aged 11, 20 and 52 weeks was cannulated, the bile duct of Car14 KO and WT livers of mice aged 3, 6, 11, 20 and 52 weeks. Biliary basal, and more so TCA-stimulated HCO3− output was significantly reduced in Car14 KO mice of all age groups, whereas bile flow and hepatic and ductular morphology were normal at young age. Interestingly, Car14 KO mice developed fibrotic and proliferative changes in the small bile ducts at advanced age, which was accompanied by a reduction in bile flow, and an upregulation of hepatic cytokeratin 19 mRNA and protein. The results suggest that the membrane-bound Car14 is essential to biliary HCO3− output, mixed micelle formation and bile acid neutralization, and that its loss results in gradual development of small bile duct disease and hepatic fibrosis. At young age, bile flow is not compromised, suggesting that Car14-knockout mice may be an optimal model to study the protective role of biliary canalicular HCO3− against luminal noxious substances to the cholangiocyte.

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The gasotransmitter hydrogen sulfide contributes to hypoxic inhibition of airway transepithelial sodium absorption

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In lung epithelial cells, hypoxia decreases the expression and activity of sodium transporting molecules, thereby reducing the rate of transepithelial sodium absorption. The mechanisms underlying the sensing of hypoxia and subsequent coupling to sodium transporting molecules remain unclear. Hydrogen sulfide (H2S) has recently been recognized as a cellular signalling molecule which is enzymatically produced by cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS) and 3-mercaptopyruvate sulfurtransferase (3MST). We have previously shown that H2S-liberating sulphur salts reduce pulmonary transepithelial sodium absorption by inhibition of Na+/K⁺-ATPase activity (1). Furthermore, airway epithelial cells endogenously produce H2S and H2S production is inversely correlated with O2 concentrations (2). Therefore it was questioned whether endogenously produced H2S contributes to hypoxic inhibition of pulmonary transepithelial sodium absorption.

Sodium absorption was measured electrophysiologically in freshly dissected porcine tracheae (obtained from a local slaughterhouse) or cultured human H441 airway epithelial monolayers (as described in (2)) in gas-lift perfusion Ussing chambers. Values are means ± SEM. Hypoxia was established by decreasing O2 concentrations of the Ussing chamber circulation gas, which resulted in an O2 concentration of 2.01 ± 0.04 mg/L (n=3) in the chambers. This hypoxia reversibly decreased normalized amiloride (10 μM)-sensitive sodium current signals from 0.94 ± 0.02 (n=8) to 0.34 ± 0.03 (n=9; p≤0.001; unpaired t-test) in porcine tracheae and from 0.90 ± 0.02 (n=14) to 0.60 ± 0.03 (n=9; p≤0.001; unpaired t-test) in H441 monolayers within 30-45 min. The decrease in sodium absorption was due to inhibition of the basolaterally located Na⁺/K⁺-ATPase. Normalized Na⁺/K⁺-ATPase currents of epithelia which were apically permeabilized with nystatin (75 μM) decreased from 1.49 ± 0.35 (n=7) to 0.64 ± 0.05 (n=8; p≤0.001; Mann-Whitney U-test) in pig tracheae and from 1.41 ± 0.10 to 0.97 ± 0.16 (n=9; p≤0.05; unpaired t-test) in H441 monolayers. Pre-treatment (30 min) with the CSE inhibitor D/L-propargylglycine (PAG; 1 mM) decreased normalized hypoxygenated sodium absorption (Ihypoxygenation) from 0.21 ± 0.02 (n=7) to 0.11 ± 0.03 (n=8; p≤0.01; unpaired t-test) in H441 monolayers, whereas inhibition of CBS (with aminooxy-acetic acid; AOAA; 0.5 mM) or 3MST (with aspartate; 1 mM) had no effect. Inhibition of all of these H2S-generating enzymes with a combination of AOAA, PAG and aspartate decreased Ihypoxygenation from 0.35 ± 0.03 to 0.03 ± 0.03 (n=6-7; p<0.001; unpaired t-test) in H441 cells and from 0.56 ± 0.03 to 0.43 ± 0.05 (n=8; p≤0.05; unpaired t-test) in pig tracheae. These data suggest that endogenously produced H2S contributes to hypoxic inhibition of transepithelial sodium absorption in airways.


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PCB100

A role for the KCa3.1 potassium channel in airway epithelial function

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KCa3.1 is a calcium activated potassium channel expressed in several tissues and cell types but its role in the function of airway epithelium is poorly understood. We have previously demonstrated that KCa3.1 activity is essential for calcium-activated chloride secretion in mouse intestine and its absence reduces water faecal content (Flores et al., 2007). We aim to understand the role of KCa3.1 in the airway epithelium using the KCa3.1−/− mouse and the selective inhibitor TRAM-34 in human and mouse cells.

We measure short-circuit currents by Ussing chamber analysis. We also determined the effect of KCa3.1 inhibition in a mouse model of chronic asthma induced by ovalbumin (0.5mg/mL) instilled intranasally during 9 weeks. All animal procedures were performed according to institutional regulations for animal welfare.

Electrophysiological measurements in freshly isolated tracheas from KCa3.1+/+ mice showed a significant reduction of ENaC-mediated sodium absorption (19.4±5 vs 3.7±4 µA cm⁻² for KCa3.1+/+ and KCa3.1−/− respectively). This reduction was not explained by decreased expression of ENaC subunits, evaluated by qRT-PCR. Reduction in sodium absorption was mimicked in human bronchial epithelial cells when incubated with TRAM-34 (3.1±0.5 vs 1.6±0.3 µA cm⁻² for control and TRAM-34 incubated cells respectively). CBF in primary epithelial airway cultures from mice was increased after UTP stimulation (1µM) in KCa3.1+/+ (28.6%) and in KCa3.1+/+ cultures treated with TRAM-34 (100µM) (16.3%) compared to KCa3.1−/− cultures (4.2%) obtained from wild type animals. Finally, we analysed the impact of KCa3.1 silencing in a mouse model of chronic asthma. We found that the development of asthmatic features such as goblet cell hyperplasia, increased collagen deposits, airway epithelium thickening and mast cell infiltration of airway tissues was attenuated in KCa3.1−/− mice. Our results demonstrate that KCa3.1 inhibition reduces sodium absorption. This effect is independent on changes in ENaC expression and might be explained by changes in cell membrane potential that do not favour the electrophysical potential for sodium entry. Increased CBF could benefit mucociliary clearance, but the mechanistics of such increased activity are being explored. All these changes on epithelial function can help to diminish asthma features in our animal model of the disease. Finally, KCa3.1 inhibition could help in the management of cystic fibrosis disease that presented increased sodium absorption. The therapeutic use of KCa3.1 is further supported by the fact that its inhibition does not produce severe phenotypes in KCa3.1−/− mice.

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PCB101

Effect of omega-3 fatty acids and its derived lipid mediators on the inhibition of sugar uptake produced by TNF-α in Caco-2 cells

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Background: During intestinal inflammation tumour necrosis factor alpha (TNF-α) level is increased (Tabas et al. 2013) and also malabsorption of nutrients may occur. In this regard, we have previously demonstrated in Caco-2 cells that TNF-α inhibits its sugar uptake by decreasing the amount of the Na⁺-glucose cotransporter SGLT1 in the plasma membrane (Barrenetxe et al. 2013). The omega-3 long-chain polyunsaturated fatty acids (n-3 PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and their lipid mediators resolvins (Rv), protectins and maresins (MaR), are able to counteract intestinal inflammation in models of Intestinal Bowel Disease (Barbalho et al. 2016; Chatterjee et al. 2014; Bento et al. 2014). The aim of the present study was to investigate in Caco-2 cells whether EPA, DHA and its derived lipid mediators RvD1, RvD2 and MaR1 are able to block the inhibitory effect of TNF-α on sugar transport and the implication of GPR120 on EPA and DHA action.

Methods: Caco-2 cells were grown on culture plates and pre-incubated for 1 hour with TNF-α (10 ng/ml) and/or 100 µM EPA; 100 µM DHA; 100 nM RvD1; 100 nM RvD2 and 100 nM MaR1 before measuring the apical uptake of 0.1 mM α-methyl-glucoside (α-MG) for 15 min. The functional implication of GPR120 was investigated using its inhibitor AH7614 (100 µM). The expression of SGLT1 in Caco-2 cells brush border membrane vesicles, under the same experimental conditions used for the functional studies, was analysed by Western blot.

Results: Both EPA and DHA prevented the inhibition of α-MG uptake induced by TNF-α. This effect was accompanied by the abolishment of SGLT1 intracellular recruitment by the cytokine. Likewise, RvD1, RvD2 and MaR1 blocked the TNF-α-induced decrease of sugar transport. The presence of the GPR120 inhibitor avoided the effects of EPA on TNF-α-induced inhibition of α-MG uptake, while did not significantly affect DHA action, suggesting that EPA produces its effects through GPR120, but DHA effect seems to be GPR120 independent.

Conclusions: n3-PUFAs and its derived-lipid mediators can reverse the decrease on sugar uptake induced by TNF-α. Therefore, these molecules could be beneficial in patients that suffer malabsorption associated with intestinal inflammation.
The TRPV4 channel regulates tight junctions and affects differentiation in a cell culture model of the corneal epithelium

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TRPV4 is a cationic channel activated by hypotonicity, moderated heat and shear stress. In non-excitable cells it is involved in cellular functions such as volume regulation, migration and differentiation. Here, we studied how the expression of TRPV4 changes during proliferation and differentiation of the rabbit corneal epithelial cell model RCE1(5T5), (RCE1) and its role in differentiation. RCE1 is a spontaneously transformed cell line that recapitulates in vitro the early stages of differentiation. In early cultures, the cells are proliferative, and after 10-14 days, a stratified epithelium of 5-6 layers is formed (García-Villegas et al., 2009). TRPV4 expression was measured by qRT-PCR and Western Blotting. Subcellular localization of TRPV4 and TRPP2 was detected by immunofluorescence. Confocal Ca2+-imaging with Fluo-4AM was used to determine the TRPV4 activity together with the TRPV4 specific activator GSK1016790A (GSK101, 100 nM), the specific blocker RN-1734 (30 µM) and EGTA (2 mM) in sodium Ringer’s solution (in mM: NaCl 145, KC1 5, CaCl2 1, KH2PO4 1, MgCl2 1, glucose 10, HEPES 10, pH 7.4). Transepithelial electric resistance (TER) was measured with an EVOM voltmeter in cultures grown on 24-wells inserts. All data are means ± SEM of at least 2 independent experiments in triplicate. TRPV4 mRNA levels remained unchanged along the proliferation and differentiation of RCE1 cells. Protein expression levels of TRPV4 in differentiated cultures decreased 70% and the channel was localized in the apical membrane of the outmost cell layer of stratified epithelia. TRPV4 channel is functional in differentiated cultures since the perfusion with GSK101 promoted an important influx of Ca2+ (2 ± 0.35 ΔF/F0; n=71), which was blunted by RN-1734 or EGTA. In our study, we analyzed the participation of TRPV4 in RCE1 differentiation by measuring the development of the TER as an indicator of tight junctions (TJ) assembly. Proliferative RCE1 cell cultures chronically treated with RN-1734 from day 7-14 exhibited only 66 % of the TER value (107 ±11 Ω•cm2) measured in control cultures (162 ± 5 Ω•cm2) and no effect was observed after GSK101 treatment. In differentiated epithelia, activation of TRPV4 increased TER by 30% (220 ± 8 Ω•cm2). EGF (10 ng/ml) up-regulates the TER of RCE1 cultures and here we also observed that TRPV4 activation mimics this EGF effect. Conversely, TRPV4 inhibition prevents the increase of TER even in the presence of the growth factor. TRPP2, an EGF-activated channel, co-localizes with TRPV4 in differentiated cultures suggesting that the EGF regulation of TJ may involve a heterotetrameric TRPV4-TRPP2 channel. Together our results demonstrate that TRPV4 activity is necessary for the correct establishment of TJ in corneal epithelia and also for regulating both the barrier function of TJ and its ability to respond to EGF.

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PCB103

Increased intestinal permeability in protein tyrosine phosphatase non-receptor type 2-deficient mice is associated with regional variations in mucosal inflammatory cytokine production

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Increased intestinal permeability plays a crucial role in the pathophysiology of inflammatory bowel disease (IBD). Select IBD candidate genes are associated with increased intestinal permeability and/or altered mucosal immune responses. Loss-of-function mutations in the protein tyrosine phosphatase non-receptor type 2 (PTPN2) gene are associated with IBD but their functional contribution to IBD pathogenesis is unknown. We identified a novel role for PTPN2 in protecting epithelial barrier function in vitro and in vivo. The aim of this study was to identify if barrier defects in Ptpn2-deficient mice are associated with altered production of barrier-modifying inflammatory cytokines in different intestinal regions.

**Methods:** In vivo intestinal permeability was assessed by oral gavage of 4 kD FITC-dextran (FD4) to 18-21 day old Ptpn2 wild-type (WT), heterozygous (Het) and knockout (KO) mice (male and female co-housed littermates) and serum collection after 4 hrs to quantify FD4. Ileum, cecum, proximal and distal colon were isolated and mounted in Ussing chambers for ex vivo studies of barrier function (FD4 permeability and transepithelial electrical resistance (TER)). Tight junction protein analysis was performed by immunohistochemistry and immunofluorescence confocal microscopy. Serum and intestinal cytokine levels were determined by Multiplex ELISA analysis.

**Results:** In vivo FD4 permeability was significantly increased in Het vs. WT (2.0 ± 0.1 fold above WT; p<0.001 by ANOVA; n=5) and further elevated in KO vs. WT (3.5 ± 0.5 fold; p<0.001; n=5). Ex vivo intestinal tissues showed increased FD4 flux and reduced TER in multiple regions in Ptpn2 KO mice compared with WT mice, while Het mice exhibited barrier defects but to a lesser extent than KO mice (n=5-11). This was consistent with increased intestinal claudin-2 expression in Het and KO mice and mislocalization of ZO-1 and occludin vs. WT (n=2). IFN-gamma, TNF-alpha and IL-6 levels were increased in KO mouse serum and large intestine with the increase in

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IFN-gamma in KO mouse serum (460 ± 260 vs. 3 ± 0.1 pg/ml in WT; mean ± SEM; n=3 in triplicate) and cecum (142 ± 42 vs. 9 ± 1 pg/ml in WT; mean ± SEM; n=2 performed in triplicate) most prominent. These cytokines were increased in Het mice in select tissue regions vs. WT (n=2-3).

Conclusion: Reduced expression of PTPN2 leads to compromised intestinal barrier function and elevated expression of inflammatory cytokines known to promote epithelial barrier defects. These data indicate additional mechanisms by which PTPN2 loss of function may contribute to decreased intestinal barrier properties relevant to IBD pathogenesis.

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PCB104

Genetic deletion of 11-β-OH-steroid-dehydrogenase type 2 causes in inappropriate activation of renal SGK1

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The effects of aldosterone are mediated via cytoplasmic mineralocorticoid receptors (MR) that control the transcriptional activity of genes such as that encoding the regulatory kinase SGK1 and, in aldosterone-stimulated cells, increased cellular SGK1 activity appears to stimulate Na+ retention via the epithelial Na+ channel (EnaC). Whilst SGK1 knock out mice do not normally display an overt phenotype, they cannot adequately increase renal Na+ retention when exposed to a low Na+ diet.

Although, the MR can also be activated by glucocorticoid hormones in vivo (cortisol in humans, corticosterone in rodents), this does not occur in vivo since aldosterone-sensitive tissues express an enzyme, 11-β-HSD2, that rapidly converts these hormones into inactive metabolites. 11-β-HSD2 thus fulfils an important physiological role by effectively protecting the aldosterone-sensitive tissues from circulating glucocorticoids. Very high levels of cortisol can, however, overwhelm this protective mechanism and, under such circumstances, glucocorticoids can evoke renal Na+ retention. This abnormal response is thought to underlie the fluid retention, oedema and hypertension seen in conditions, such as Cushing’s syndrome, that are characterised by glucocorticoid excess.

The role of 11-β-HSD2 has recently been clarified by studies of rats that have been genetically modified by the deletion of the gene encoding this enzyme (3). These animals express a complex phenotype that involves overt hypertension that is worsened by high Na+ intake and is associated with increased renal Na+ retention (3). Since abnormalities in SGK1 are known to disturb whole body Na+ balance, we have now explored the effects of 11-β-HSD2 gene deletion upon renal SGK1 activity. Kidneys removed from 11-β-HSD2 nul rats and wild type animals were therefore homogenised and extracted proteins subject to Western analysis / densitometry. Analysis of these proteins showed that the Thr365/Ser366 phosphorylated from of the protein encoded by n-myc downstream regulated gene 1 (NDRG1) was ~2.5 fold more abundant in the tissue from 11-β-HSD2 nul rats (Fig 1). There was, however, there was no difference in the overall NDRG1 expression level (Fig 1) and analyses using an antibody against b-actin confirmed that equal amounts of protein had been loaded onto each gel (Fig 1). Deletion of the 11-β-HSD2 thus causes increased phosphorylation of NDRG1-Thr365/Ser366 and, since these residues are phosphorylated by SGK1 and not by other, closely related kinases (4), these data show that deletion of the 11-β-HSD2 gene deletion is associated with an abnormally high level of renal SGK1 activity. Abnormal regulation of this kinase may therefore underlie the physiological abnormalities that have recently been documented in these animals (3).

Conclusion: Reduced expression of PTPN2 leads to compromised intestinal barrier function and elevated expression of inflammatory cytokines known to promote epithelial barrier defects. These data indicate additional mechanisms by which PTPN2 loss of function may contribute to decreased intestinal barrier properties relevant to IBD pathogenesis.

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Short Palate and Nasal Epithelial Clone 1 (SPLUNC1) is cleaved by neutrophil elastase present within mucopurulent material from CF lungs

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With approximately 70,000 sufferers and 8 million carriers worldwide, cystic fibrosis (CF) is one of the most common lethal genetic diseases. CF is caused by mutations in the gene encoding the cystic fibrosis transmembrane regulator (CFTR). These mutations result in altered anion (Cl- and HCO3-) secretion across airway epithelia which in combination with Na+ hyperabsorption via the epithelial sodium channel (ENaC)(1), results in reduced airway surface liquid (ASL) volume, impaired mucociliary clearance, chronic inflammation and bacterial colonization. As such, CF airways become obstructed due to the presence of excessive mucopurulent material (MM) which contains numerous proteases. We have previously reported that the secreted protein, SPLUNC1, regulates ENaC activity and hence, ASL hydration levels and that SPLUNC1 regulation is defective in CF airways(2). We hypothesize that proteases present within the MM can cleave SPLUNC1 and may alter its ability to regulate ENaC. Post-mortem MM was harvested from Pseudomonas aeruginosa- and Staphyloccocus aureus-positive CF lungs. MM was incubated with SPLUNC1 at 37°C and SPLUNC1 levels were determined by western blot. Significant breakdown of 10 µM SPLUNC1 was observed after incubation with MM diluted 1:2 with PBS (t 1/2 = 1.17 hours; N = 3). To determine which enzymes were responsible for SPLUNC1’s cleavage, MM was pre-incubated with protease inhibitors including aprotinin, EDTA, E64 and leupeptin. These compounds failed to inhibit SPLUNC1 cleavage (all N = 3). Sivelstat, a neutrophil elastase (NE) inhibitor, significantly prevented SPLUNC1 cleavage (10µM * * * * * p < 0.0001, N = 4). Given that SPLUNC1 regulates ENaC activity and SPLUNC1 is cleaved by MM, the effects of MM and SPLUNC1, alone and in combination, on ENaC protein levels in normal human bronchial epithelial cells (HBECs) were investigated. Our preliminary data (N = 3) indicated that MM proteolytically cleaved γENaC and increased levels of cleaved γENaC subunit expression were observed. Data from normal HBECs incubated with SPLUNC1 alone or SPLUNC1 and SMM in combination suggests decreased γENaC protein levels, consistent with ENaC degradation and reduced ENaC activity. The effect of SPLUNC1 and MM in CF HBECs remains to be determined. In conclusion, we have shown that MM readily cleaves SPLUNC1, and that cleavage can be prevented by sivelstat. MM cleavage of SPLUNC1 may alter SPLUNC1’s ability to regulate innate defense in CF airways.


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Little cigar exposure induces CFTR dysfunction and airways surface liquid dehydration in human bronchial epithelial cultures

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Tobacco inhalation is one of the major causes of mortality and morbidity worldwide. Tobacco can be smoked in many different ways, including cigarettes, cigars, pipes and hoo-kah. Cigars are defined as “any roll of tobacco wrapped in leaf tobacco or any substance containing tobacco” and cigars that weigh less than three pounds per one thousand units are identified as “Little Cigars” and often have less stringent regulation that cigarettes. Although the deleterious effects of cigarette smoking have been widely documented, little is known about the health effects of related tobacco products such as little cigars. We exposed well differentiated, primary human bronchial epithelial cells cultured at the air liquid interface to air or freshly generated smoke from either Kentucky research cigarettes, two leading brands of little cigars (Swisher Sweets and Captain Black) and one brand of cigar (Cheyenne; that is of similar size to little cigars) and evaluated general cytotoxicity and CFTR-dependent airway hydration. All experiments were performed on replicate cultures obtained from a minimum of 5 separate donors. Neither chronic cigarette smoke nor chronic cigar smoke exposure altered gross cellular morphology, but caused cytotoxic effects that were significantly greater with little cigar exposure than with Kentucky cigarette exposure, as evident by significantly greater LDH release (Air, 2.5±0.7, Kentucky, 5.6±1.2, Swisher, 11.7±2.3; n=12). Airway surface liquid height and CFTR protein levels were also significantly decreased in chronic little cigar-exposed cultures compared to Kentucky cigarette or air exposed cultures (Air, 6.9±0.2, Kentucky, 5.4±0.2, Swisher, 4.9±0.1; n=12). However, epithelial sodium channel levels were unaffected (n=5). A significantly greater decrease in transepithelial resistance also occurred with little cigar exposure (Air, 5.3±0.5, Kentucky, 438±31, Swisher, 355±32; n=12). Our GC-MS analysis indicated that compounds present in all groups were found in higher quantity in cigars relative to Kentucky cigarettes. Additionally, many more unique compounds were identified in cigars that were not present in Kentucky cigarettes, suggesting that cigars expose the lung to different and potentially more harmful toxicants. In conclusion, our data indicate that little cigar/cigar smoking causes severe airway dehydration and ciliary dysfunction that is predicted to trigger pulmonary disease that may be more harmful than that seen with cigarette smoking. These changes are caused by the increased number of chemical species present in the tar phase of cigars.

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Leptin mediates downregulation of intestinal SGLT1 and hyperplasia in obese type 2 diabetic mice

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The intestine is critical in maintenance of euglycemia. We showed that intestinal SGLT1 is decreased in hyperleptinemic db/db mice, but not in leptin-deficient ob/ob mice. Only hyperleptinemic mice exhibit increased mucosal mass with longer villi and deeper crypts compared to controls. Catestatin (CST), a fragment of chromogranin A, can modulate leptin signaling. We conducted molecular dynamics simulations and found that CST mimics the AB-loop of leptin’s binding site-III. Pairwise sequence alignment between CST and the AB-loop showed >40% similarity. Both leptin and CST exhibit similar modes of binding to the leptin receptor by targeting the Ig-like domain. We hypothesized that CST treatment would affect leptin signaling in db/db mice resulting in better glycemc control and normalization of intestinal epithelial cell turnover. To understand the increased mucosal mass in hyperleptinemic mice, we quantified proliferation and apoptosis in the jejunum of db/db, ob/ob, and control (db/con, ob/con) mice (n=4-5/genotype). Compared to controls, db/db mice had more proliferating cell nuclear antigen-positive cells in the crypts (2,361±110 vs 1,453±211 cells; P<0.05) indicating an increased proliferative response, while cleared caspase-3 staining, a marker for apoptosis, showed reduced apoptosis in the crypts (2.5±0.5 vs 9.5±0.5 cells; P<0.05) and villi tips (5±1 vs 9±1 cells; P<0.05). Proliferation and apoptosis were not different between control and ob/ob mice. Glucagon-like peptide (GLP)-2 is an intestinoendocrine hormone that increases cell proliferation. We found that GLP-2 plasma levels are ~35% higher in db/db versus db/con mice (1.4±0.1 vs 1.0±0.1 pg/ml, P<0.001), and may contribute to the intestinal hyperplasia. In a separate cohort, we treated db/db (n=4) and db/con (n=5) mice for 7 days with CST (5 mg/kg/day i.p.). CST treatment not only restored intestinal SGLT1 expression but also normalized villus length and intestinal proliferation to levels found in controls. To study if there is a functional consequence of elevated leptin levels, we performed oral glucose tolerance tests (OGTT) by administering glucose (2 g/kg, 1% of bw via oral gavage) without or with phlorizin, an SGLT1/2 inhibitor (0.5 g/kg), to db/db and db/con mice (n=4-8/genotype). Blood glucose was measured at 0, 15, 30, 45, 60 and 120 min and the area under the curve (AUC) was calculated. Compared to vehicle, phlorizin significantly improved glycemic control in db/con mice (AUC: 13,857±4,051 vs 36,483±7,747; P<0.05) but was without effect in db/db mice (AUC: 12,794±4,675 vs 13,714±2,648; P=NS). Further, phlorizin treatment mimicked the OGTT profile of SGLT1 knockout mice (AUC: 6,325±2,535). In conclusion, CST may be a novel regulator that interferes with leptin signaling in hyperleptinemic mice and may lead to beneficial effects on intestinal turnover and restoration of SGLT1 abundance.

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PCB109

The role of Na⁺ in establishing the membrane potential in cancer cells

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The transmembrane potential (V_m) is established as a functionally instructive biophysical cue in non-excitable cells and has been shown to be a key modulator of cell volume, differentiation, migration and proliferation. Numerous studies have demonstrated a depolarised V_m phenotype as a determinant of cell proliferation across a broad range of cancer cell types (1). The transmembrane potential is generated by ionic gradients and the associated permeability of the membrane to said species. Due to the ubiquitous expression of a vast array of background/leak potassium (K⁺) channels in normal mammalian cells, K⁺ is the predominantly permeable ion, and V_m tends towards the reversal potential for K⁺ (E_K) resulting in a negative membrane potential. The reversal potential for sodium (Na⁺) is positive and increased plasma membrane permeability to Na⁺ would result in a more depolarised phenotype. However, the role of Na⁺ in the contribution to V_m in cancer is still poorly understood. The aim of this study was to systematically investigate the Na⁺ permeability and the existing Na⁺ gradient independently in MCF-7 breast and SKOV-3 adenocarcinoma cancer cell lines. Sodium permeability was investigated with the whole-cell current clamp (I=0) technique by replacement of 135 mM Na⁺ (5 mM K⁺) in external physiological salt solution (PSS) with 135 mM N-methyl-D-glucamine (NMDG), 5 mM K⁺). The cytosolic Na⁺ concentration was determined in MCF-7 cells by live cell imaging where we combined a cytosolic pH sensitive dye (5-carboxy-SNARF-1) with a Na⁺/H⁺ exchanger to manifest a pH change that is dependent on Na⁺ gradient. In MCF-7 cells, the V_m in normal PSS was -8.38 ± 4.95 mV (n=5) (mean ± standard error of mean, compared by paired t test). Upon replacement with Na⁺-free solution, V_m hyperpolarised to -23.5 ± 3.93 mV (n=5) (p<0.05). In SKOV-3 cells V_m in normal PSS was -2.34 ± 0.95 mV (n=9), compared with -15.53 ± 4.65 mV (n=9) (p<0.05) upon replacement with Na⁺-free solution. The cytosolic Na⁺ concentration was determined to be 8.43 ± 0.75 mM (n=3) (mean ± standard error of mean). In two distinct cancer cell lines, there exists a persistent permeability to Na⁺ and thus a contribution to the plasma membrane potential. In MCF-7 cells cytosolic Na⁺ concentration is comparable to that of non-cancerous non-excitable cells in contrast to values reported using more invasive energy dispersive x-ray microanalysis methods (2). These data indicate that there is a contributory role of Na⁺ in the establishment of a persistently depolarised V_m in cancer cells, suggesting that sodium dynamics may play a central role in modulating cancer cell proliferation.


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PCB110

Role of tubular NHE3 for Na⁺ homeostasis and blood pressure regulation

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The Na⁺/H⁺ exchanger isoform 3 (NHE3) facilitates Na⁺ absorption and H⁺ secretion. NHE3 is expressed in the intestine, proximal tubule and thick ascending limb of the kidney. Conventional NHE3 knockout mice show severe signs of dehydration or die when deprived of NaCl for 3 days. However, the importance of kidney-specific NHE3 inactivation in this context is unknown. To test the role of kidney-specific NHE3 knockout in Na+ homeostasis we studied tubulus-specific NHE3 knockout mice which have been previously generated by us. NHE3loxlox (Con) and NHE3loxloxPanCre (NHE3loxloxCre) mice were randomized and studied at baseline and for 10 days on low (<0.01%) or high (4%) NaCl intake (N=5-7/group). At baseline and the end of each diet regimen blood was collected and analyzed for Na⁺, K⁺, aldosterone as well as glomerular filtration rate (GFR) determined via FITC-Sinistrin clearance. Body weight was recorded daily. Under baseline conditions there was no difference between genotypes in body weight, fluid or food intake, plasma Na⁺ or K⁺, or blood pH. Plasma aldosterone (259±30 vs 237±28 pg/ml, NS) was not significantly different in NHE3loxloxCre compared to Con mice while urinary pH was significantly elevated (8.1±0.1 vs 7.2±0.1, P<0.05). NaCl intake had no impact on plasma Na⁺ in Con mice; however, plasma Na⁺ in NHE3loxloxCre mice was susceptible to the effects of low (-3.9±1.0 mM, P<0.05) and high NaCl (+2.2±0.6 mM, P<0.05) compared to baseline. Low NaCl decreased plasma K⁺ in Con (+0.5±0.2 mM, P<0.05) but to a significant greater amount in NHE3loxloxCre mice (-1.2±0.1 mM, P<0.05). Aldosterone was not significantly different between Con and NHE3loxloxCre under low (345±96 vs 498±78 pg/ml) and high NaCl intake (60±19 vs 86±20 pg/ml). Possibly as a consequence of impaired proximal tubular Na⁺ reabsorption and consequently activation of tubuloglomerular feedback, GFR was lower in NHE3loxloxCre mice under baseline conditions (457±20 vs 358±17 µl/min, P<0.05). Low NaCl decreased GFR in Con (-110±13 µl/min, P<0.05) and NHE3loxloxCre (-99±8 µl/min, P<0.05) mice, whereas high NaCl was without effect on GFR in either genotype. No significant differences in blood pressure were detected between Con mice after 10 days on low or high NaCl intake. In contrast, NHE3loxloxCre mice show lower systolic blood pressure under low and high NaCl intake (20 and 10 mmHg, respectively, P<0.05). Of note, blood pressure in NHE3loxloxCre mice was salt-sensitive, with a 10 mmHg increase upon high NaCl diet, while heart rate was not affected by dietary NaCl intake or genotype. The results indicate that maintenance of plasma Na⁺ requires functional NHE3 in the proximal tubule; otherwise dietary NaCl directly impacts total body NaCl possibly by disturbing glomerulo-tubular balance. In addition, the role of renal NHE3 for overall Na⁺ homeostasis is less detrimental than previously reported for NHE3 knockout mice.

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MCT1 transporter abundance in carcinoma tissues from different colonic regions

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MCT1 transporters are crucial for the trans-epithelial transport of bacterially-derived short chain fatty acids within the healthy human colon (1). In normal colonic tissue, MCT1 is located in the basolateral membranes of epithelial cells (2). However, MCT1 transporters also play a key role in lactate transport in tumour cells, although both increases (3) and decreases (4) in MCT1 abundance have been reported in colonic carcinoma. Our previous study showed significant regional differences in healthy colon MCT1 abundance (5), suggesting an explanation for these conflicting reports. This current study hence investigated MCT1 abundance changes in carcinoma tissue from different colonic regions.

Immunolocalization studies were performed using microarray slides containing matching samples of carcinoma and normal resection tissue from the same patient. Tissue sample pairs were taken from either the ascending, transverse, descending or sigmoid colon. Immunoperoxidase staining was performed using an anti-MCT1 antibody and the extent of MCT1 staining scored. Overall, MCT1 staining was not significantly different between normal and carcinoma paired samples from ascending (NS, N=16, paired t-test), transverse (NS, N=7, paired t-test) or descending colon (NS, N=5, paired t-test). However, in sigmoid colon there was a significant five-fold increase in MCT1 staining in carcinoma compared to normal tissue (P<0.05, N=21, paired t-test).

In conclusion, our data showed changes in MCT1 abundance observed in colonic carcinoma tissue compared to normal resection tissue were region-dependent. Future research is now required to establish if this finding is repeated for other carcinomas. In conclusion, our data showed changes in MCT1 abundance observed in colonic carcinoma tissue compared to normal resection tissue were region-dependent. Future research is now required to establish if this finding is repeated for other carcinomas.

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In vitro NHERF adaptor-specific modulation of different ion transporters versus in vivo intestinal fluid secretory response to the heat-stable Eschericia coli enterotoxin (STA) analogue linaclotide in NHERF- and transporter knockout mice

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The different PDZ-adaptors of the NHERF family differentially associate with intestinal electrolyte transporters and modulate their activity in an agonist-specific fashion. STA is one of the most common causative agents in travellers diarrhea as well as diarrheal disease in livestock. It induces secretory diarrhea via binding to the guanylate cyclase C, eliciting cGMP production, resulting both in a stimulation of CFTR-mediated anion secretion and an inhibition of NHE3-mediated fluid absorption, and these effects are modulated by different NHERF adaptor proteins in cell lines. We investigated whether and how well the in vitro mediation of STA action on NHE3, and CFTR by different NHERF proteins and by the cyclic GMP kinase II (cGKII) in murine intestine correlates with their in vivo involvement in modulation of fluid transport. 10^{-7}M of the STA analogue linaclotide was applied to the luminal bath as chambered isolated jejunal mucosa of CFTR-, NHE3-, cGKII-, and NHERF1-3 deficient and WT mice, and short circuit current (Isc) response was measured. The same concentration of linaclotide was added to the jejunal perfusate during single pass perfusion of a jejunal segment in isoflurane-anesthetized, acid/base- and blood pressure controlled KO and WT mice, and net fluid balance before and after linaclotide application assessed gravimetrically. The peak Isc response to linaclotide was absent in CFTR KO, reduced by 88% in cGKII KO, by 40% in NHERF1 KO, and not significantly in NHERF2 and NHERF3 KO isolated mucosa. The magnitude of change from jejunal fluid absorption to secretion was reduced by 51% in cGKII KO, 49% in CFTR KO, 45% in NHERF1 KO, 34% in NHERF2 KO, and not significantly in NHERF3 KO anesthetized mice. NHE3 deletion (48%) or pharmacological NHE3 inhibition (50%) reduced the magnitude of change to a similar degree as CFTR deletion. The results demonstrate that Isc measurements in isolated small intestinal mucosa are excellent models to selectively study agonist-mediated CFTR activation, but do not accurately predict in vivo secretory responses. Apart from direct interference with guanylate cyclase C activation, potential pharmacological strategies to prevent STA-mediated fluid loss may be targeting both NHERF1 and NHERF2.

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Dietary nitrite induces occludin nitration in the stomach

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Dietary nitrate, from green leaf vegetables, has raised a great deal of interest in the recent few years as the main source of nitric oxide (+NO) in the human gut via nitrate-nitrite-nitric oxide pathway. The physiological significance of this pathway is connected to the non-enzymatic reduction of nitrite to •NO and other bioactive nitrogen oxides in the stomach and ensuing impact in human health and therapeutics. The bioactive nitrogen oxides are acknowledged for their oxidizing and nitrating capacity, modifying the function of lipids and proteins. This study shows a nitrate-dependent nitrating pathway targeting a tight junction protein in the stomach, occludin. This transmembrane protein is located in the most apical position of epithelial cell membrane and exhibits two extracellular loops containing up to 60% of tyrosine residues. Hence, both the chemical structure and location of occludin make it a potential target for nitration.

All animal experiments were performed according to the ARRIVE guidelines and the European Community Council Directive for the Care and Use of Laboratory Animals (86/609/ECC). Male Wistar rats (n=5, per group) were used in this study. Inorganic nitrate (10 mM), nitrite (2 mM) or human saliva collected following lettuce ingestion were administered by oral gavage. In order to ensure a completed enterosalivary cycle, the nitrate circulation occurred for 4 hours. After this period, the rats were anesthetized (halothane) and euthanized by cervical dislocation. The stomachs were then collected.

Nitrated occludin was detected by immunoprecipitation in the gastric epithelium upon inorganic nitrate administration (p<0.05, through a t-student test). No significant nitration was observed in the case of inorganic nitrate or human saliva. Accordingly, salivary ascorbate, urate and thiocyanate promote nitrite reduction to •NO in the acidic pH of the stomach diverting the production of nitrated species. Furthermore, different •NO production rates were observed when using inorganic or salivary nitrite. Using an electrochemical approach, we observed that sodium nitrite produced lower steady state concentrations of •NO (0.12±0.03 µM) as compared to salivary nitrite (0.37±0.01 µM). These findings indicate that the production of nitrating agents or other stable oxides could be affected by competing reactions at acidic pH.

The biological impact of occludin nitration remains elusive. Considering, that the nitrate-nitrite-nitric oxide pathway, by generating •NO in the gastric lumen, could modulate gut barrier function, the implications for gastrointestinal disorders such as inflammatory bowel disease or other leaky disorders should be further investigated.

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Dietary spray-dried plasma (SDP) supplementation promotes anti-inflammatory mediators in mice challenged with S. aureus enterotoxin B

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In rodents, dietary supplementation with spray-dried porcine plasma (SDP) attenuates lymphocyte activation in Peyer’s patches and mesenteric lymph nodes and improves the intestinal protective effects of SDP. To perform the experiments, male C57BL/6 mice (n=24) were used. Experimental diets were administered for 14 d starting at weaning (day 19). Mice were challenged with a single SEB dose (25 µg/mice, i.p) at day 32 and killed 24 h later. Mice were randomly distributed in three groups: CTL group, fed a control diet (in which SDP was substituted by milk proteins); SEB group, fed a control diet and administered with SEB; and SDP group, fed a diet supplemented with 8% SDP and challenged with SEB.

MLN lymphocytes were stained and analysed by flow cytometry. The expression of cytokines (IL-10 and TGFβ1), adhesion molecules (Madcam-1 and ICAM-1) and transcription factors (Smad2/3 and NFκB) in the intestinal mucosa were determined by real-time PCR and by Western blot. Results (means ± SEM; n=8) were analysed by one way ANOVA. SEB administration increased the MLN cell recruitment (SEB: 34.9±10³ ± 3.0±10⁸ vs CTL: 22.9±10³ ± 1.7±10³, p<0.05), the percentage of activated Th lymphocytes (SEB: 8.3 ± 0.5 vs CTL: 3.6 ± 0.1, p<0.05) and the activated to regulatory Th lymphocytes ratio (SEB: 3.0 ± 0.2 vs CTL: 1.3 ± 0.1, p<0.05). SDP diet prevented all these effects (p<0.05). The enterotoxin administration did not change the mucosal expression of IL-10 and TGFβ1, but SDP supplementation increased the expression of both cytokines when compared to the SEB group (SDP: 5.5 ± 1.0 vs SEB: 1.2 ± 0.2; SDP: 2.6 ± 0.4 vs SEB: 1.0 ± 0.1, p<0.05 respectively).

The administration of enterotoxin increased 6-fold the expression of Madcam-1 and ICAM-1 (p<0.001) and these effects were attenuated by SDP supplementation (p<0.05). SDP also decreased Smad2/3 phosphorylation and augmented NFκB phosphorylation (SEB: 0.8 ± 0.0 vs CTL: 1.0 ± 0.1; SEB: 1.5 ± 0.1 vs CTL: 1.0 ± 0.1, p<0.05, respectively) without affecting their total expression. SDP prevented the effects of SEB on both transcription factors (p<0.05). Our results indicate that SDP modulates the immune response in challenged animals through regulation of transcription factors and adhesion molecules that, in turn, will reduce intestinal cell infiltration and the magnitude of the inflammatory response.

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In vitro studies of impact of repeated hypoxic stress on transendothelial transport mediated efflux pumps Pgp and MRP-1 of blood-brain barrier co-culture model

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The blood brain barrier (BBB) is a physical and metabolic barrier, composed of multicellular unit, separating the central nervous system from blood circulation. One of BBB’s roles is to protect the brain of potential cytotoxic compounds. This protection is ensured by tight junction proteins (TJs), which create a physical blockade to paracellular diffusion, and by efflux transporters proteins like P-glycoprotein (Pgp) or multidrug resistance proteins (MRPs) (transendonethelial pathway). These proteins acted as a barrier system by pumping compounds out of endothelial cells. The structural and functional integrity of transporters and TJs are neccessary for an intact BBB. Repeated hypoxia linked to some neurological disorders, like obstructive sleep apnea syndrome, is less understood, in particular on BBB integrity mediated efflux transporters protein expressions. In this study we investigated the impact of repeated hypoxic stress on two efflux transporter proteins (Pgp and MRP-1) of an in vitro BBB model. For that, we have developed an in vitro BBB model composed of the immortalized mouse brain endothelial cells (bEnd.3) which was co-cultured in contact with the rat malignant glioma cells (C6). Hypoxic stress was induced by chemical agent: hydralazine (a mimetic agent of hypoxia pathway) during 2h and repeated during 24h or by physical hypoxia (2% O2). We assessed the functionality of Pgp and MRP-1 by measuring the transport of BCECF-AM, a fluorescent compound after repeated hypoxic stress. Then we investigated Pgp and MRP-1 expressions of our BBB model after hypoxic stress, by cell-ELISA method. We showed that endothelial cells increased their efflux transporter’s activity after repeated hypoxic stress. Indeed the level of extracellular BCECF (BCECF which was efflux by Pgp and MRP-1) was significantly increased after hypoxic stress (p<0.001). The concentration of extracellular BCECF varied from 4.43 ± 1.01 to 8.84 ± 0.47 µg/ml after the first exposition and to 11.13 ± 0.72 µg/ml after the third exposition. This increase of transport was associated to a significant increase of Pgp and MRP-1 expression (p<0.01). We observed that Pgp and MRP-1 expressions doubled between controls and cells exposed to repeated hypoxic stress (3.99 ± 1.14 versus 8.72 ± 0.27 µg/ml for Pgp, 9.21 ± 3.14 versus 19.97 ± 7.35 for MRP-1). No studies have specifically addressed the effect of repeated hypoxic stress on transporters, in brain endothelial cells. Ours results showed that brain microvascular endothelial cells had set up defence mechanism by increasing Pgp and MRP-1 activities. This increase of activity was associated to an increase of expression. In conclusion endothelial cells of the BBB increased the expression of Pgp and MRP-1 to fight against the entry of potential cytotoxic compounds in the brain.

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**PCB119**

Regulation of pro-inflammatory cytokine release from monocytes by the secondary bile acid, ursodeoxycholic acid

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Inflammatory Bowel Disease (IBD) is a group of disorders that are characterised by chronic intestinal inflammation. While the etiology of IBD is not yet fully understood, infiltration of monocytes to the mucosa and the release of proinflammatory mediators is thought to play an integral role in disease progression. Among the mediators released from monocytes is IL-8, a known neutrophil attractant that drives mucosal inflammation. Ursodeoxycholic acid (UDCA) is a naturally-occurring secondary bile acid that is well-established to exert cytoprotective and anti-inflammatory effects. Here, we investigate the effects of UDCA in regulating IL-8 release from monocytes. IL-8 release from U937 monocytes was induced by treatment with either lipopolysaccharide (LPS) [1 μg/mL], or the pro-inflammatory cytokine, tumour necrosis factor α (TNFα) [5 ng/mL], in the absence or presence of UDCA. Supernatants were analysed for IL-8 release by ELISA. IL-8 mRNA levels were measured by qPCR. Levels of phospho-p38 MAPK, phospho-p65 and phospho-TRAF2 were measured by western blotting. Cellular toxicity was determined by LDH release. Statistical analyses were performed by one way ANOVA with the Newman Keul's post-test.

Treatment of U937 monocytes with either TNFα or LPS induced levels of IL-8 from 195 ± 82 pg/ml to 2,343 ± 282 (n=3) and 26,017 ± 394 pg/ml (n=3), respectively. Co-treatment with UDCA [100 μM] reduced TNFα-driven IL-8 release from 2,343 ± 282 to 1,684 ± 243 (n=5) but did not have an effect on LPS-driven IL-8 release (n=7). Similarly, UDCA was found to reduce TNFα but not LPS-induced IL-8 mRNA levels (n=4). At the concentrations employed, UDCA did not exert toxic effects, as determined by LDH release (n=3). Analysis of the signalling pathways involved revealed that both TNFα and LPS induced phosphorylation of p38 MAPK. However, UDCA did not alter these responses (n=3). Both TNFα and LPS induced phosphorylation of p65, a marker of NFκB activation, while the NFKB inhibitor, BMS-345541 [10 mM], attenuated IL-8 release in response to both agonists (n=5). Interestingly, co-treatment with UDCA attenuated TNFα, but not LPS-, induced phosphorylation of p65 (n=4). Finally, we examined TRAF2, a protein involved in mediating TNFα-induced actions in monocytes. We found that TNFα, but not LPS, induced phosphorylation of TRAF2, while treatment with UDCA attenuated this response (n=7).

UDCA specifically inhibits TNFα-induced IL-8 release from monocytes by a mechanism that appears to involve inhibition of TRAF2 and NFKB activation. In vivo, such effects would be expected to dampen mucosal immune responses and therefore suggest that UDCA may be useful as a new approach for treatment of IBD.

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**PCB120**

CFTR potentiator PG-01 and corrector KM-11060 can rescue hERG mutations trafficking

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Type II congenital long QT syndrome (LQT2) is due to genetic mutations in hERG channel. Genetic or pharmacological factors could potentially affect hERG channel biogenesis and contributes to LQTS, for example, disease mutations G601S and T473P result in hERG trafficking deficiency [1,2]. Various rescue strategies for hERG dysfunction are being developed. Some correctors for CFTR channel have been reported to act indirectly on proteostasis pathways to promote folding and correction on hERG trafficking deficiency [3]. In this study, we tested the hypothesis that the CFTR corrector KM-11060 and the potentiator PG-01 may correct hERG mutation trafficking diseases. We use HEK293 cell line expressing a well-studied trafficking disease mutation G601S-hERG channel [4]. We treated cells with CFTR potentiator PG-01 and corrector KM-11060, which function through different cellular mechanisms, and assessed whether correction occurred via immunoblotting. Whole cell proteins from HEK 293 cells expressing hERG channels were used for analysis [5]. Proteins were separated on 8% SDS-polyacrylamide electrophoresis gels for 1 hour, transferred onto PVDF membrane, and blocked for 1 h with 5% nonfat milk. The blots were incubated with the primary antibody (Santa Cruz Biotechnology) for 12-16 h at 4C temperature and then incubated with a donkey anti-goat horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology). Actin expression was used for loading controls. The blots were visualized using the ECL detection kit (Genshare). Results were deemed significantly different from controls by a one-way ANOVA (p < 0.05).

Our results show that both KM-11060 (5, 10, 20uM) and PG-01 (5, 15 uM) can correct G601S mutant alleles of hERG protein trafficking (Fig 1, 2). KM-11060 (20uM) but not PG-01(15 uM) enhance protein expression of wild type hERG channel (Fig 2). Further treatment on cells at low temperature with different drug concentration will be tested. Functional studies are also needed to test whether the drugs can correct the function of hERG mutation channel. These results could potentially provide novel insight into the correction mechanism of CFTR potentiator and also help to develop new treatment for LQT2.
Glucose can enter the ASL via paracellular and transcellular routes, and evidence indicates that it is removed from the ASL by glucose transporters located within the cell membrane. Intracellular glucose is maintained at low concentrations by glucose metabolism. Upon entering the cell, glucose is phosphorylated to glucose-6-phosphate (G-6-P) by hexokinase in the glycolytic pathway. G-6-P is then modified by a variety of enzymes into further metabolic products which are either utilised or expelled from the cell. This aids the maintenance of a glucose concentration gradient across the epithelial cell membrane. When intracellular glucose rises, the concentration gradient is altered which may prevent uptake of glucose from the ASL and drive glucose excretion into the ASL.

Therefore, we investigated the effect of extracellular glucose on intracellular glucose concentrations to determine whether phosphorylation of glucose by hexokinase is a potential rate-determining step in maintaining ASL glucose concentrations. Data is presented as mean ± SEM and analysed by ANOVA.

Using the intracellular Förster resonance energy transfer (FRET) sensor Gluconic2, a glucose binding protein flanked by donor and acceptor fluorophores cyan and yellow fluorescent proteins (CFP and YFP respectively), transfected into H441 airway epithelial cells we analysed changes in intracellular glucose by measuring absolute FRET, which increases with glucose concentration.

Changing extracellular glucose concentrations from 5mM D-Glucose +10mM L-Glucose (osmotic control) to 15mM D-Glucose increased intracellular glucose as evidenced by rise in absolute FRET by 17.4 ± 2.2% (P<0.01; n=6). Addition of the GLUT transporter inhibitor Phloretin (1mM) prevented this rise in the presence of 15mM glucose.

Inhibition of hexokinase II with bromopyruvic acid (100µM) after elevation to 15mM D-glucose resulted in a further increase in absolute FRET of 7.8 ± 3.7% (P<0.05; n=6) compared to 15mM glucose alone. The effect of bromopyruvic acid (100uM) when added to cells in 5mM D + 10mM L-glucose was not significant (P=0.14; n=3).

These data indicate that elevating extracellular glucose concentrations results in an increase of intracellular glucose and that glucose moves into the cell via GLUT transporters. In addition, hexokinase II is important in maintaining low intracellular glucose which would promote uptake from the ASL over efflux. However, when glucose is elevated the rise in intracellular glucose could reverse this process driving more glucose into the ASL.


Takanaga H, Chaudhuri B, Frommer WB. GLUT1 and GLUT9 as major contributors to glucose influx in HepG2 cells identified by a high sensitivity intramolecular FRET glucose sensor. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2008;1778(4):1091-1099.

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Trichostatin A (TSA) disrupts the aldosterone-induced activation of the epithelial Na\(^+\) channel (ENaC) in mouse cortical collecting duct cells by impairing the control of serum and glucocorticoid-inducible kinase 1 (SGK1)

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Aldosterone evokes ENaC-mediated Na\(^+\) retention in the cortical collecting duct by activating mineralocorticoid receptors (MR) that promote expression of the kinase SGK1 (1). However, the transcriptional activity of the MR seems to be restricted by acetylation (2), a post-translational modification that involves the attachment of acetyl groups to lysine residues. The acetylation status of the MR seems to be determined by lysine deacytelyases (KDACs), enzymes that remove acetyl groups from such modified proteins (2). The present study therefore explores the effects of TSA, a broad spectrum KDAC inhibitor, upon the responses to aldosterone in mCCD\(_{11}\) murine cortical duct cells. Electrometric studies showed that aldosterone (3 nM, 3 h) augments amiloride-sensitive (10 \(\mu\)M) short circuit current (\(I_{\text{amil}}\)) (Fig 1A) whilst Western analysis of protein extracted from these cells showed that aldosterone also increased the abundance of the Thr\(^{346/356/366}\)-phosphorylated form of the protein encoded by n-myc downstream regulated gene 1 (NDRG1) without altering overall NDRG1 abundance. Aldosterone thus promotes NDRG1-Thr\(^{346/356/366}\) phosphorylation (Fig 1B) and, since this provides a read out of SGK1 activity, the electrometric response to aldosterone is accompanied by activation of this kinase. Aldosterone also increased the abundance of SGK1 protein (Fig 1C) and these findings confirm that this hormone stimulates ENaC-dependent Na\(^+\) absorption via a mechanism that involves sgk1 gene expression.

TSA (1 \(\mu\)M, 2 h pre-incubation) caused a slight (~5%) reduction of basal \(I_{\text{amil}}\) and substantial (~90%) inhibition of the electrometric response to aldosterone (Fig 1A). TSA also abolished the effects of aldosterone on the cellular SGK1 activity (Fig 1B) and abundance (Fig 1C). However, separate experiments (n = 6) showed that TSA did not alter the electrometric response to insulin (20 nM). Since insulin activates SGK1, and ENaC, without evoking gene transcription (3), the effects of TSA upon the response to aldosterone (Fig 1) cannot be attributed to non-specific effects. TSA also increased in the abundance of the hyperacetylated forms of histone 3 (13.2 ± 1.4 fold) and 4 (10.6 ± 3.9 fold) and these effects (n = 4) peaked after 6 – 12 h. Since these proteins are archetypical KDAC substrates, these data confirm that TSA blocks these enzymes under the present conditions. Previous work has shown that a broad spectrum KDAC inhibitor can lower blood pressure in an animal model of hypertension (1) and the present data provide a physiological basis for this by showing that such compounds also block aldosterone-dependent Na\(^+\) transport by disrupting MR-dependent control over SGK1 gene expression.

This work was funded by Kidney Research UK

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Electrophysiological characterisation of chondrocytes exposed to pro-inflammatory cytokines in an in vitro model of osteoarthritis

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Chondrocytes from articular cartilage have been shown to express a wide range of ion channels and transporters. These proteins control a number of cellular functions, including maintenance of membrane potential and regulation of cell volume. Various transcript studies have identified changes in ion channel gene expression with osteoarthritis (OA). In vitro studies have shown that pro-inflammatory cytokines can
induce significant chondrocyte apoptosis, one of the hallmarks of OA (Lopez-Armada et al., 2006). Increasing evidence suggests that pro-inflammatory cytokines can also directly modulate ion channel activity (Viviani et al., 2007). Here, we investigate the electrophysiological characteristics of both healthy chondrocytes and those from an in vitro inflammatory model of osteoarthritis, using patch clamp techniques and dielectrophoresis.

Chondrocytes were isolated from canine articular cartilage by standard methods (Lewis et al., 2011), from dogs euthanased for unrelated clinical reasons. The in vitro model consisted of treatment with 10ng/ml TNFα and IL-1β for 72h in culture. Cells were used up to and including the third passage. Whole cell patch clamp electrophysiology was used to determine the membrane potential of the cells. 3-dimensional dielectrophoresis (DEP) technology was used to determine the DEP spectrum of the cells, the electrical properties of the plasma membrane and the cytoplasm including membrane conductance and capacitance, as well as cytoplasmic conductivity. Data are expressed as mean ± standard error, p-values are from unpaired t-tests.

Healthy chondrocytes exhibit depolarised resting membrane potentials (~−10mV) so we investigated if membrane potential was affected in cells from the in vitro model, using a whole cell patch clamp configuration. Compared to the healthy cells, cells from the in vitro model had a whole cell reversal potential (Vr) 16±2mV more positive (n=5, p<0.05). Healthy cells also possessed a greater whole cell conductance than cells from the in vitro model (1.8±0.6nS vs 0.9±0.1nS; p<0.05). Healthy chondrocytes had a significantly smaller capacitance than the cytokine-treated cells (4.1±0.2pF in healthy cells vs 8.7±0.5 pF/m² in cytokine treated cells; n=35 vs 32, p<0.001) as determined by DEP.

These results show significant changes in membrane properties between control cells and cells stimulated with pro-inflammatory cytokines. Future studies are necessary to determine which ion channels are contributing to these altered membrane potentials and if these changes ultimately result in altered cell function.


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PCB124

Exploring the effect of tacrolimus on the renal kinome: identification of novel phosphoproteins

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Tacrolimus is a calcineurin inhibitor (CNI), and the main immunosuppressant used in solid organ transplantation. However, it causes complications such as hypertension, acidosis, hyperkalaemia, diabetes mellitus and hypercalcemia, mirroring the metabolic syndrome, which may be mediated by altered renal tubular transport mechanisms (Jain et al., 1999; Kim et al., 2004). As calcineurin is a protein phosphatase, and tacrolimus has been shown to alter the activity of serine/threonine kinases, we used quantitative phosphoproteomics to identify novel phosphoproteins involved in pathways that lead to the adverse effects of tacrolimus.

Male C57BL/6J mice of 6-8 weeks of age (23.4-25.7g) were administrated 2mg/kg/day tacrolimus or vehicle by intraperitoneal injections for two weeks. At the end of the treatment period they were euthanized by cervical dislocation, under Schedule 1, and the kidneys were isolated and removed. The cytoplasmic and membrane fraction of the renal cortices were separated by centrifugation. The proteins were reduced, alkylated and digested by trypsin. Peptides were then labelled with isobaric Tandem Mass Tags, followed by phosphopeptide enrichment. Vehicle and tacrolimus treated samples were mixed together and injected into the liquid chromatography-mass spectrometry (LC-MS/MS) for analysis. LC-MS/MS detected 415 unique phosphopeptides in the cytoplasmic fraction and 622 in the membrane fraction. In both fractions of the renal cortices, ~21% of the phosphopeptides showed a minimum of 10% increase in phosphorylation and ~26.5% showed a minimum of 10% decrease. Several cortical calcium-related proteins were identified in this study and were shown to be highly phosphorylated following tacrolimus treatment. These include adenylyl cyclase 6 (+23.41%), calnexin (+3.54-16.98%), CACNA1E (Voltage-dependent R type Calcium channel) (+22.2%) and Cdhr5 (Cadherin-related family member 5) (+21.64-360.54%). Other distal calcium- transporting proteins, plasma membrane Ca2+ ATPase (PMCA) and the renal sodium-calcium exchanger (NCX1) were quantified using western blot analysis. Values are presented as means ± S.E.M.; statistical significance was calculated using unpaired t-test. Both PMCA and NCX1 showed a significant increase following tacrolimus treatment (NCX1: 1±0.18 vs. 2.76±0.27 tacrolimus treated, n=5, p<0.001), (PMCA: 1±0.14 vs. 3.02±0.24 tacrolimus treated, n=5, p<0.0001).

These data suggests that the effects of calcineurin inhibition on renal calcium transport and their potential regulators are complex, profound and may relate to hypercalcemia. Further investigation into the role of these novel candidates in the adverse effects of CNIs is warranted.


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**PCB125**

**Effect of SGLT1 inhibitor phloridzin, metformin and azithromycin on glucose homeostasis in the fluid-filled adult rat lung**

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Maintaining a low glucose concentration in airway surface liquid (ASL) is important for defence against infection. The Na+-coupled glucose cotransporter SGLT1 is present in the lung and may aid in removing glucose which diffuses across the epithelium into the ASL¹. Metformin decreased glucose diffusion across airway in vitro² and azithromycin is thought to have similar effects by increasing transepithelial electrical resistance³. Metformin has also been shown to alter ion transport processes across epithelia⁴. Thus, we tested the hypothesis that glucose concentration in ASL is modulated by inhibitors of SGLT1, metformin and azithromycin.

Wistar rats were given terminal anaesthesia with intra-peritoneal (IP) injection of 0.5 ml Hypnorm/0.5 ml Hypnovel /1 ml water at a final dose of 2.7 ml/kg. The circulation to the lungs was isolated by cardiac bypass and perfused with a modified Ringer’s solution + 5mM glucose. The lung lumen was filled with the same solution (10ml/kg)⁵, without glucose, but with Blue Dextran (5mg.ml⁻¹); an impermeant tracer to determine lung liquid absorption rate (Jv). The SGLT inhibitor phloridzin (PZ) 100μM, was instilled into the lungs after a period of control sampling. Metformin (MET) 20mg was given IP on 2 consecutive days prior to the experiment, and azithromycin (AZ) 10mg.kg⁻¹ was given IP for 1 day prior to the experiment. PZ increased glucose concentration in the lung lumen from 0.053 ± 0.008 mM to 0.362 ± 0.045 mM over a period of 70 minutes (P<0.01; n=6). There was no significant effect of treatment on final lung lumen glucose concentration, compared to vehicle control (0.298 ± 0.14 mM to 0.915 ± 0.29 mM; P<0.09; n=7) with MET (0.185 ± 0.09 mM to 0.663 ± 0.18 mM; P<0.59; n=3) or AZ (0.175 ± 0.09 mM to 0.7890 ± 0.23 mM; P<0.79; n=3) on the PZ induced change in glucose concentration. PZ decreased Jv from -0.024 ± 0.003 ml.min⁻¹.dry lung weight⁻¹ to -0.012 ± 0.003 ml.min⁻¹.dry lung weight⁻¹ (P<0.02; n=6) consistent with inhibition of SGLT1 mediated Na⁺ uptake which drives fluid absorption. Treatment with MET or AZ, produced the same effect as PZ alone. AZ -0.015 ± 0.001 ml.min⁻¹.dry lung weight⁻¹ (P=0.61; n=3); MET -0.016 ± 0.001 ml.min⁻¹.dry lung weight⁻¹ (P<0.03; n=3); AZ and MET vehicle control -0.009 ± 0.002 ml.min⁻¹.dry lung weight⁻¹ (P=0.43; n=7).

In conclusion, transport via SGLT1 removes glucose that diffuses across the lung epithelium into the ASL and this process also contributes to lung fluid absorption. Thus SGLT1 maintains a low glucose concentration in ASL. There was no effect of metformin or azithromycin on glucose diffusion into ASL or on SGLT function using this treatment regimen.

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**PCB126**

**Intestinal absorption, bioavailability and metabolism of maslinic acid, a pentacyclic triterpene from Olea europaea L**

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Scope: The intestinal epithelium is a selective barrier that facilitates the absorption of essential compounds from the diet. In this sense, prediction of the oral bioavailability of bioactive compounds, such as maslinic acid, has to consider not only the uptake but also the subsequent metabolism. Therefore, the aim of the present work was to investigate in the rat: 1) the mechanism of transport of maslinic acid in the apical membrane of enterocytes, 2) the bioavailability after oral and intravenous administration and 3) the metabolism in plasma and urine.

Methods: Maslinic acid absorption was studied with an in vivo perfusion technique. Overnight fasted Sprague-Dawley rats (272-300 g) were anesthetized with ketamine (90 mg/kg) and xylacine (10 mg/kg). A 20-cm jejunal segment was perfused with a phosphate buffer containing 13 concentrations of maslinic acid from 0.01 to 20 μM. In each experiment, five different concentrations were used in triplicate at a flow rate of 3.6 mL/min for 5 minutes with re-circulation. For the bioavailability study, maslinic acid was administered orally (50 mg/kg) and intravenously (1 mg/kg). Blood was withdrawn at different time points over 24 hours through the saphenous vein. Plasma was extracted with ethyl acetate before being injected to an LC-APCI-MS. Metabolite profile was characterized by LC-APCI-LTQ-Orbitrap-MS in plasma and urine obtained at 45 min after oral administration.

Results: Kinetic analysis with the Enzfillter software showed that data was best adjusted to a first order equation with an apparent diffusional constant (Kd) of 7.01 µL/5 min/mg dry weight, without the participation of an active transporter, as evidenced with perfusions with dinitrophenol (200 μM). Plasmatic data analyzed with the WinNonlin software was best fitted to a bicompartamental model with first order absorption and linear elimination processes. The pharmacokinetic parameters indicated a relatively rapid absorption (Ka = 0.52 1/h) with a maximum concentration of 4.03 µM at 30 min, and an oral bioavailability of 5.13%. Screening for metabolites in plasma yielded four monohydroxylated derivatives


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ter (V1/2) relates to the Na/K pump’s affinity for Na+ cations. Voltage dependence of the charge (Q) moved in these processes is given by the equation: Q = ±0.21 ± 0.03 (n=11) for wild type (WT), 0.23 ± 0.05 (n=6) for N333K, 4.06 ± 1.4 (n=8) for N785K and 0.32 ± 0.08 (n=7) for N333K/N785K. In the presence of 125 mM Na+o, Na/K pumps undergo voltage-dependent transitions between Na+-bound and Na+-free states that produce transient currents. Voltage dependence of the charge (Q) moved in these transitions follows a Boltzmann distribution where the center (V1/2) relates to the Na/K pump’s affinity for Na+o (~25 mV shift per 2-fold affinity change (2)). The V1/2 of these Q-V curves was (in mV): -46 ± 1.3 (n=9) for WT, -120 ± 8 (n=8) for N785K and -92 ± 1 (n=8) for N333K/N785K, indicating a reduction in Na+o affinity; ~10-fold in single mutants and ~4-fold in N333K/N785K. Measurement of the mutants’ apparent affinity for intracellular Na+, by following the [Na+]o dependence of total Q moved in inside-out patches as previously described (3), is underway. Non-additive effects of the mutations demonstrate coupling between these residues.

Molecular dynamics simulations (150 ns) of the mutations in ion-bound crystal structures explain why the two mutations occur concurrently. In particular, the reduced ion affinity induced by K785S is “rescued” by the presence of K333R, as the latter repels K785, forcing it inside the ion-binding pocket where it substitutes one Na+ or K+. Indeed, simulations of N333K/N785K with one ion removed from the structures increased ion-binding site stability, consistent with a proposed altered stoichiometry. To determine the stoichiometry of N333K/N785K we measured 86Rb+ (K+ congener) uptake in oocytes under TEVC and found that, as expected, wild-type pumps imported 2.11 ± 0.07 (n=40) Rb+ per charge extruded, while N333K/N785K imported 1.01 ± 0.05 (n=13) Rb+ per charge. Thus, this reduced 2 Na+:1 K+:1ATP stoichiometry enables the shrimp to maintain a much larger Na+ gradient than other animals.

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PCB127

Molecular mechanism by which brine shrimp Na/K pumps confer high-salinity adaptation

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In most animal cells, the Na/K pump maintains the electrochemical gradient for Na+ across the plasmalemma by extruding 3 Na+ and importing 2 K+ per ATP molecule hydrolyzed. Brine shrimp (Artemia franciscana) adapted to high salinity express a Na/K pump α subunit that has two Asn-to-Lys substitutions within the normally conserved ion-binding region (1). To address the molecular mechanisms by which these substitutions are advantageous, we expressed Xenopus α1β1ε3 pumps in Xenopus oocytes and used two-electrode voltage clamp (TEVC) and giant inside-out patches to evaluate the functional effect of introducing these substitutions (N333K and N785K), individually and concurrently.

TEVC was performed on Na+-loaded oocytes to study external ion binding. The K0.5 for activation of Na/K pump current by external K+ (Ko+) at 0 mV, without Na+j, was (in mM): 0.21 ± 0.03 (n=11) for wild type (WT), 0.23 ± 0.05 (n=6) for N333K, 4.06 ± 1.4 (n=8) for N785K and 0.32 ± 0.08 (n=7) for N333K/N785K. In the presence of 125 mM Na+o, without K+o, Na/K pumps undergo voltage-dependent transitions between Na+-bound and Na+-free states that produce transient currents. Voltage dependence of the charge (Q) moved in these transitions follows a Boltzmann distribution where the center (V1/2) relates to the Na/K pump’s affinity for Na+o (~25 mV shift per 2-fold affinity change (2)). The V1/2 of these Q-V curves was (in mV): -46 ± 1.3 (n=9) for WT, -120 ± 8 (n=8) for N785K and -92 ± 1 (n=8) for N333K/N785K, indicating a reduction in Na+o affinity; ~10-fold in single mutants and ~4-fold in N333K/N785K. Measurement of the mutants’ apparent affinity for intracellular Na+, by following the [Na+]o dependence of total Q moved in inside-out patches as previously described (3), is underway. Non-additive effects of the mutations demonstrate coupling between these residues.

Molecular dynamics simulations (150 ns) of the mutations in ion-bound crystal structures explain why the two mutations occur concurrently. In particular, the reduced ion affinity induced by K785S is “rescued” by the presence of K333R, as the latter repels K785, forcing it inside the ion-binding pocket where it substitutes one Na+ or K+. Indeed, simulations of N333K/N785K with one ion removed from the structures increased ion-binding site stability, consistent with a proposed altered stoichiometry. To determine the stoichiometry of N333K/N785K we measured 86Rb+ (K+ congener) uptake in oocytes under TEVC and found that, as expected, wild-type pumps imported 2.11 ± 0.07 (n=40) Rb+ per charge extruded, while N333K/N785K imported 1.01 ± 0.05 (n=13) Rb+ per charge. Thus, this reduced 2 Na+:1 K+:1ATP stoichiometry enables the shrimp to maintain a much larger Na+ gradient than other animals.

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Elevated serum uric acid (SUA) or urate, causing gout, has been linked to cancers, especially prostate cancer (PCa) (1). Activins, inflammatory cytokines of the TGFβ superfamily, act as negative growth regulators in the prostate, and activin insensitivity is considered a hallmark of PCa progression. However, the underlying molecular mechanisms of activin insensitivity and ‘cellular uric acid homeostasis’ (CUAH) in PCa are unknown. This study aimed to determine how a disturbance of CUAH counters activin growth inhibitory effects of activins in PCa, and to identify the transporter facilitating this.

Expression of activin A and B (ActA, ActB), urate transporter GLUT9 (GLUT9-kd) were tested on LNCaP cell growth. Finally, [14C]-urate transport studies were conducted on LNCaP cells using probenecid.

ActA expression was increased in low-grade PCa (normal: 5.6 ± 1.1 vs GS4-5: 9.0 ± 1.8, n=11-12, p<0.05), whereas ActB expression was reduced in high-grade (normal: 5.9 ± 1.1 vs GS4-5: 3.9 ± 1.6, n=20 and 11, p<0.05) and extra-capsular spread (5.9 ± 1.1 vs 3.2 ± 1.4, n=20 and 24, p<0.05) PCa. Intracellular urate levels decreased in all prostate pathologies, while expression of GLUT9 decreased in benign prostatic hyperplasia (BPH, normal: 7.6 ± 0.9 vs BPH: 2.9 ± 0.9, n=12 and 26, p<0.05), prostatitis (7.6 ± 0.9 vs 3.0 ± 0.7, n=12 and 9, p<0.05) and high-grade PCA (7.6 ± 0.9 vs 5.4 ± 0.7, n=12 and 20, p<0.05, Mann-Whitney U test). Activin responsive LNCaP cells had higher intracellular (90.1% ± 9.7 vs 32.53% ± 12.53) and lower secreted (9.9% ± 9.7 vs 67.47% ± 12.53, n=3, p<0.05, ANOVA) urate levels than activin insensitive PC3 cells. LNCaP and DU145 cells showed a decrease of GLUT9 mRNA consistent with prostate disease tissue and PCA cell protein expression. Normal and high extracellular urate (300 and 500µM) was growth promoting in vitro (122% and 165%, n=3-5, p<0.05, ANOVA), and it antagonised the growth inhibitory effects of ActA and ActB. This was abolished by the gout medication probenecid and reduced by a GLUT9-kd (100% ± 12.5 vs 84% ± 16.6, n=3, p<0.05, ANOVA). [14C]-urate transport in LNCaP cells was inhibited by probenecid (40%, n=3, p=0.0001, t-test) and matched by a similar inhibition of LNCaP cell proliferation under high extracellular urate.

Implications: Changes of CUAH facilitated by GLUT9 significantly impact prostate cancer cell growth, and lowering SUA levels in PCa could be of therapeutic benefit (2).
Measures of the peripheral chemoreflex do not predict oxygenation in the setting of acute steady-state hypoxia

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The carotid bodies, which detect changes in arterial PO2 and elicit a peripheral chemoreflex (PCR), are particularly important in their role in acclimatization to altitude. Ventilatory acclimatization increases the PCR magnitude at altitude, improving oxygenation, and potentially protecting against acute mountain sickness (AMS). The PCR magnitude can be tested through steady-state techniques that require sophisticated equipment and lack portability, decreasing their utility in field studies at altitude. The transient hypoxia test is simple and more portable, but making participants more hypoxic while at altitude may be uncomfortable and is potentially dangerous. We tested the feasibility of using a transient hyperoxic ventilatory withdrawal (TT-HVV) test of the PCR while in the setting of simulated high altitude (normobaric hypoxia), and compared these responses to (a) a previously characterized transient hypoxic ventilatory response (HVR) test (TT-HVR) in room air and (b) a poikilocapnic hypoxia test during acute steady-state hypoxia (SS-HVR), within-individuals. Participants were recruited (n=15; 28.5±6.5 yrs; BMI 24.7±3.1 kg/m²; six males; SD) and were positioned in a dentist chair in semi-recumbent position in a dark room with white noise fed through ear buds. Participants were instrumented with a pneumotachometer and end-tidal gases were sampled from the mouth-piece in percent using a dual O2 and CO2 gas analyzer, and expressed in mm Hg using dialing atmospheric pressure (BTPS). While breathing room air, participants underwent five consecutive trials of a TT-HVR (three-breaths of 100% N2) to calculate HVR magnitude. Participants were then exposed to a fraction of inspired (Fi)O2 of 13.5-14% (simulated 4500-5000m in Calgary; Patm≈665 mm Hg) for 25-30 minutes until steady-state was achieved, where an additional index of the poikilocapnic HVR was calculated by comparing ventilation (L/min) and calculated oxygen saturation (ScO2) between baseline and steady-state hypoxia. Three consecutive trials of a TT-HVV (one-breath 100% O2) were then administered and quoted. The TT-HVR response (0.37±0.04 L/min/Δ%ScO2) was larger than the TT-HVV (0.22±0.03 L/min/Δ%ScO2; P<0.001) and the SS-HVR (0.1±0.01 L/min/Δ%ScO2; P=0.003; all ±SEM; all one-f RM ANOVA). No test of the PCR was correlated with any other test (r<0.32, P>0.25), nor was any test of the PCR correlated with indices of oxygenation while in steady-state hypoxia: partial pressure of end-tidal (PET)O2 (r<0.2, P>0.47) or ScO2 (r<0.2, P>0.48; all Pearson r correlations). Our results demonstrate that any test of the PCR at low altitude does not predict indices of oxygenation while in steady-state hypoxia, and may have minimal utility in predicting ventilatory acclimatization and protection from hypoxia during gradual ascent to altitude, where individuals are variably susceptible to AMS.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Hypoxic ventilatory response magnitude is not correlated with breath-hold duration during voluntary apnea

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The control of voluntary breath-holding is multifactorial, with contributions from cognitive factors, sex hormones, initial lung volume, lung stretch and stimulation of respiratory chemoreceptors. Breath-holding elicits chemoreceptor stimulation through increases in CO2 and decreases in O2, which change in proportion to metabolic rate. The peripheral respiratory chemoreceptors (PCRs) detect decreases in O2 (hypoxia), eliciting an increase in drive to breathe (i.e., hypoxic ventilatory response: HVR). We know that breath-hold duration is positively related to prevailing inspired oxygen. However, how HVR magnitude influences breath-hold duration (BHD) is unclear. We hypothesized that (a) voluntary BHD would be positively correlated to the initial oxygen status prior to breath-holding and (b) the HVR magnitude would be negatively correlated with voluntary BHD, within-individuals. In 16 healthy participants (25±8 yrs; BMI 24.2±3.5 kg/m²; six males), three voluntary breath-holds were performed: (a) following five breaths of 100% O2 (hyperoxia), (b) following breathing room air (normoxia), and (c) after >30 min of breathing steady-state fraction of inspired (Fi)O2 of 13.5-14% (hypoxia). In a subset of participants (n=12) we performed a transient test of the HVR, comprised of five trials of three consecutive breaths of 100% N2, where the change in ventilation was indexed against change in calculated oxygen saturation (Severinghaus transform; ΔL/min/Δ%ScO2). Subsequent analysis indicated that BHD was positively related to initial oxygen status: 89.9±9.5 sec in hyperoxia, 53.8±4.1 sec in normoxia and 40.4±3.5 sec in hypoxia (P<0.001). Interestingly, hyperoxia increased BHD by 65.1±10.9% compared to normoxia, but only reduced it by 24.5±3.6% in hypoxia compared to normoxia, and almost three-fold difference in the influence of background oxygen status. However, the HVR magnitude (0.38±0.04 L/min/Δ%ScO2) was not correlated with BHD in hyperoxia (r=0.46, P=0.13), normoxia (r=0.537, P=0.07) or hypoxia (r=0.45, P=0.14). Our data suggest that (a) although PCRs are likely activated during breathing-holding, they contribute differentially to BHD, within-individual, (b) the magnitude of the normoxic HVR does not predict BHD, and (c) other physiological and psychological factors are more important in determining BHD.

NSERC

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Effects of aerobic exercise on quality of life, depression and cognition in subjects with Multiple Sclerosis

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Multiple Sclerosis (MS) is a progressive neurological disorder of the central nervous system (CNS) characterised by episodes of inflammatory demyelination and axonal deterioration. Neurological damage can result in a spectrum of symptoms including spasticity, depression and cognitive abnormalities.

Physical activity is proposed to target multiple clinical manifestations in MS, and may improve overall quality of life in MS patients. In this study, we investigate whether a cycle ergometry training programme has therapeutic potential in individuals with MS by improving quality of life and depressive symptomatology, while ameliorating cognitive disturbances.

Healthy volunteers (n=10) and MS patients (n=10) were recruited and informed consent was obtained from each participant. Participants cycled for 30 minutes at 65-75% VO₂max on a recumbent ergometer, and this session was repeated twice a week for 8 weeks. Assessments were performed pre- and post-training, including the Quick Inventory of Depressive Symptomatology and the MS Quality of Life questionnaire. Cognitive performance were assessed using CANTAB and the Symbol Digit Modality Test (SDMT). Data were analysed using Student’s t-test and two-way ANOVA.

We determined that quality of life was reduced in MS patients, compared to healthy subjects, with a reduction in physical (90.6±2.2% con vs. 47.8±4.0 MS; p<0.001) and mental (90.3±0.5% con vs. 55.1±0.6 MS; p<0.001) health, observed. Exercise improved both physical (47.8±4.0% pre-exercise vs. 69.9±3.2% post-exercise) and mental (55.9±7.6 pre-exercise vs. 76.3±4.8 post-exercise) health in MS patients. In support of this, exercise was shown to reduce depressive symptomatology in MS patients (1.4±0.5 pre-exercise vs 0.3±0.2 post-exercise; p<0.01). We determined lower SDMT scores in MS patients pre-exercise (45.3±3.4) versus healthy individuals (58.8±3.0; p<0.05), and importantly, exercise was associated with an improvement in SDMT score (48.6±4.5) in MS patients, indicating that exercise was associated with an improvement in information processing speed. Exercise was also associated with an improvement in visual sustained attention (rapid visual information processing; RVP). Pre-exercise, healthy subjects had higher RVP hits (40.6±1.8 con vs. 33.0±4.1 MS), and demonstrated a faster mean latency (390.4±31.3ms con vs. 432.1±29.8ms MS) than MS patients. Exercise improved RVP hits (37.0±4.1 post-exercise) and RVP latency (393.5±25.9ms post-exercise) in MS subjects. Our findings indicate that ergometry training is associated with improvements in quality of life and depression indices in MS patients. Further analysis will correlate cognitive improvements with inflammatory signatures in peripheral blood.

Grant support from the Physiological Society. Ethical approval from the Clinical Research Ethics Committee of Cork Teaching Hospitals.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Paired corticospinal-motoneuronal stimulation increases maximal voluntary activation of human adductor pollicis muscle

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Paired corticospinal-motoneuronal stimulation (PCMS) is a technique in which transcranial magnetic stimulation (TMS) of motor cortex is repeatedly paired with stimulation of motor axons in a peripheral nerve to produce spike-timing-dependent plasticity at corticospinal-motoneuronal synapses in the human spinal cord. TMS evokes presynaptic volleys in corticospinal terminals and antidromic activation of motoneurones supplies postsynaptic depolarisation. Studies have shown that, depending on the interstimulus interval of the paired stimuli, delivery of 50-100 pairs can facilitate or depress both evoked and voluntary motor responses (Taylor & Martin 2009; Bunday & Perez 2012; Fitzpatrick et al 2016). However, it is not known if such stimulation can increase maximal voluntary output. Hence, this study aimed to determine whether PCMS increases maximal voluntary activation of the hand muscle, adductor pollicis. On two days, subjects (n=14) performed isometric maximal voluntary contractions (MVCs) of the thumb adductor before and after PCMS or a control protocol. PCMS comprised 100 pairs of TMS (figure 8 coil, 60-95% stimulator output) and supramaximal ulnar nerve stimulation at 0.1 Hz.

Interstimulus intervals (3.3-7.8 ms) were calculated so that presynaptic volleys were estimated to arrive at the synapse 1.5 ms prior to postsynaptic depolarisation. The control protocol comprised TMS at 0.1 Hz. Five brief 2-3 s MVCs were performed (2 min apart) prior to and starting at 0, 20 and 40 min after the intervention. Single supramaximal ulnar nerve stimuli were delivered during and 2-4 s after each MVC to evoke superimposed and resting twitches from adductor pollicis. Voluntary activation (%) was calculated as 100 x (1 - superimposed twitch/resting twitch). Adductor pollicis EMG was also recorded. Changes from baseline were calculated for each parameter and were compared for PCMS and control protocols with 2-way repeated measures ANOVAs (protocol X time [0, 20, 40 min post]). The superimposed twitch was reduced and hence, voluntary activation increased by 4.4% [1.4, 7.5] (mean [95%CI]) after PCMS compared to TMS alone (F₁,26=10.2, p=0.007). Maximal EMG was non-significantly higher (8.1% [0.6, 16.8], F₁,26=4.014, p=0.066) and the resting twitch did not differ (0.1% [5.7, 5.9] smaller). Thus, there was a small improvement in the ability of subjects to drive the muscle maximally after PCMS. This suggests that PCMS-induced changes in the transmission of corticospinal input are effective for high threshold motoneurones as well as the low threshold ones recruited by evoked responses and weak contractions. If PCMS can be applied reliably, it may have therapeutic potential to improve strength in patients with muscle weakness caused by impaired corticospinal drive.


This work was funded by the National Health and Medical Research Council of Australia.

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**PCB135**

L-Arginine supplementation does not impact cerebrovascular function in young adults

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**Background:** L-Arginine (LA) is an amino acid, which when combined with oxygen, catalyses nitric oxide (NO), a potent vasodilator that is important for vascular regulation (1). Due to the anti-athrogenic properties of NO (2), there has been increasing interest in the potential therapeutic use of LA to treat vascular disease. Both long (3) and short-term (4) supplementation have been linked to improvements in vascular function in the systemic circulation. However, less is known about its influence on the cerebral vasculature.

**Methods:** Eight healthy males (age: 21 ± 1 years; body mass index: 24.3 ± 3.4 kg.m⁻²) participated in this single-blinded randomised placebo-controlled trial. Middle cerebral artery velocity (MCAv; transcranial Doppler ultrasound), mean arterial pressure (MAP; photoplethysmography) and end-tidal carbon dioxide (CO₂; capnography) were continuously recorded throughout each testing session, which was preceded by either a 3-day oral supplementation of LA (x7 500mg capsules per day) or placebo powder (flour). Cerebrovascular resistance (CVR) and cerebrovascular conductance (CVC) were calculated as MAP/MCAv and MCAv/MAP respectively, following a 7-day “washout” period between trials. Exhaled nitric oxide (ozone-based chemiluminescence) was measured on the morning of each visit. Following confirmation of distribution of normality (Shapiro-Wilk Tests), data were analysed using paired samples t-tests. Significance was established at P < 0.05 and data are expressed as mean ± SD.

**Results:** Supplementation with LA elevated exhaled NO (Figure 1; P < 0.05). However, this did not confer any changes in cerebrovascular function (Table 1).

**Conclusion:** These findings indicate that short-term oral supplementation of LA does not impact cerebrovascular function in healthy young adults. Whether LA supplementation influences cerebrovascular function in clinically relevant populations remains to be fully elucidated.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>L-Arginine</th>
<th>P Values</th>
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</thead>
<tbody>
<tr>
<td>MCAv (cm.s⁻¹)</td>
<td>60.1 ± 6</td>
<td>59.0 ± 5</td>
<td>0.11</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>93 ± 7</td>
<td>92 ± 6</td>
<td>0.56</td>
</tr>
<tr>
<td>CVR (mmHg.cm⁻¹.s)</td>
<td>1.45 ± 0.22</td>
<td>1.47 ± 0.30</td>
<td>0.79</td>
</tr>
<tr>
<td>CVC (mmHg.cm⁻¹)</td>
<td>0.75 ± 0.15</td>
<td>0.69 ± 0.15</td>
<td>0.70</td>
</tr>
<tr>
<td>CVR_CVC</td>
<td>5.37 ± 1.03</td>
<td>5.54 ± 0.93</td>
<td>0.90</td>
</tr>
<tr>
<td>CVR/100/CVC</td>
<td>2.38 ± 0.32</td>
<td>2.62 ± 0.86</td>
<td>0.40</td>
</tr>
<tr>
<td>CVR/CVC*</td>
<td>0.57 ± 1.43</td>
<td>5.96 ± 4.60</td>
<td>0.42</td>
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</tbody>
</table>

Values are mean ± SD

**Figure 1.** Changes in exhaled NO following a 3-day supplementation of either placebo or L-Arginine.

Values are mean ± SD; *P < 0.05 vs. placebo.


The present research was supported by the JPR Williams Trust, in co-ordination with the University of South Wales.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

**PCB136**

Measuring skeletal muscle oxidative capacity to assess the underlying physiology of reduced exercise performance with aging

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**Background**

The physiological mechanisms underlying reduced exercise performance in older adults are poorly understood. Maladaptation’s of skeletal muscle oxidative function with age have been implicated but are difficult to measure non-invasively during exercise. Therefore, reliably measuring muscle oxidative capacity alongside traditional markers of cardiorespiratory fitness is an important step towards better understanding exercise physiology with age.

**Method**

Participants (n=96; 72=male, mean age=71.5±6.3years) undertook a 6-minute stepper test. Oxygenated(HbO₂) and deoxygenated haemoglobin(Hb) were monitored in the calf muscle using near infrared spectroscopy(NIRS) and peak
Effects of knee joint angle and contraction intensity on quadriceps and hamstring coactivation in healthy young humans

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Introduction: The coordination of agonist and antagonist muscles is essential for the correct execution of movements. For instance, the simultaneous activation of agonist-antagonist muscles (coactivation) is integral to the maintenance of knee joint stability (1). In this regard, it is logical to hypothesize that the coactivation of the quadriceps and hamstring muscles could depend on knee joint angle and contraction intensity. Therefore, we investigated the neural activation of the quadriceps and hamstring muscles during knee extension performed at different knee joint angles and contraction intensities in healthy young humans.

Methods: 13 men (24.3 ± 2.8 years) and 13 women (23.4 ± 2.4 years) participated. Maximal voluntary isometric contraction (MVIC) of knee extensors (KE) and flexors was assessed at two knee joint angles (90° and 60°, 0° = full extension). At each angle, participants performed a 5 second isometric contractions at 20, 50 and 80% of KE MVIC in a random order. Surface EMG was recorded from vastus lateralis (VL) and biceps femoris (BF) muscles and the root mean square (RMS) amplitude calculated. To quantify the agonist (VL) and antagonist (BF) neural activity, at each contraction intensity, EMG RMS values for each muscle were normalized to the RMS amplitude for that muscle when acting as an agonist during MVIC at the same angle (2).

Results: For BF coactivation, interactions for intensity × sex (p<0.01) and intensity × angle (p<0.01) were observed. Regardless of angle, women exhibited a higher level of BF coactivation with increasing contraction intensity than men (Fig. 1A). Additionally, irrespective of sex, the coactivation level was higher at 90° than at 60° with a more evident difference at higher intensities (Fig. 1B). VL activation increased with contraction intensity at 90° and 60° for both sexes with a more marked increment at 90° than 60° for women but not for men (Table 1). Conclusion: The main findings were: 1) a more flexed knee joint angle (90°) was associated with an augmented coactivation level; 2) a higher level of BF coactivation was observed at higher contraction intensities; 3) women exhibited a higher antagonist coactivation than men especially at the highest contraction intensity (80%MVIC). Overall, the observed increment in antagonist coactivation as a function of joint angle and contraction intensity may play an important role in maintaining joint stability by providing synergistic action to counteract the agonist’s increasingly destabilizing influence. This is of particular relevance for women.

Table 1: VL activation values (mean ± SD). *p<0.05, **p<0.01, ***p<0.001 significant different from 60°

<table>
<thead>
<tr>
<th>Contraction Intensity</th>
<th>Men 60°</th>
<th>Men 90°</th>
<th>Women 60°</th>
<th>Women 90°</th>
</tr>
</thead>
<tbody>
<tr>
<td>20%MVIC (%)</td>
<td>24.1 ± 19.5</td>
<td>14.1 ± 5.3</td>
<td>21.6 ± 2.7</td>
<td>16.5 ± 2.8</td>
</tr>
<tr>
<td>50%MVIC (%)</td>
<td>56.3 ± 29.2***</td>
<td>39.9 ± 9.7</td>
<td>50.7 ± 7.8**</td>
<td>43.2 ± 14.5</td>
</tr>
<tr>
<td>80%MVIC (%)</td>
<td>88.5 ± 14.1***</td>
<td>70.6 ± 11.0</td>
<td>99.6 ± 15.9***</td>
<td>72.2 ± 12.0</td>
</tr>
</tbody>
</table>

Fig. 1: BF coactivation (mean ± SEM) across three intensities for both sexes (A) and angles (B). *p<0.05, **p<0.01 significant difference between men and women (A) and between 90 and 60° (B). #p<0.01, ##p<0.001 significant difference from 20%MVIC up to 80%MVIC.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Acknowledgements of thanks to the participants of the Southall And Brent REVisted (SABRE) study and the funding bodies; Wellcome Trust & British Heart Foundation.

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Impaired critical speed in mice with sickle cell anemia: Implications for therapeutic development

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Sickle cell anemia results in vascularopathy, organ damage, and impaired cardiorespiratory function and exercise tolerance likely due to a combination of central and peripheral abnormalities stemming from deranged hemoglobin (Hb). A transgenic mouse model of sickle cell anemia has been developed to help elucidate the mechanisms of vascular and organ damage, but a valid and reproducible measurement of exercise capacity and the severity of impaired physical function have yet to be determined in this model. Therefore, the purpose of this investigation was to measure the speed/duration relationship, known as critical speed (CS), and the anaerobic work capacity (AWC, the finite work capacity available above CS) in healthy wild type mice (WT) and mice expressing human HbSS (BERK). Following ethical approval from the institutional animal care and use committee (University of Colorado, Denver), six young-adult female mice (WT, n=3 and BERK, n=3) performed 5 constant-speed treadmill tests that resulted in fatigue within the range of 1.5 to 20 min. Additionally, the speed at lactate threshold (T_lac) was determined ("5 μl blood sample, Lactate Pro Analyzer) during an incremental exercise test consisting of 9x2 min runs separated by 20 second intervals at speeds corresponding to 70-110% of maximal CVC (%CVCmax) and 20 second intervals. Data are presented as cutaneous vascular conductance (CVC; flux/MAP) and CVC expressed as a percentage of maximal CVC (%CVCmax). For all protocols, linear mixed models (main effects of intervention and time) were used to examine the effect of acute tea ingestion on forearm cutaneous vascular perfusion.

Introduction. Dietary flavonoids, such as those present in black tea, are associated with reduced cardiovascular disease (CVD) risk. Tea ingestion is linked to reduced blood pressure(1) and improved nitric oxide-mediated, endothelium-dependent dilation of conduit arteries.(2) The potential impact of tea ingestion on microvascular function has not been explored. The aim of this study, therefore, was to examine the effect of acute black tea ingestion on cutaneous microvascular function.

Methods. Twenty healthy participants (58±5 yr, BMI 26±4, 9 men) attended two experimental trials (tea and placebo), 7 days apart in a randomised, controlled, double-blind, crossover design. Participants ingested 200ml black tea or placebo, followed by assessment of forearm cutaneous perfusion using laser-Doppler flowmetry (LDF) and 3 distinct local heating protocols: 1. rapid 42°C,(3) 2. rapid 39°C(4) and 3. gradual 42°C.(5) On the contralateral arm, full-field laser perfusion imaging (FLPI) was used to assess forearm perfusion during gradual 42°C.(5) Mean arterial blood pressure (MAP) was measured at 5-min intervals. Data are presented as cutaneous vascular conductance (CVC; flux/MAP) and CVC expressed as a percentage of maximal CVC (%CVCmax). For all protocols, linear mixed models (main effects of intervention and time) were used to examine the impact of acute tea ingestion on forearm skin perfusion.

Results. Baseline MAP was not different between interventions (P>0.05) and showed no change across time (P>0.05). Rapid local heating demonstrated no effect of tea for flux, CVC or %CVCmax(all P>0.05), either for rapid 39°C or rapid 42°C. Gradual local heating to 42°C produced a higher skin blood perfusion following black tea ingestion for absolute CVC (P<0.04) when measured by LDF, and higher absolute flux (P<0.001) and CVC (P<0.001) measured with FLPI. No effect of tea was found for %CVCmax when assessed by either LDF or FLPI.

Conclusion. Acute tea ingestion enhanced cutaneous vascular responses to gradual local heating to 42°C in healthy, middle-aged subjects, possibly through a mechanism related to activation of endothelium-derived chemical mediators, such as nitric oxide. These improvements may contribute to the cardiovascular health benefits of regular tea ingestion.

Funding: NIH-NHLBI T32HL007171 and NIH-R01HL125642

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Acute black tea consumption improves cutaneous vascular function in healthy middle-aged humans

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1Research Institute for Sport & Exercise Science, Liverpool John Moores University, Liverpool, UK, 2Unilever Research & Development, Vlaardingen, Netherlands and 3Department of Physiology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands

Introduction. Dietary flavonoids, such as those present in black tea, are associated with reduced cardiovascular disease (CVD) risk. Tea ingestion is linked to reduced blood pressure(1) and improved nitric oxide-mediated, endothelium-dependent dilation of conduit arteries.(2) The potential impact of tea ingestion on microvascular function has not been explored. The aim of this study, therefore, was to examine the effect of acute black tea ingestion on cutaneous microvascular function.

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Funding: NIH-NHLBI T32HL007171 and NIH-R01HL125642

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Ca²⁺ uptake and ROS production. Here we test the hypothesis that statin-induced myopathy, the most common side-effect, is paramount. We have previously shown that statin treatment in vivo increases sarcoplasmic reticulum (SR) Ca²⁺ leak from skeletal muscle linked with mitochondrial Ca²⁺ uptake and ROS production. Here we test the hypothesis that ROS-dependent post-translational modifications of the ryanodine receptor 1 (RyR1) and changes in the RyR1 molecular complex contribute to statin-induced leak.

White gastrocnemius (GAS) was dissected from male Wistar rats given simvastatin (40 mg/kg/day) or saline by oral gavage for 4 weeks. Human vastus medialis samples were obtained from patients at St James’ University Hospital. All animal experimentation was carried out in accordance with the Directive 2010/63/EU of the European Parliament. Human data was approved by the Local Research Ethics committee and complies with the principles outlined in the Declaration of Helsinki. Individuals taking statins were age- and sex-matched with control subjects. Data from n=10-11 rats and n=13-13 patients were compared with the Student’s t-test.

In RyR1 immunoprecipitated from GAS homogenates, statin treatment had no effect on RyR1 nitration (P>0.05)) but increased phosphorylation at Ser²⁸⁴⁰ (P<0.01), linked with a loss of FKBP12 (P<0.01), but not calmodulin (P>0.05), from the complex. Data from human vastus medialis RyR1 confirm results obtained with rodent GAS i.e. marked loss of FKBP (P<0.01) in the statin-treated group. FKBP normally acts to hold the RyR1 in a closed state; its loss will promote SR Ca²⁺ leak. One of the effectors of phosphorylation at the Ser²⁸⁴⁰ site of RyR1 is CaM kinase II (CaMKII), whose activity is regulated by ROS via oxidation of the regulatory domain. Statin treatment promoted an increase in oxidised CaMKII (P<0.05) without any change in CaMKII expression (P>0.05). Our data suggest that statin treatment increases ROS which activates CaMKII, phosphorylating the RyR and increasing its open probability. We propose that SR Ca²⁺ leak creates a vicious cycle whereby Ca²⁺² taken up by the closely apposed mitochondria triggers further production of ROS which acts to maintain the leak. Apoptosis is another consequence of mitochondrial Ca²⁺ overload and ROS production, and in both rodent GAS and human vastus medialis, statin treatment produced an increase (P<0.05) in the cleavage of caspase-3, the end-effector of apoptosis. SR Ca²⁺² leak is a mechanism common to several types of skeletal myopathy. Here, for the first time, we describe a mechanism for the SR leak induced by statin.

Defining the cellular process that underlies statin-induced myopathy is the first step in the development of co-therapies to improve statin compliance.

Supported by the BHF

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Acute dietary nitrate supplementation decreases muscle sympathetic nerve activity at rest and attenuates the response to static handgrip

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Acute dietary nitrate (NO₃⁻) supplementation with beetroot juice can decrease blood pressure (BP) at rest (1) and during cycling exercise (2) in healthy normotensives. The mechanism responsible for these hypotensive responses is attributed to increased systemic levels of the potent vasodilator, nitric oxide (1–3). However, evidence also supports a central sympathoinhibitory role of nitric oxide (4). We hypothesized that acute beetroot juice supplementation would decrease BP at rest and during exercise, coincident with reductions in muscle sympathetic nerve activity (MSNA). Fourteen healthy normotensive participants (7 female; 25 ± 5 years) with normal blood pressure (BP) (Finometer) and MSNA during a 3 minute baseline and 2 minute static handgrip exercise at 30% maximal voluntary contraction. All participants that acute NO₃⁻ supplementation, whereas diastolic BP (Δ± 5 vs. -3 ± 5 mmHg, p<0.05) and heart rate (Δ± 4 vs. 0 ± 4 bpm, p<0.05) were unchanged. During static handgrip, systolic (Δ± 8 vs. 11 ± 7 mmHg, p<0.05) and diastolic (Δ± 4 vs. 11 ± 4 mmHg, p<0.05) BP and heart rate (Δ± 12 vs. 13 ± 10 bpm, p<0.05) responses were similar between placebo and NO₃⁻ supplementation. In contrast, compared to placebo, MSNA burst frequency (Δ 11 vs. 10 vs. 6 ± 6 bursts/min, p<0.05) and burst incidence (Δ 8 ± 9 vs. 1 ± 8 bursts/100 heartbeats, p<0.05) responses were attenuated following NO₃⁻ supplementation. Collectively, these data demonstrate in healthy normotensive participants that acute NO₃⁻ supplementation with beetroot juice can decrease central sympathetic outflow to skeletal muscle at rest and during exercise. This provides the first evidence for a potential neural contribution to the reported improvements in resting hemodynamics, endothelial function, and exercise tolerance. Dietary NO₃⁻ supplementation may provide a novel treatment for targeting exaggerated sympathoexcitation in cardiovascular disease states (e.g. heart failure).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.


diagram
Stress during simultaneous interpreting: a pilot experiment

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Simultaneous interpreting is more than often labelled as one of the most stressful tasks. We tried to study how work conditions affect the level of stress that simultaneous interpreters have to go through while doing their job and training. Using telemetric heart rate recording, we set up a pilot experiment hoping to compare, first, how interpreters react at their booth partners’ performance, and second, if one direction of the translation is more energy-consuming than the other. 6 students took part in the experiment (4 interpreted into and from German and 2 into and from English) where they had to complete 4 tasks: shadowing in foreign language and mother tongue and interpreting into and from mother tongue. Four of the students performed at a high level, the other two were not so successful. The measurements were performed via Zephyr HxM Smart Heart Rate Monitor in the context of event-related telemetry technology, while the psychophysiological tests before and after performance measurements included an original emotional disadaptation test, campimetry, sensomotor activity test, laterometry and Stroop test.

In general students who performed at high level tended to show more stress during their partners less qualified performance along with increased parasympathetic control and wider heart rate variability, while the latter found themselves calmer in the same situation and more stressed during their own performance.

Based on the heart rate variability analysis, the first most stressful activity among the two participants turned out to be interpreting into mother tongue. It was also quite peculiar that for one of the participants shadowing in her mother tongue was surprisingly more difficult than interpreting into a foreign language.

The sensomotor activity test showed that students with good performance showed high level of cognitive control as opposed to unsuccessful ones. The majority of the subjects demonstrated right hemispheric asymmetry when measured before the experimental task and left hemispheric domination afterwards. Half of the participants revealed reverse interference in Stroop test after interpreting implying verbal satiation. Based on campimetry results analysis, all of the participants except one showed emotional comfort both before and after the task despite its obvious psychological challenges.

Meanwhile, in the emotional disadaptation test four out of six participants reported 3 to 5 times increased emotional discomfort showing a clear disconnection between subjective and objective evaluation of their functional condition. Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB144

GPCM3 gene variant protects against the development of Rheumatoid Arthritis

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Background - Genome-wide association studies identified two single-nucleotide polymorphisms (SNPs) rs204989 and rs204991, that are significantly less prevalent in individuals with Rheumatoid Arthritis (RA). We addressed whether GPCM3 SNPs result in a detectable phenotype explaining their inverse association with RA.

Methods - A WVU IRB-approved study (#1304033165) recruited 200 volunteers for blood samples. SNP genotypes were determined using TaqMan probes for rs204989 (GPCM3), rs204991 (GPCM3), rs2812378 (CCL21) and rs6457620 (HLA gene region). 3.5 kb of sequence 5′ to the GPM3 transcription start site from select volunteers was subcloned into a luciferase reporter vector to assess promoter strength as a function of genotype. THP-1 and NB4 cell lines were transfected with lentivirus expressing shRNA directed toward the GPM3 transcript to mimic decreased GPM3 expression as observed in volunteers bearing the minor allele of rs204989. Resultant cell lines were assessed in Transwell migration assays for chemotactic responsiveness.

Results - The presence of the SNP rs204989 was seen to reduce GPCM3 expression. Reduced GPCM3 expression in turn was observed to reduce THP-1 and NB4 cell line migration towards relevant chemottractants.

Conclusions - The GPM3 gene variant rs204989 may protect against RA development by decreasing GPCM3 expression and, thereby, reducing neutrophil and/or monocyte trafficking to the inflamed joint.


Giguère PM, Billard MJ, Laroche G, Buckley BK, Timoshchenko RG, McInnis MW.
OBJECTIVES: The purpose of this study was to verify the physiological and psychological effects of HM. The effect on the autonomic nervous system activity was evaluated using the visual analogue scale (VAS), (HRV), systolic and diastolic blood pressure (SBP/DBP), and nasal skin thermogram. In addition, relaxation and anxiety were evaluated using the visual analogue scale (VAS).

METHODS: The participants included 1 female and 5 male patients, who received palliative care for pain. Five of them had been diagnosed with high blood pressure, of whom 4 had achieved good blood pressure control with medicine. Five of them were evaluated using the visual analogue scale (VAS).

RESULTS: The major findings of this study are as follows: (1) the HR trendy decreased during HM (vs. HM1 p=0.050, vs. HM2 p=0.058); (2) nasal skin temperature significantly increased after HM (p=0.027); (3) SBP significantly decreased after HM (p=0.034); (4) relaxation levels significantly increased after HM (p=0.031); (5) anxiety levels significantly decreased after HM (p=0.002). The frequency analysis of HRV revealed that HM does not increase autonomic nervous system activity, ratio of low frequency to high frequency LF/HF; sympathetic nervous system activity did not reveal any significant effect of HM. CONCLUSION: The decrease in HR and SBP, and the increase in nasal skin temperature reveal the physiological relaxation effect of HM. The decrease in relaxation and anxiety show that physiological and psychological responses exist in parallel. Therefore, we believe that HM would be effective in inducing a physiological and psychological relaxation effect in a patient in need of palliative care. The frequency analysis of HRV revealed that HM does not increase autonomic nervous system activity significantly, and hence, is safe for patients with cardiovascular disease. Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB146

The relaxation effects of hand-massage therapy on autonomic nervous system function and emotions among patients receiving palliative care

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BACKGROUND: In integrative oncology, it has been suggested that massage can activate the immune function, and alleviate pain and anxiety. However, the level of evidence regarding the effectiveness of massage therapy is not high; our study aims to provide such evidence. One of the basic concepts of the WHO Definition of Palliative Care (2002) is to increase the quality of life (QOL) by relieving suffering, regardless of the stage of cancer. Therefore, investigating and reporting the relaxation effect of massage, aimed at providing palliative care, is a pressing need at present.

OBJECTIVES: The purpose of this study was to verify the changes in autonomic nervous activity and emotions resulting from the application of our hand-massage method (HM) to a patient in need of palliative care, as well as to clarify the physiological and psychological effects of HM.

METHODS: The participants included 1 female and 5 male patients, who received palliative care for pain. Five of them had been diagnosed with high blood pressure, of whom 4 had achieved good blood pressure control with medicine. Our HM was a modified version of Tatsumura’s pair-hands-healing method, consisting of a 15-min HM session (7.5 min per hand, HM1 and HM2), both preceded and followed by a 15-min rest period, while the patient lay in a supine position. The effect on the autonomic nervous system activity was evaluated by measuring the heart rate (HR), heart rate variability (HRV), systolic and diastolic blood pressure (SBP/DBP), and nasal skin thermogram. Additionally, relaxation and anxiety were evaluated using the visual analogue scale (VAS). Data were analyzed using the paired t-test.

RESULTS: The major findings of this study are as follows: (1) the HR trendy decreased during HM (vs. HM1 p=0.050, vs. HM2 p=0.058); (2) nasal skin temperature significantly increased after HM (p=0.027); (3) SBP significantly decreased after HM (p=0.034); (4) relaxation levels significantly increased after HM (p=0.031); (5) anxiety levels significantly decreased after HM (p=0.002). The frequency analysis of HRV revealed that HM does not increase autonomic nervous system activity, ratio of low frequency to high frequency LF/HF; sympathetic nervous system activity did not reveal any significant effect of HM. CONCLUSION: The decrease in HR and SBP, and the increase in nasal skin temperature reveal the physiological relaxation effect of HM. The decrease in relaxation and anxiety show that physiological and psychological responses exist in parallel. Therefore, we believe that HM would be effective in inducing a physiological and psychological relaxation effect in a patient in need of palliative care. The frequency analysis of HRV revealed that HM does not increase autonomic nervous system activity significantly, and hence, is safe for patients with cardiovascular disease. Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB147

Body ownership and representation when holding a fake finger

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Grasping a hidden finger-like object induces perceived ownership of the object and a change in perceived position of the hands such that they appear much closer together (Héroux et al. 2013). We investigated whether object characteristics (shape, firmness, texture or temperature) influence this “grasp illusion”. Thirty healthy subjects held a finger-like object (control finger) between their left index and thumb, and their right index finger was clamped, 12 cm directly below the left. After holding the object passively for 3 min, the perceived vertical spacing between the index fingers was only ~4 cm. The object was then changed to one with a different shape (rectangular or oddly shaped), firmness (squishy or firm), texture (smooth or rough) or temperature (hot or cold [55 or 12 deg C]). The order of presentations was randomised. Perceived ownership of the held object was assessed with a 7-point Likert scale. Perceived vertical spacing between the index fingers was also measured. Judgements were made immediately after the object was changed. Compared to when subjects held the control finger, perceived ownership decreased by 1.2 [0.6-1.8; mean, 95% CI] points with the cold finger, by 1.2 [0.6-1.7] points with the rough finger, and by 1.1 [0.5-1.7] points with the oddly shaped finger. Compared to holding the control finger, perceived vertical spacing was further reduced by 1.0 [0.2-1.9] cm with the smooth finger. There was no difference in perceived spacing when holding any of the other objects. In summary, once a grasp illusion has been induced by 3 min of holding a fake finger, changing the finger’s physical characteristics can decrease the extent to which subjects feel it is part of their body. However, the distorted representation of the hands caused by the grasp illusion resists changes in object characteristics.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB148

3D representations can be useful to characterize skin’s biomechanical profile

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Elasticity is an important feature of skin physiology, basically responsible for allowing all movements, in all directions, without breaking. Several measuring devices have been developed to assess and quantify this property and recently, the CutiScan® system (CK Electronics, FRG) was presented as an advance, providing its quantification in 360 degrees. By applying a negative pressure the system optically measures the displacement of the skin caused by suction, over time, for each of the 360 degrees. Considering this innovative approach, we explore, in the present study, new parameters based on different time-angle-displacement 3D representations to quantify in vivo skin elasticity, and compare them with the descriptors obtained by the device software. 20 female subjects (mean age 37.0 ± 18.7 were selected after informed written consent, and divided in two age groups (group 1: mean age 22.0 ± 1.3 years old; group 2: mean age 52.0 ± 13.7 years old). The elasticity descriptors were measured with the CutiScan® in three regions – forearm, leg and forehead, being V1 – the maximal displacement during suction, V2 – returning rate during the relaxation time, and V3 – the ratio of V2 and V1. Additionally we constructed 3D-based representations of the displacement curves, and calculated their surface area and the volume under the surface. Previous studies could not establish a relationship of the CutiScan® descriptors with age except an age related statistically significant increase found for the V2 value in the forehead, and a negative age related statistically significant relationship found for the V1 descriptor in the forearm. However, our 3D-based representations were found to be more pronounced in group 2, although only significant in the forehead (significant differences were also detected when comparing the forearm with the forehead and the leg with the forehead values). So, these results suggest that these 3D-based representations might be very useful to complement the analysis provided by this new device, in particular to visualize and describe age-related and regional differences in skin biomechanical behavior.


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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB149

The IL-6 signalling pathway in human melanoma cells of different stages

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The interleukin-6 signal transducer glycoprotein 130 (gp130) is a co-receptor for cytokines of the interleukin (IL)-6 family and heavily N-glycosylated. While gp130 is expressed ubiquitously the IL-6 receptor is present only in hepatocytes, leukocytes, but also in cancer cells. In particular, circulating IL-6 levels are elevated in late stage melanoma patients and have unfavourable prognosis. We have recently shown that the cholesterol lowering drugs, the statins trigger apoptosis in metastatic melanoma cells, while radial growing melanoma cells are virtually insensitive (Minichsdorfer et al., 2015) to statin induced apoptosis. Moreover, statins affect the glycosylation machinery in the endoplasmic reticulum by a reduction of dolichol, but it is unclear whether a statin like simvastatin alters the glycosylation status of gp130 and impairs IL-6 signalling in human melanoma cells in a stage dependent manner. Experiments were carried out with various human melanoma cell lines reflecting early radial growth phase (WM35) and advances metastatic stages (WM793b, A375 and A518A2) of the disease. The melanoma cells were analysed for cell surface expression of the IL-6 receptor and gp130 by FACS and confirmed by Western blot, including STAT3 and phosphorylated STAT3. IL-6 levels were determined with ELISA, qPCR and activation of STAT3 was monitored by a luciferase reporter assay. Treatments were analysed with ANOVA and post hoc Dunnett test for statistical significance at a p-value of <0.05. The prototypical statin simvastatin induces a concentration and time dependent induction of the glycoprotein gp130. Upon incubation times longer than 24 hours accelerate migration of gp130 in SDS-PAGE, shifting the 130 kDa band to 100 kDa. Interestingly, such a shift is not obtained in WM35 melanoma cells from the early disease stage. Irrespective of the melanoma cell type a deglycosylation pattern is obtained with tunicamycin, an inhibitor of N-linked glycosylation. It is known that deglycosylation of gp130 does not impair IL-6 receptor signalling, IL-6 dependent activation of STAT3 is confirmed by Western blot, including STAT3 and phosphorylated STAT3. Significant activation of the transcription factor STAT3 by already 1 μM simvastatin is further confirmed by a luciferase reporter assay in metastatic melanoma cells. Accordingly, simvastatin treated metastatic melanoma cells secrete significantly more IL-6 into the medium compared to melanoma cells from the early stage. Taken together, our data show that the HMG-CoA reductase inhibitor simvastatin impacts the IL-6 pathway in a stage dependent manner with possible consequences for the tumour microenvironment.


This work was supported by Herzfelderscher Familienstiftung and the Funds of the major of Vienna (No. 15023).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
A 5-yr longitudinal study of quadriceps physiological cross sectional area, voluntary activation and in vivo specific force of older men and women

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Background: Loss of strength is one of the most recognisable features of advancing older age and has been termed dynapenia. It may be due to lower muscle mass (sarcopenia), reduced voluntary activation, or lower specific force (force per physiological cross-sectional area (PCSA)). Methods: To investigate, 12 older men and 13 older women (mean ± SD age at baseline 71±4 yrs) who were all relatively active and healthy provided written informed consent and completed measurements at baseline and again 5 yr later. Maximal voluntary isometric knee extension force (MVC) was measured at 90 degree knee angle. The interpolated twitch technique was applied to assess the level of voluntary activation and ultrasound was used to measure fascicle length and pennation angle of each of the quadriceps muscles during MVC. The volume of each quadriceps component muscle and the patella tendon moment arm were estimated from magnetic resonance imaging. PCSA for each muscle was estimated from muscle volume/fascicle length. Quadriceps specific force was estimated from: (MVC/patellar tendon moment arm) / (quadriceps PCSA * the cosine of the fascicle pennation angle). Results: Compared with baseline, MVC (12%, p<0.001), voluntary activation (6%, p<0.003) and PCSA (5%, p<0.0005) were reduced and the in vivo specific force was 7% lower at follow-up compared with baseline (p=0.027). After accounting for the reduced voluntary activation by estimating the force that could be produced if the muscle was fully activated, there was no difference in specific force between baseline and follow-up (p=0.693). Conclusion: These results show that strength was lost at around 2% per year, while PCSA and voluntary activation declined at rates of around 1% per year. The change in muscle size (PCSA) and the reduced neural activation equally contributed to the loss of strength and there was no apparent loss of ‘muscle quality’, as evidenced by the specific force when corrected for activation levels.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Assessment of pesticide exposure in migrant agricultural workers in the United Arab Emirates: Baseline findings from the Al Ain Agricultural Cohort Study

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The United Arab Emirates (UAE) is one of the leading agricultural producers in the Middle East. Organophosphates and carbamates are two types of pesticides commonly used to control pests that affect crop quality and production. Both types of these pesticides inhibit acetylcholinesterase activity leading to an accumulation of acetylcholine in the synaptic cleft, hyper-stimulation of nicotinic and muscarinic receptors, and disrupted neurotransmission that can cause serious neuropsychological and neuromuscular disorders. Agricultural workers may be at a high risk of inhalation, transdermal and ingestion exposure during the transportation, mixing, application and disposal of pesticides. The aim of this study was to quantify the occupational exposure to pesticides amongst agricultural workers in the city of Al Ain (UAE) prior to the start of the spraying season. Following ethical approval, erythrocyte acetylcholinesterase activity (AChE) was used to assess the extent of organophosphate and carbamate exposure in agricultural workers (n=119) currently employed as either sprayers (SPRAY; n=73) or supervisors, drivers and warehouse managers (SDWM; n=46). Participants in the SPRAY and SDWM group were involved in the handling, mixing, storage and disposal of pesticides. The SPRAY group was also responsible for pesticide application. The majority of the SPRAY group (94.5%) were from South Asia (i.e. Bangladesh, India, Pakistan) and the SDWM group were predominantly (69.6%) from other Arab countries (i.e. Egypt, Jordan, Sudan, Yemen). Mean (± SD) age of the SPRAY and SDWM groups was 37.6 ± 11.9 years and 44.3 ± 9.8 years, respectively. Mean (± SD) AChE activity was significantly lower for the SDWM group (4574 ± 1587 UI/l) versus the SPRAY group pre-spraying season (6206 ± 2309 UI/l); independent t-test t(116.06)=4.568, p<0.001, 95% confidence intervals for the mean difference 925, 2341 UI/l). A greater proportion of the SDWM group (73.9%) had a baseline AChE value classified as abnormal (normal range 5.320–12.920 UI/l) compared to the SPRAY group at baseline (34.2%; Pearson Chi-Square test χ2(1)=17.761, p<0.001). Contrary to our hypothesis, pesticide exposure (indexed by lower AChE values) was greater amongst supervisors, drivers and warehouse managers compared to pesticide sprayers before the start of the spraying season. This may be due to the SPRAY group having better knowledge, attitude and practices (KAP) towards pesticides and/or the SDWM group experiencing greater occupational exposure during the transportation, storage, mixing and disposal of pesticides during the previous spraying season. Subsequent time points in the cohort study and a nested questionnaire-based KAP study will help clarify the baseline findings.

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The effects of ascorbic acid supplementation on endothelial dysfunction following SCUBA diving

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During dives with compressed air divers are exposed to environmental stress that leads to impaired endothelial function. Vascular changes linked to hyperoxia induced oxidative stress could be prevented by antioxidants. We wanted to complete a comprehensive observation on the endothelial function and isolate the impact of hyperoxia from other diving related stress factors in light of vitamin C supplementation. Fourteen male divers (age 37.6±8.5 years) with 15±10 years of diving
experience participated in two studies. In the field study they completed a dive at 18 m seawater (msw), 49 minutes bottom time. At the laboratory they were exposed to ambient pressure gas mixture of 60% O₂ balanced with pure N₂ (same pO₂ that occurs while breathing compressed air at 18 msw). Prior to both studies, half of the participants ingested ascorbic acid (1g) twice a week for six days and two hours before diving or O₂ exposure, while the other half ingested placebo powder (1g). After two weeks of study intervention subjects switched groups and ingested the opposite type of powder before the studies. Endothelial function was assessed by measuring NO dependent flow-mediated dilation (FMD) pre- and post-intervention. All divers experienced a significant decrease in FMD% following placebo trials (from 9.18±1.35 to 5.97±1.36 post-dive, from 9.50±1.54 to 6.80±1.84 post hyperoxic breathing; p<0.001). FMD% did not change after 60% O₂ breathing in the vitamin C trial (from 9.76±1.80 to 8.98±1.66; p>0.05), but was significantly decreased post-dive (from 9.88±1.53 to 7.90±1.64; p<0.001). There was no difference between SCUBA and hyperoxic conditions and no interaction effect. The data suggest that vitamin C supplementation can prevent hyperoxia induced decrease in endothelial function. Diving induced vascular dysfunction can only in part be explained by hyperoxia, as an important stress factor in diving. Yang M et al. (2015). Am J Physiol Regul Integr Comp Physiol 309, R338-R344.


Orthostatic hypotension, but not orthostatic symptoms, is a marker of cerebral hypoperfusion in older adults
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Falls affect one in three older adults annually with orthostatic hypotension (OH) and its variants significant and treatable risk factors particularly for unexplained and injurious falls. OH when combined with self-reported orthostatic symptoms and a positive falls or syncope is considered a clinical marker of impaired cerebral autoregulation. Age and cognitive impairment reduce the perception of such symptoms and recall of such events, affecting the validity of this approach. Here we examine if self-reported orthostatic symptoms in combination with OH variants is a marker of cerebral hypoperfusion. A self-selected sample of N=80 older adults (age 87(6.1) years;73.5% female) were recruited from a nursing home population living in Ontario, Canada. The protocol was approved by the local ethics committee. All participants underwent a supine-stand transition (AS). Orthostatic symptoms were quantified using an 8-point orthostatic symptoms scale. Beat-to-beat blood pressure (mmHg) was recorded using a calibrated volume clamp method, while near-infra red spectroscopy (NIRS) measured relative changes in regional cerebral tissue oxygen saturation (tSO₂ - %), oxyhemoglobin (OxHb - µmol/l) and deoxyhemoglobin (Hb – µmol/l) concentration. 9.3% reported a positive falls history, 24.4% had OH 40 seconds after standing with 6.4% having sustained OH at up to 3 minutes after standing. 51.3% reported one or more orthostatic symptoms. 41.3% reported a feeling of unsteadiness during standing, 16.3% a feeling of light-headedness/dizziness. After adjusted multivariate analyses (SPSS, V22) orthostatic symptoms were not associated with relative changes in tSO₂, [OxHb] or [Hb], while the presence of OH at 40 seconds after standing was associated with a decrease in tSO₂ (B =-4.562; P=0.011) and percent [OxHb] (B=-1.88.; P=0.017). Combining OH and symptoms did not strengthen these associations. OH accounting for 20% of model variance. Current medical practice combines peripheral measurements of BP during AS stand, and orthostatic symptoms to identify those at risk of cerebral hypoperfusion. Our results suggest that postural symptoms are an inadequate surrogate marker of cerebral perfusion in older adults which maybe related to a broader range of cognitive and/or sensory mechanisms, while orthostatic BP changes are a better, yet still limited surrogate marker of cerebral perfusion. A direct measure of cerebral perfusion should be considered to assess cerebral hypoperfusion in older adults and will likely play an emerging role in identifying future syncpe and falls risk.

We would like to acknowledge the residents and staff of the Schlegel Village Ontario and the Schlegel-Waterloo Research Institute for Ageing. Dr Finucane was supported by the Royal Irish Academy Charlemount Mobility Grant.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Effects of 21-day bed rest on Skeletal Muscle Mitochondrial Function
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Background: Bed rest represents an extreme model of physical inactivity and leads to age-related changes in cardiovascular and metabolic function. In particular, reduced skeletal muscle mitochondrial capacity and intrinsic mitochondrial function in response to physical inactivity may have broad implications for human disease. The aim of this study was to determine if 21-days of bed rest decreased total and intrinsic mitochondrial respiration and if changes could be mitigated by exercise sessions performed during bed rest.

Methods: Subjects (n=9) completed 21-days bed rest without (CON) and with resistive vibration exercise (RVE) using a randomized crossover design. Fat mass was maintained by adjusting dietary intake based on body composition and resting metabolic rate measurements. The physiological response to inactivity was measured by VO₂ max, a hyperinsulinemic euglycemic clamp, resting energy expenditure and body composition. The O₂ flux capacity of saponin permeabilized skeletal muscle fibres from the vastus lateralis was measured using a carbohydrate and lipid substrate-uncoupler-inhibitor-titration (SUIT) protocol. Respirometry data was normalized to wet weight and citrate synthase activity. Mitochondrial protein content was determined by Western blot analysis.

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Results: There was a significant reduction in body mass and lean tissue mass (p<0.05) but no change in fat mass following the CON and RVE trials. Insulin sensitivity and VO₂max, relative to fat free mass, decreased significantly in the CON but not RVE group (p<0.05). There was a significant reduction in LEAK, OXPHOS and ETS capacity, normalized to wet weight, in the CON but not RVE group (p<0.05). Skeletal muscle citrate synthase activity was significantly lower in both groups (p<0.05) and when used to normalize the respiratory data, only LEAK respiration remained significantly reduced. The change in LEAK significantly correlated with the change in VO₂max (p<0.05) in the control group. Mitochondrial proteins representative of complex III and IV are also reduced in both groups.

Conclusion: Bed rest is associated with a decrease in skeletal muscle mass, insulin sensitivity and whole body oxygen consumption, independent of fat mass. Many of these changes can be prevented by resistance vibration exercise. The decrease in OXPHOS and ETS were not significantly different when normalised to citrate synthase suggesting the changes could be related to mitochondrial content or function. However, the change in LEAK respiration was independent of citrate synthase and exercise training. This may be due to reduced uncoupling of substrate oxidation and ATP synthesis and is supported by a reduction in the coupling control ratio. In conclusion, a decrease in LEAK respiration is one of the earliest changes associated with physical inactivity and may be important for long term changes in metabolic function.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB155

Temporal and spatial changes in the fibroblast and myogenic cell content of human skeletal muscle during regeneration after experimentally induced muscle injury

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Satellite cells, the resident stem cells of skeletal muscle, are indispensable for muscle repair following injury and increasing evidence also points to an important role for other cell types, such as macrophages and fibroblasts, in orchestrating various stages of the repair process (Saclier et al., 2013, Murphy et al., 2011). However, the fibroblast content of human skeletal muscle at rest and during regeneration remains unknown. The purpose of this study was to determine temporal and spatial changes in the skeletal muscle content of satellite cells and fibroblasts during regeneration. The Regional Scientific Ethical Committees of Copenhagen approved this study (Ref: HD-2008-074), and all procedures conformed to the Declaration of Helsinki. 7 healthy males (age 21±4 yrs; height 1.79±0.03 m; weight 69±8 kg (mean±SD)) were subjected to 200 electrical stimulation (stim) induced eccentric contractions of the thigh muscles of one leg (Mackey et al., 2016). Muscle biopsies were collected from the vastus lateralis muscle of the stimulated leg 2, 7, and 30 days (d) post and from the unstimulated leg as a control. Fibroblast (TCF7L2+) and satellite cell (Pax7+) number were determined from immunohistochemically stained frozen sections. Data were analysed by repeated measures ANOVA and are reported as means ± SEM. In the control muscle, the number of satellite cells per fibre was 0.07±0.004 and the number of fibroblasts 0.13±0.02, resulting in a ratio of fibroblasts to satellite cells (f:sc) of 1.8±0.2. Increases were observed on d7 and d30 for both cell types (p<0.05). Satellite cell number peaked on d7 at 0.23±0.05 cells/fibre with a corresponding fibroblast number of 0.27±0.04 cells/fibre. The proportion of damaged fibres on d7 was 25±4%, which correlated strongly with the number of fibroblasts at this time point (Pearson’s r = 0.92, p = 0.003), where 57±6% of fibroblasts were found to be located immediately around these fibres. D30 fibroblast values were 0.45±0.05 cells/fibre, a ~4-fold increase from control, where the f:sc ratio peaked at 2.7±0.3, representing a 0.6-fold increase from control. The different temporal responses of satellite cells and fibroblasts observed during regeneration, with satellite cells peaking in number on d7 and fibroblasts during the later stages of repair (d30), is in line with persistent myogenic cell differentiation and extensive muscle extracellular matrix (ECM) remodelling at this stage (Mackey et al., 2016, Mackey et al., 2011) and warrants further investigation into the role of fibroblasts in regulating myogenic cell activity, as well as restorative of muscle ECM architecture, during the regeneration of human skeletal muscle.


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PCB156

Physiological tremor reveals how thixotropy adapts skeletal muscle for posture and movement

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People and animals can move freely, but they must also be able to stay still. How do skeletal muscles economically produce both movement and posture? Humans are well known to have motor units with relatively homogeneous mechanical properties. Thixotropic muscle properties can provide a solution by providing a temporary stiffening of all skeletal muscles in postural conditions. This stiffening is alleviated almost instantly when muscles start to move. In this paper, we probe this behaviour. We monitor both the neural input to a muscle, measured here as extensor muscle EMG, and its output,
measured as tremor (finger acceleration). Both signals were analysed continuously during smooth transitions between posture and movement. Fifteen human volunteers performed a simple tracking task using the middle finger. This involved a gradual transition from static posture to movement, and back again, lasting 120s in total (see figure 1). We recorded vertical finger position and acceleration (tremor), along with extensor EMG. Changes in the frequency and amplitude of these signals were monitored over time using a continuous wavelet transform. The results show that movement caused up to 7-fold increases in tremor amplitude, accompanied by a reduction in frequency (from 18 to 8Hz approx.). These changes correlated primarily with finger velocity, rather than position ($r^2 = 0.87$; $p<0.05$). By contrast, EMG changed little and reflected muscle force requirement rather than movement speed. The altered tremor reflects naturally occurring thixotropic changes in muscle behaviour. Our results suggest that physiological tremor provides a useful and hitherto unrecognized insight into skeletal muscle’s role in posture and movement (Vernooij et al, 2016).


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PCB157

An investigation of the magnitude and temporal change in contractile function in young, resistance exercise-trained male and female humans, following an ecologically valid resistance exercise -training session

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High force, resistance exercise (RE) is used to promote muscle hypertrophy and improved contractile function. When performed unaccustomed, or in excess, RE results in exercise-induced muscle damage (EIMD), muscle pain and decreased contractile function that may persist for several days. An appropriate model of muscle damage and repair EIMD is a non-ecologically valid model of RE training in RE-trained athletes. This study investigated the magnitude and temporal change in static and dynamic contractile function in RE-trained male and female athletes following an ecologically valid RE-training session. Subjects were RE-trained young (18-35y) male (MEN, n=11) and female (WOMEN, n=8) athletes. Isometric maximal voluntary contraction (IMVC) and angle of peak torque (APT) of the knee extensors (Con-Trex Mj; CMV AG, Dubendorf, Switzerland) and dynamic counter movement jump (CMJ) was measured prior to (BASAL) and 4, 24, 48 and 72h following an ecologically valid RE-training bout comprising ~ 50 repetition of a fixed mass barbell back squat (> 80% 1RM). Creatine kinase (CK) activity and perceived muscle soreness (visual analogue scale, VAS) were used as an analog of EIMD. Data are reported as % change from BASAL, mean [lower : upper] 95% confidence intervals and P-values adjusted for posthoc contrasts by time or sex. Minor perturbation in post-RE CK activity (MEN 24h, 126 IU.l$^{-1}$ [76 : 176] ; WOMEN 24h, 89 IU.l$^{-1}$ [55 : 123] confirmed the non-overt-EIMD RE-training bout. A significant decrease in IMVC (MEN -10.2% [15.0 : -5.5], P = 0.003 ; WOMEN -14.8% [25.0 : -14.6], P = 0.011) and CMJ (MEN -13.5% [18.3 : -8.7], P < 0.001 ; WOMEN -21.8% [30 : -13.5], P < 0.001) was observed 4h post-RE. IMVC recovered by 24h and CMJ recovered by 72h for both sexes. The magnitude of decrease for the CMJ was greater for MEN than WOMEN (24h; MEN -9.6% [-13.4 : -5.7] vs. WOMEN -20.3% [-29.6 : -11.3], P = 0.046, $d = 1.31$; 48h; MEN -6.2% [-10.4 : -2.0] vs. WOMEN -24.9% [-35.2 : -14.6], P < 0.001, $d = 2.05$). There was no change in APT over time (P > 0.05, $ω^2 = 0.02$). Muscle soreness increased throughout recovery. WOMEN reporting higher initial scores than the MEN (4.4 mm [3.5 : 5.3] vs. 6.0 mm [4.8 : 7.2], P = 0.036, $d = 1.14$). These data find a significant decrease in static and dynamic contractile function following ecologically valid RE training in RE-trained men and women and a differential recovery rate for static vs. dynamic function that was marginally greater in women. It is speculated that mechanical disruption to the excitation-contraction coupling accounted for the immediate force loss. Latent calcium-mediated myocellular disruption/ remodelling may in part explain the delayed recovery of function and, potentially, the minor increase in CK activity.

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PCB158

Circulating myostatin is reduced with aging in humans but not altered by short-term, high intensity training

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Introduction: Ageing involves a loss of muscle mass and function. The rate of decline is associated with negative health outcomes and increased mortality (1). Muscle atrophy is observed at a predictable rate from 30 years of age (2), however maintenance of function is seen in masters athletes > 60 years of age (3). Myostatin acts as a negative regulator of muscle mass (4) and underlies hypertrophy with chronic resistance training (5) and atrophy in chronic conditions (4). Experiment 1: Declared healthy participants (n = 83, 18 – 75 years of age, 36 male, 47 female) were recruited. Body composition, metabolic rate,
grip strength and 6-minute walk test were recorded. Venous blood was collected and total myostatin concentration (herein referred to as myostatin) quantified by enzyme-linked immuno-sorbent assay. Total myostatin was lower in females compared with males (2176.1 [135.3] vs. 2788.7 [180.2] pg.mL$^{-1}$ ($p = 0.007$)). Stepwise regression observed that myostatin concentration is best predicted firstly by gender, then by age ($r = 0.399$, $p = 0.02$), and was not further improved by the addition of measures of metabolism, muscle mass or function.

Experimental 2: A cohort of aged sedentary (SED) males ($n = 14$; 63.9 [5.6] years of age) and masters athletes (lifelong exerciser [LEX]; $n = 10$, 61.1 [5.8] years of age) completed 6 weeks of high intensity interval training (HITT). Two way ANOVA suggested no group (SED, LEX) by time (pre, post) interaction on myostatin concentration ($p = 0.649$), nor a main effect of time ($p = 0.757$), however there was a trend towards increased myostatin in the LEX group relative to SED ($p = 0.083$). Discussion: Loss of muscle mass and function occurs at a predictable rate from ~30 years of age, however the rate of loss differs between active and inactive populations. Here we demonstrate that total circulating myostatin decreases as age increases, and differs significantly between males and females. Total circulating myostatin negatively correlates with increasing age, however alterations in myostatin do not appear after short term training interventions. Longer term activity may alter myostatin, thus our next work will follow up experiment 2 with a 3 year longitudinal analysis.


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PCB159

The variability of urinary albumin excretion and the optimal sample collection method to assess albuminuria

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Increased excretion of albumin in urine (albuminuria) is an early indicator of kidney disease in patients with diabetes, and cardiovascular disease in healthy individuals. The relationship between urinary albumin levels and the risk of vascular disease is a continuous one and commences within the clinically defined “normal” albuminuria range. To fully investigate these relationships it is important to understand the variation of albumin excretion within the normal range.

Clinically, the “gold-standard” measure of albuminuria is albumin excretion rate (AER) from a timed overnight collection. Due to variability of AER, multiple collections are needed for diagnosis. Timed collections are burdensome for patients and are often incomplete causing unreliable results. Therefore, spot (untimed) samples are often used that report the albumin to creatinine ratio (ACR). Creatinine is used to account for differences in urinary concentration. This work investigates the variability of albumin excretion in different collections and will determine which collection, first morning void (FMV), second morning void (SMV) or at a random time, is the best surrogate for timed collections in individuals with albuminuria within the normal range.

Methods: 17 healthy participants collected their urine over a 36-hour period in different containers on 3 separate occasions. For each collection method the mean of three repeats was calculated for each participant and then the median value [interquartile range] among the participants was determined. Intra-individual reproducibility of different collection methods (coefficient of variation calculated from 3 collections) and the correlation of the ACR in spot samples with timed overnight AER (Spearman’s rank) were determined.

Results: The median AER was 4.5 µg/min [2.7 - 6.9] during the day and 3.2 µg/min [1.2 - 4.0] overnight, although not significantly different ($p=0.105$). The median ACR in the FMV, SMV and random samples was 3.1 mg/g [2.3 - 4.5], 4.8 mg/g [2.8 - 6.6] and 4.8 mg/g [2.8 - 7.2] respectively. The intra-individual reproducibility of albumin excretion in the FMV, SMV and random spot samples was 15.9%, 22.1% and 22.6% respectively. The correlation of the ACR in FMV, SMV and random spot samples with timed overnight AER was 0.88, 0.54 and 0.59 respectively.

Discussion: Albumin excretion is lower overnight than during the day, most likely the result of lower activity which influences albumin excretion. The FMV has the best reproducibility and the strongest correlation with the “gold-standard” timed overnight collection. A FMV sample is the best untimed surrogate of an overnight collection when investigating the “normal” albuminuria range.

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Functional and biological changes in supraspinatus muscle after rotator cuff tear

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Rotator cuff (RTC) tears, particularly in the supraspinatus muscle (SS), are a common clinical problem that affects the quality of the muscle (muscle atrophy and lipid infiltration). Muscle quality is the strongest predictor of functional and surgical outcomes, but surgical repair often fails to reverse it, leading to high re-tear rates and poor shoulder function. Tenotomy of RTC muscles is an established animal model for RTC tear, but currently no published studies have assessed muscle function or susceptibility to injury after a tear. PURPOSE: 1) To develop a method to assess RTC contractility and assess functional
changes in SS after RTC tear. 2) To describe an in situ method to induce injury in the supraspinatus muscle (SS) through eccentric contractions and assess susceptibility to injury in torn versus healthy RTC muscle. METHODS: RTC tears were performed by surgical release of the SS and infraspinatus tendons in anesthetized Sprague Dawley rats (2% isoflurane via nosecone using precision vaporizer). Rats were monitored and allowed normal cage activity. Contractile testing was performed in anesthetized animal in situ 2 days (2D) and 15 days (15D) after tears, with contralateral side serving as control, followed by euthanasia through cardiac puncture. Muscle was harvested to measure wet weight, assess neuromuscular junction (NMJ) morphology, and fiber cross-sectional area (CSA). In a second set of experiments we induced injury to the SS in situ. The tendon was released and tied to a lever arm attached to a stepper motor in line with a load cell. The muscle was set at optimal length (L_o) followed by 30 eccentric contractions with a stepper motor in line with a load cell. The muscle was set at optimal length (L_o) followed by 30 eccentric contractions with the muscle lengthened 15% of L_o. Muscles were harvested, weighed, snap frozen in liquid nitrogen, and sectioned for labeling of structural proteins. RESULTS: Maximal isometric force was lower in 2D (2.2 ± 0.4 N vs. 2.9 ± 0.4 N), but not 15D. A decrease in CSA and altered NMJ morphology were present for 2D only. After contracture-induced injury, the loss of force in 2D was not different to control (43.5% ± 2.3), but it was significantly greater in 15D (51.8% ± 2.5). Moreover, desmin content was reduced in injured 15D only compared to injured control SS. CONCLUSIONS: This is the first study to assess susceptibility to injury after RTC tear. Shortly after a RTC there is a loss in contractile function with morphological changes to the muscle and NMJ that recover over time. However, SS muscle actually becomes more susceptible to injury over time, with corresponding loss of the intermediate filament protein, desmin, which could contribute to the poor quality of muscle seen clinically after RTC tears. All protocols were approved by the University of Maryland Institutional Animal Care and Use Committee.

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PCB161

Mitochondrial DNA haplogroups as predictors of athlete status, mitochondrial copy number, VO2 max and strength in Lithuanian athletes and controls

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Background: Genetic variation accounts for ~50% of the variation in endurance performance (1). There is a large maternal component to this contribution (1). Mitochondria have a unique matrilineal inheritance pattern making them a candidate for this component. Previous studies have investigated the association between mitochondrial haplogroup and endurance athlete status (e.g. 2-3). However, the results have been inconsistent. Furthermore, they include associations between haplogroup and strength athlete status. No studies have simultaneously investigated associations between mitochondrial haplogroup, athlete status, mitochondrial DNA (mtDNA) copy number and quantitative measures of endurance and strength.

Methods: The major European haplogroups were determined for 407 Lithuanians (84 END, 126 SSP and 197 CON). Haplogroups were classified according to (4) using TaqMan genotyping at positions 4580 G/A, 7028 C/T, 8251 G/A, 9055 G/A, 10398 G/A, 12308 A/G and 13368 G/A. Primer/probe sequences have been previously published with the exception of 9055 G/A for which a custom assay was designed. Individuals with haplogroups atypical for European populations, or with a rare combination of genotypes found in more than one European haplogroup, were classified as others. VO2 max and isokinetic peak torque were assessed using standard protocols. Mitochondrial DNA copy number was assessed by qPCR as a proxy for mitochondrial number. Haplogroup frequencies were compared using Genepop.

Results: Overall haplogroup frequencies (Table 1) were similar to published frequencies for Lithuanian populations (5). Haplogroup U was significantly different between END and SSP (p=0.008). Carriers of haplogroup U were 2.5 times more likely by odds ratio to be END than SSP (p=0.005). Haplogroup was significantly associated with mtDNA copy number by ANOVA both before (p=0.014, r^2 adj=2.64%) and after (p=0.015, r^2 adj=2.62%) a correction for athlete group. Haplogroup U had the highest mtDNA copy number. However, no associations were observed with VO2 max or isokinetic peak torque.

Conclusions: Mitochondrial haplogroup is significantly associated with END status and mtDNA copy number but not with more direct measures of whole body physiology. The high mtDNA copy number of haplogroup U may explain its over-representation in END.

Frequency of major European haplogroups in Lithuanian athletes and controls

<table>
<thead>
<tr>
<th>Haplogroup</th>
<th>Overall</th>
<th>Expected (%)</th>
<th>END</th>
<th>CON</th>
<th>SSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>127 (43.5%)</td>
<td>48.7%</td>
<td>10 (9.1%)</td>
<td>3 (3.0%)</td>
<td>4 (4.2%)</td>
</tr>
<tr>
<td>T</td>
<td>29 (9.7%)</td>
<td>7.8%</td>
<td>15 (7.8%)</td>
<td>4 (4.4%)</td>
<td>10 (7.9%)</td>
</tr>
<tr>
<td>V</td>
<td>30 (7.6%)</td>
<td>10.0%</td>
<td>16 (8.1%)</td>
<td>4 (4.1%)</td>
<td>9 (6.3%)</td>
</tr>
<tr>
<td>U</td>
<td>60 (21.4%)</td>
<td>18.4%</td>
<td>47 (21.5%)</td>
<td>20 (24.3%)</td>
<td>22 (17.7%)</td>
</tr>
<tr>
<td>X</td>
<td>9 (2.9%)</td>
<td>2.2%</td>
<td>3 (2.9%)</td>
<td>1 (1.2%)</td>
<td>5 (0.0%)</td>
</tr>
<tr>
<td>K</td>
<td>24 (8.5%)</td>
<td>4.0%</td>
<td>11 (5.8%)</td>
<td>3 (3.0%)</td>
<td>10 (7.7%)</td>
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<tr>
<td>Other</td>
<td>23 (7.6%)</td>
<td>5.6%</td>
<td>10 (5.1%)</td>
<td>6 (7.0%)</td>
<td>7 (5.1%)</td>
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</tbody>
</table>


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Poster Communications
PCB162

Evaluating of the intellectual quality coefficient of young adults with various types of functional asymmetry

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Introduction. In these days due to the development of brand new information technologies number, strength and impact of both biological and social factors on the human body are increasing. However an increasing intellectual intensity has influence not only on the development of cognition but also on the emotional and volitional sphere, it affects motivation and needs of a person. Cognitive abilities of a person determine his learning ability and memory. There is a positive relationship between the level of intelligence and information processing speed, specified by one or more genes, which approximate position is set in the chromosomes.

Studying of general resistance mechanisms to physical activity, depending on the condition of the functional asymmetry of the human, is very important because it causes more effective and oriented prevention of disadaptation violations. Objective: to find the possible relationship between individual characteristics of functional asymmetry and of the intellectual quality coefficient that determines the adequacy and effectiveness of adaptive responses.

Materials and methods: The research involved 136 second-year medical students who have been examined. Control group comprised 48 persons with the right type of functional asymmetry (RTFA). Comparison group involved 42 persons with the left type of functional asymmetry (LTFA), 26 persons with the mixed type of functional asymmetry (MTFA) and 20 persons with socio-modified type of asymmetry (SMTA). The intellectual quality coefficient (IQC) was evaluated according to the formula.

Results: The results of bicycle ergometer testing show that people with LTFA (142,1 sec), have the highest physical toughness, people with MTFA and SMTA got approximately equal results (125,1 and 125,3 accordingly), and people with RTFA ranked the lowest place (111,5 sec).

The IQC of students with different types of functional asymmetry when undisturbed is established to be the following: 3,5 RTFA; 4,3 LTFA; 4,6 MTFA and 4,0 SMTA. People with RTFA have the lowest index (4,0 and 4,1 accordingly). People with RTFA have the lowest IQC in all 3 conditions (3,5; 4,0 and 4,1 accordingly).

Conclusion:
1. Young adults with LTFA demonstrate high physical endurance.
2. People with MTFA has the most significantly increasing quality of intellectual activity under the effect of physical exercises. People with RTFA shows the lowest quality.
3. Individuals quickly demonstrate the strong adaptive reaction as a result of the dominating excitative process. However, the vegetal “price” of such adaptation combined with high anxiety and aggression is too high, which rapidly exhausts the adaptive resources of the human body.


PCB163

Intensive physical activities induce changes in innate immunity and perforin-mediated cytotoxicity

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Physically active lifestyle affords healthier life while sessile way of life reduces quality of life. Many different factors may change the immune functions: young or elderly people, chronic diseases or exhausted people and person after intensive physical exertion. Exercise-induced factors such as oxidative stress, increased metabolic rate, heat shock proteins, catecholamine, cortisol and insulin-like growth factor can influence pathogen recognition by altering expression of recognition molecules such as Toll-like or scavenger receptors, cell trafficking by altering hematopoiesis, cell death and adhesion molecule expression. Some evidence has shown that athletes included in heavy training programs were susceptible to infection. Regulatory T cells (Tregs) play a crucial role in peripheral T-cell tolerance. Tregs represent a subpopulation of suppressor T cells that mediate immune tolerance by suppressing autoreactive T cells. Immune system’s capacity to distinguish between innocuous and harmful foreign antigens is controlled by mechanisms of central and peripheral tolerance. Mechanisms of peripheral tolerance involve induction of cell death or the development of a non-responsive state (anergy) of T cells. Lymphatic cells could be stimulated to release perforin causing induction of apoptosis. An important mechanism for activation of Tregs is by immature dendritic cells. The aim of this study was to investigate the percentage of innate immune cells and perforin positive cells in lymphocyte subpopulations of peripheral blood of professional athletes. Subjects were selected from a stratified population sample of adults of both sexes during a routine examination of professional athletes, as well as, with “recreational” or noncompetitive athletes. The study was approved by the ethical committee and all subjects gave their written informed consent. The phenotypic profiles of peripheral blood lymphocytes were done by flow cytometry. Our preliminary data showed that the percentage of cells of innate immunity: NK (CD3-CD56+), NKT (CD3+CD56+) and regulatory T cells (Tregs: CD4+CD25+FoxP3+) in professional athletes included in heavy training programs were significantly elevated in professional athletes. Total perforin positive cells and double positive (perforin+ NK+cells) in professional athletes were increased in comparison with blood donors. Percentage of B cells was increased in trained athletes. Intensive physical activity in professionally trained athletes had a positive impact on immune response, particularly intensive in innate immunity, which is the first line of defense in the body against the effects of adverse factors. Tracking changes in the percentage of T regulatory cells may contribute to better understanding the interdependence of hormonal and immunological network during physical activity.
PCB164

The effects of isometric handgrip (IHG) training of one forearm on reactive and exercise hyperaemia in the untrained contralateral arm: Differences between young White European (WE) and South Asian (SA) men.


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IHG training reduces arterial blood pressure (ABP), particularly in hypertensive subjects and was shown to enhance reactive hyperaemia in the trained arm (1, 2). We hypothesised that IHG training may have remote effects on endothelial dilator function. Since the prevalence of cardiovascular disease (CVD) is greater in White Europeans (WEs) and South Asians (SAs) men, we tested whether IHG training would similarly affect reactive and exercise hyperaemia in the untrained arm of young SA and WE men. Experiments were performed on 10 recreationally active young (18-25 years) WE and 10 SA men (1st or 2nd generation UK residents originating from the Indian subcontinent). IHG training comprised 4x3min contractions at 30% Maximum voluntary contraction (MVC) at 5min intervals, 4 days/week for 4 weeks with the dominant arm. Forearm blood flow (FFB) was recorded by venous occlusion plethysmography at rest and at intervals after 3-min rhythmic handgrip contractions at 60%MVC (exercise hyperaemia) and 3-min arterial occlusion (reactive hyperaemia) before and after training. As expected, MVC was increased after IHG training in the trained arm (WE: 29.0 ± 1.3 vs 33.5 ± 1.5 Kg; SAs: 26.1 ± 0.9 vs 26.8 ± 0.9 Kg). Resting arterial blood pressure was not different between WEs and SAs and not changed by IHG training: WEs 124 ± 1.8/70±1.7 vs 125±1.8/72±1.6 mmHg; SAs: 116±3.3/76±4.4±1.13 vs 115±9.3/75±1.7 mmHg. FBF in the untrained arm was not different between WEs and SAs and not changed by IHG training: WEs 4.8±0.9 vs 6.6±0.8; SAs 5.3±1.0 vs 6.8±1.1 ml/100ml/min. However, peak exercise hyperaemia in the untrained arm was increased by IHG training, from 77.8±9.4 to 101.1±2.9 ml/100ml/min in WEs and from 86.1±3.0 to 98.2±3.3 ml/100ml/min in SAs (P<0.01 RMANOVA). Furthermore, reactive hyperaemia increased from 41.4±1.9 to 50.8±2.0 ml/100ml/min in the untrained arm of WEs and from 45.2±2.1 to 49.8±2.1 ml/100ml/min in SAs. These results indicate that in healthy young WE and SA men, IHG training of one arm for 4 weeks not only improves peak muscle power in the trained arm but induces a concomitant increase in exercise and reactive hyperaemia in the untrained trained arm in the absence of a change in muscle power. The mechanisms underlying these effects are not yet clear, but we propose IHG training does have remote, beneficial effects on endothelial dilator function, which are mediated by increased shear stress. These effects may be more pronounced in young WE than SA men, reflecting the latter’s predisposition to CVD.

Alexander S. Onassis Public Benefit Foundation

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB165

Peripheral muscle fatigue is linked to type-II cross sectional area in the elderly

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Background: Muscle strength is influenced by muscle cross-sectional area (CSA) and fiber type composition. Especially in the elderly, it is unclear to which extent these muscle characteristics influence volitional and non-volitional muscle force. To address this, quadriceps twitch contractile properties prior to (pre) and after (post) a fatiguing knee-extension task were related to fiber type CSAs. Methods: Thirty healthy, untrained elderly men (63.9 ± 2.6 years) participated in the study and all agreed to a vastus lateralis biopsy to assess fiber-type specific CSAs. Total quadriceps muscle CSA (CSAQ) and fiber-type specific muscle CSA (CSAI and CSAI) were estimated by modeling thigh lean mass by means of a DXA scan. Potentiated quadriceps twitches were elicited by supramaximal magnetic femoral nerve stimulation before and immediately after fifty maximal, isokinetic knee extensions at 180°/s. Results: Significantly lower post-exercise values were observed in twitch amplitude (Qtw: pre 47.8 ± 13.0, post 39.8 ± 12.2 Nm, p<0.01), rate of torque development (RTD: pre 1416 ± 645, post 1216 ± 575 Nm s-1, p<0.05), relaxation rate (RR: pre -488.5 ± 160.5, post -394.6 ± 138.3 Nm s-1, p<0.01) and maximal voluntary torque (MVT: pre 183.5 ± 38.9, post 167.2 ± 33.0 Nm, p<0.001) while voluntary activation (VA: pre 88.9, post 86.6 %, p>0.05) remained unchanged. Single twitch characteristics pre exercise were not related to CSAQ, CSI and CSII, but pre-MVT showed a significant relationship with CSAQ (pre: r=0.73, p<0.05) as well as CSII (r=0.71, p<0.05). Fatigue-related twitch characteristics, i.e. pre-to-post

Poster Communications
differences (\( \Delta \)) in oxygen uptake, 

\[ \text{RTD and RR, were significantly associated with} \quad \Delta \text{CSA}_{\text{pot}} \quad (r = -0.78, \ p < 0.01), \quad \Delta \text{RTD} \quad (r = -0.64, \ p < 0.05) \] 

\( \text{and} \quad \Delta \text{RR} \quad (r = -0.77, \ p < 0.01), \) while a trend was observed for 

\( \Delta \text{MVT} \quad (r = -0.51, \ p = 0.01). \) A stepwise regression model to predict 

\[ \Delta \text{CSA}_{\text{pot}} \quad (dependent \ variable) \quad including \quad \Delta \text{Q}_{\text{tw}}, \ \Delta \text{RTD}, \ \Delta \text{RR} \] 

and \( \Delta \text{MVT} \) as predictive parameters resulted in 

\[ \Delta \text{CSA}_{\text{pot}} \quad (r^2 = 0.91, \ r \text{ of} \quad 0.91, \ r^2 = 0.58). \] Conclusion: The change in single twitch characteristics in an intense fatiguing task may potentially serve as a non-invasive tool to predict \( \Delta \text{CSA}_{\text{pot}} \) in untrained, elderly men.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**PCB166**

**Effects of iron deficiency and intravenous iron on human skeletal muscle metabolism**

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Background

Skeletal muscle dysfunction is a major contributor to morbidity in chronic cardiac and respiratory diseases. Iron deficiency is deleterious in these conditions but the underlying mechanisms are poorly understood. Animal studies suggest a direct effect of iron deficiency on skeletal muscle but human studies are inconsistent. We hypothesised that iron deficiency would disturb human skeletal muscle metabolism, and that intravenous iron supplementation would have beneficial physiological effects.

Methods

We performed a prospective, double-blind, randomised, controlled, clinical physiology study of the effects of iron status on human skeletal muscle metabolism. Otherwise healthy individuals with absolute iron deficiency (ID), and an iron-replete (IR) control group, underwent \( ^{31} \text{P-MRS} \) of exercising calf muscle followed by carboxymaltose, 15 mg/kg, maximum 1g) or saline at the end of the first study morning. Identical assessments were performed around a week later. Mixed-effects modelling was used to examine the physiological effects of iron status and iron supplementation.

Results

Thirteen ID individuals completed the study and were matched with controls. Baseline group characteristics were similar. \( \text{VO}_{\text{2max}} \) did not differ significantly between groups (ID 38.5 v. IR 40.5 ml/kg/min; 95% CI for difference -0.9 to 4.5; \( \text{P} \) = NS). Despite similar venous blood lactate concentrations at volitional fatigue, net lactate clearance during submaximal exercise was impaired in the ID group (ID 4.8 v. IR 7.5 mmol/L/h; 95% CI for difference 0.7−4.7; \( \text{P} \) < 0.05). A significant interaction between baseline iron status and the effect of intravenous iron on lactate clearance was detected (\( \text{P} \) < 0.05). Additionally, intravenous iron significantly raised the lactate threshold irrespective of baseline iron status (\( \text{P} \) < 0.05).

\( ^{31} \text{P-MRS} \) revealed more marked intracellular acidosis during small muscle mass exercise in the ID group, despite similar phosphocreatine kinetics.

Conclusion

This study provides clear evidence of an effect of iron bioavailability on human metabolism. Iron deficiency appears to promote anaerobic glycolysis, as evidenced by abnormal lactate kinetics during whole body exercise, and myocyte acidosis during light small muscle mass exercise. Administration of intravenous iron corrects this to an extent, and appears to have an effect even in iron-replete individuals. These observations have implications for the pathophysiology of skeletal muscle dysfunction in chronic cardiopulmonary diseases, as well as for human athletic performance.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**PCB167**

**Effect of frequent interruptions of prolonged sitting on metabolic flexibility in overweight adults**

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Although public-health recommendations to engage in moderate-to-vigorous physical activity (> 30min, 5 d/wk) have been widely promulgated by the governments, most people in contemporary societies are physically inactive. Sedentary behaviors, like time spent sitting, are associated with serious implications on metabolic health, indicating that even in individuals who regularly exercise a reduction in time spent sitting can confer health benefits. Metabolic flexibility, defined as the ability to adjust substrate use to substrate availability and energy demand, has been recognized as a core component of metabolic health. This study tests the effect of interruptions of prolonged sitting by microbursts of activity on metabolic flexibility. We are comparing the metabolic effects of 4 days of activity microbursts (5min of moderate intensity walking every hour for 9h) to an isocaloric single 45-min bout of moderate intensity walking and a sedentary control condition in overweight adults. In this ongoing study, eleven (age=32±7 yrs, BMI=31.0±1.9 kg/m²) subjects out of 24 were studied under 3 different conditions in random order. Each condition consisted of 3 days in a free living state followed by a 24h stay in a whole room calorimeter to measure total energy expenditure and substrate utilization. Energy intake was controlled and matched across days and conditions by design. Protein oxidation was estimated by urinary nitrogen excretion. Blood samples were collected every hour from 8am to 10pm, at 3am and the following day at 7am to measure plasma insulin, glucose and free fatty acids. Metabolic flexibility is assessed by the daily changes in plasma insulin, glucose and free fatty acids concentration and non protein respiratory quotient (NPRQ). As previously published, a metabolic inflexible state is characterized by a large daily variance in insulin for a small variance in NPRQ, i.e. a small shift in fuel mix being oxidized at a high insulin signal. We expect the metabolic effects of
microbursts of activity to be mediated through enhanced insulin sensitivity and a greater shift in substrate use in response to meal consumption. Plasma samples are under analysis. We believe the results from this study will provide an initial evidence base for the health benefits of breaking up prolonged sitting with short bursts of activity. A finding that short bouts of activity promote metabolic flexibility will be of importance for individuals who have difficulty complying with traditional physical activity recommendations that promote structured exercise to be done in one longer bout.

Healy GN et al. Diabetes Care 2008 31:661-666


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PCB168

Oxygen consumed in response to a standardised exercise challenge is markedly increased by the prior consumption of a carbohydrate-rich meal

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It has been shown in overfeeding studies of animals and humans that weight gain is highly variable but often small. Whilst there is an enhancement of oxygen consumption in the hours after eating, this diet-induced thermogenesis is too small to account for ability of some individuals to avoid weight gain during over feeding [1]. In 1967, Miller and colleagues reported that oxygen consumed by adult humans during a standardised exercise test was increased following a meal and that the effect was proportional with the calorie load [2]. We have reproduced and extended this study. Thirteen healthy male undergraduate students aged 20 to 23 years were recruited to the study. Oxygen consumption was measured at rest (seated) and during exercise (Human Respiratory Kit, LabChart v8, AD Instruments). Exercise consisted of stepping up and down (30cm) every 2 seconds. Measurements were taken during the last minute of five minutes of rest or exercise. Experimental interventions were fasting, carbohydrate-rich meal and a protein-rich meal. The target for each meal was 1650 kcal which is two-thirds of the recommended dietary intake. Subjects arrived following a 5 hour fast, completed both resting and exercising measurements and then returned one hour after completing their meal intervention to repeat the measurements. Three interventions were scheduled for the same time and day of consecutive weeks. The order of the interventions was random and the experiments were blind to the intervention until all data analysis was complete.

Mean oxygen consumption (ml. min⁻¹ STP) at rest did not differ significantly between the interventions, either BEFORE or AFTER the intervention (BEFORE [AFTER]; fasting 204 [214.2]; carbohydrate 217.5 [276.6]; protein 206.9 [225]). Mean oxygen consumption during exercise did not differ for the fasting or protein-rich interventions (BEFORE [AFTER]; fasting 806 [898]; protein-rich 727 [1020], p=1 and 0.071, respectively – one-way ANOVA with post-hoc Bonferroni). However, oxygen consumption during exercise after a carbohydrate-rich meal was markedly and significantly increased. Before the meal the average (n=13) was 786 ml O₂·min⁻¹ (range 270 to 1214) but after the meal the average was 1220 O₂·min⁻¹ (range 720 to 1740, p<0.0001 – one-way ANOVA with post-hoc Bonferroni).

In summary the findings of this study are consistent with those reported by Miller and colleagues [2]. Oxygen consumption measured in the last minute of exercise performed at least one hour after a carbohydrate-rich meal was on average 62% than before the meal. Oxygen consumption at rest for the same intervention was higher, but not significantly. The findings could have value for those who wish to combine diet and exercise to control their weight.


The technical support of Mr David Gee is gratefully acknowledged

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PCB169

Diet, functional performance and muscle quality of independent-living men and women aged 65-75 years

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Age-related sarcopenia is a syndrome characterised by progressive decline in skeletal muscle mass and strength (von Haehling, Morley, & Anker, 2010). The European Working Group on Sarcopenia in Older People recommends the measurement of muscle mass and function as means of diagnosing sarcopenia (Cruz-Jentoft et al., 2010) since sole focus on measurement of muscle mass may be of limited value. The age-associated loss of muscle strength (Dynapenia) cannot be only explained by reductions in muscle size since reductions in strength are more rapid than reductions in muscle (Clark & Manini, 2012). Cawthon et al. (2014) developed cut points for appendicular lean mass (ALM) that would identify individuals with clinically significant weakness considering both ALM and strength. Since sarcopenia is a multifaceted syndrome with potentially modifiable factors such as dietary intakes, the aim of this pilot study was to explore the interrelationships between dietary intakes, ALM, and strength. Twenty-five healthy older adults including both female (n=15, age: 68.8 ± 2.9 years) and male (n=10, age 69.5 ± 2.5 years) participants completed a 7-day diet diary before having their handgrip strength and body composition (dual energy X-ray absorptiometry) measured. Males with ALM<19.75 kg and females with ALM<15.02 kg were defined as having low lean muscle mass, whilst cut points of <30 kg and <20 kg (Campbell & Vallis, 2014) were used to identify males and females with low strength. Participants received guidance on recording food and drink by household measures. EI was calculated using the World Health Organization/Food and Agriculture Organization equation (Frankenfield, Roth-Yousey, & Compher, 2005) for resting energy expenditure and an activity factor of 1.5. Forty percent (40%) of the females displayed low muscle strength while their male counterparts were all above 335P
the 30 kg cut point. ALM was 25.6±3.7 and 15.9±1.7 kg for males and females respectively. Twenty-seven percent (27%) of the females were below the cut point for low lean mass whilst males were all above the equivalent cut point. Energy intake (EI) was 1753±366 kcal for males and 1376±270 kcal for females corresponding to an EI deficit of 27.8±21.7 % and 27.7±6 % for males and females respectively. EI was significantly (P<0.05) lower than recommended EI. Protein intake was 0.97±0.3 g/kg/d for the males and 0.95±0.2 g/kg/d for the females representing 18.8±3.1 and 17.8±2.4 % of EI for males and females respectively. Our findings suggest that females in early retirement years are at greater risk of sarcopenia and dynapenia than their male counterparts. Inadequate energy intake and protein consumption which was below current research led recommendations of 20 % suggest that females may benefit from dietary interventions that would address energy and protein deficits.


Theocharis Ispoglou
Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Central apelin effects on fluid intake involves carbon monoxide function in water deprived rats

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Apelin plays an important role in fluid homeostasis. Both apelin and its receptor have been demonstrated within the circumventricular organs and supraoptic nucleus (SON) of the hypothalamus, important areas involved in the regulation of drink behavior and hormone release. There is evidence that apelin acts inhibiting and counteracting vasopressin actions. However, few studies revealed inconclusive apelin effects on water and salt intake. Additionally, we recently showed that carbon monoxide (CO) acts as an excitatory gas molecule in hypothalamic neurons, which the haeme-oxigenase (HO, a CO synthesizing enzyme) is up-regulated during water deprivation. The goals of this study were to determine whether central application of apelin affect fluid intake and hypothalamic neuronal activity in water deprived rats. In addition, we also evaluated the potential CO functions to regulate apelin responses. Wistar male rats were anaesthetized with 2.5% tribromoethanol (1ml/100g, i.p.) and intracerebroventricular (icv) cannulas were implanted. Water deprived rats (48h, n=8/group) were treated icv with vehicle (0.15M NaCl) or apelin-13 (0.1µg/5µl), and drink behavior of water or hypertonic solution (0.30M NaCl) were measured during 60min. Tin protoporphyrin IX chloride (SnPP, 20µM/5µl, icv) was used as HO inhibitor 20min before apelin. In another set of animals (n=4/group), 90min after icv application of apelin, rats were anaesthetized and perfused transcardially with paraformaldehyde to collect brains for immunofluorescence protocol. Results are reported as means±SEM, and data were analyzed by Two-Way ANOVA followed by Newman-Keuls post-hoc test (p<0.05). In the current study, we report that water deprivation induced an increase in water intake (9.4±0.4 vs. 0.3±0.1ml, p<0.05), which was increased by icv application of apelin (10.6±0.6ml, p<0.05). Important, the inhibition of the HO production by SnPP reduced water intake induced by apelin (8.7±0.5ml, p<0.05). Similar response was observed in salt intake, in which apelin increased (8.4±0.7 vs. 7.1±0.7ml, p<0.05), and SnPP diminished hypertonic saline intake induced by apelin (6.6±0.5ml, p<0.05). We also found a proportion of apelin immunoreactive neurons in SON to be co-express with HO-1 immunoreactive neurons in SON to be co-express (25.2±1.9%).% of neuronal cell bodies were co-express, which was increased by icv application of apelin (10.6±0.6ml, p<0.05). Important, the inhibition of the CO production by SnPP reduced water intake induced by apelin (8.7±0.5ml, p<0.05). Similar response was observed in salt intake, in which apelin increased (8.4±0.7 vs. 7.1±0.7ml, p<0.05), and SnPP diminished hypertonic saline intake induced by apelin (6.6±0.5ml, p<0.05). We also found a proportion of apelin immunoreactive neurons in SON to be co-express with HO-1 neurons (25.2±2.6%), which was increased in water deprivation rats (33.5±3.8%, p<0.05). Moreover, c-Fos immunoreactivity revealed that in water deprivation increased neuronal activation in co-expressed apelin and HO-1 neurons in the SON. Taken together, our results suggest that apelin plays an important role in the hypothalamic regulation of drink behavior. Moreover, apelin increases water and salt intake during water deprivation, in which those effects are dependent on CO function.
Financial Support: CNPq 401281/2014-0 to JAR and 314766/2014-6 to WLR.

Experimental procedures were approved by Ethical Committee for Animal Use of the School of Medicine of Ribeirao Preto (015/2015-1).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB173

Mechanism of childhood overweight and obesity at high altitude

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Problem:

Childhood overweight and obesity is emerging as a serious health problem in developing countries worldwide. It has immediate and long term health risks. There is an increased risk of diseases such as hypertension and diabetes mellitus. The most significant long-term consequence is its persistence into adulthood along with its numerous associated health risks. In the Kingdom of Saudi Arabia, several studies addressed the problem of childhood overweight and obesity. However, few studies have dealt specifically with the Southwestern heights where environmental factors and the population genotypes differ widely from the other studied areas.

Methods:

The study was a cross-sectional study of 145 Saudi children born and living permanently at high altitude (3100m) and 154 Saudi children born and living permanently at relatively low altitude (500m). For each child selected information regarding birth weight and breast feeding were taken from his or her file. Anthropometric measurements were then performed. Body mass index was calculated and fat mass and fat free mass percentages were derived from triceps skinfold measurement. Physical activity level was determined using the short form of international physical activity questionnaire (IPAQ-SF).

Results:

The percentage of fat and the overall prevalence of overweight and obesity among children aged 10-15 years were significantly greater among highlanders than among lowlanders (P<0.01 and < 0.004 respectively), while the percentage of fat free mass showed an opposite trend (P<0.01). The average birth weight of highland children was found to be significantly lower than their respective at lowland (P<0.01). Highland children reported lower level of physical activity than lowland ones. Birth weight was found to be positively and significantly associated with the percentage of fat free mass and negatively and significantly with the percentage of fat mass and the prevalence of overweight and obesity. Physical activity level was found to be inversely and significantly related to overweight and obesity in boys at both altitudes but there was no clear trend for girls at either altitude.

Conclusion:

Highland children of Southwestern Saudi Arabia were found to be significantly fatter, significantly less leaner and have significantly higher prevalence of overweight and obesity when compared with their respective at lowland. These differences were attributed to low birth weight and low physical activity level among children living at high altitude.

PCB174

Role of high fat diets on AngII and oxytocin metabolism in hypothalamus

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Obesity is one of the most important nutritional problems in developed countries. Usually, it is associated with an increase in the prevalence of hypertension and cardiovascular diseases, the primary cause of worldwide mortality. Obesity and hypertension have been widely related to the intake of high fat diets, and both seem to be associated with changes in brain Renin Angiotensin System (RAS), specifically with an increase in hypothalamic Ang II levels (de Kloet et al, 2014), and changes in others related peptides, as oxytocin. However, not all fat sources seem to show the same effects on health, and Virgin Olive Oil (EVOO), the main fat source in the Mediterranean Diet, have demonstrated beneficial effects on cardiovascular disease and hypertension (Villarejo et al 2015). On the other hand, dietary fatty acids are able to change the brain fatty acid composition, and modulate the activity of different peptidase activities (Segarra et al., 2011). The main objective of this work was to analyse the possible effects of two high fat diets, with different fat source, on AngII and oxytocin metabolism, and their relationships with body adiposity and arterial blood pressure.

Male Swiss Webster mice were divided in three groups (n=9). One group was fed with a standard chow diet (Control group), and the other two with a high fat diet, but supplemented with different sources at 20%: butter (Butter group) or Virgin Olive Oil (EVOO group). Animals were fed during three months in ad libitum conditions and, at the end of the experimental period, were sacrificed under anaesthesia, and samples of hypothalamic were taken out. Only Butter group showed high levels of BP and visceral adipose tissue amount. When the hypothalamus samples of these animals were analysed, the expression of Aminopeptidase A (1,71±0,279 relative RNA level; p<0,05) and IRAP (2,75±0,438 relative RNA level; p<0,01) were both increased, but the activity was lower for Aminopeptidase A(3256±207,5 vs 3579±159,8 pmol/mg prot/min; p<0,05). We did not find significant differences in AngII (31,6±3,36 vs 26,8 ±
Antioxidative enzymes activity during gut inflammation and hyperbaric treatment in DSS-induced colitis in BALB/c mice

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INTRODUCTION: ROS generated by the inflammatory cells during an immune response create oxidative stress considered as important factor contributing to the pathogenesis of inflammatory bowel disease. Hyperbaric oxygenation (HBO2) treatment frequently applied in medical practice increases tissue oxygen content and also leads to enhanced ROS production. Positive therapeutical effects of HBO2 have been attributed to its antioxidative and anti-inflammatory action. In the present study DSS-induced colitis has been employed in BALB/c mice as an experimental model of gut mucosa inflammation to investigate the effects of HBO2 on the antioxidative enzymes and hydrogen peroxide and peroxynitrite production during the colonic inflammation.

METHODS: BALB/c mice at the age of 10-12 weeks were randomized into 4 groups (m=20-25g, n=5 mice/group): control mice, control mice undergoing HBO2, mice receiving dextran sodium sulfate (DSS) and DSS treated mice undergoing HBO2. In drinking water ad libitum for 7 consecutive days mice were drinking 5% w/v of DSS. HBO2 started at day 1 and was administered until the end of experiment (60 min/2.4 ATM, 2x/day, days 1-8). After last HBO2 session, mice were sacrificed by cervical dislocation and colon, mesenteric lymph node (MLN) and spleen were collected for antioxidative enzymes activity, and MLN and spleen for determination of H2O2 and peroxynitrite production (basal level and after PMA activation). Results are presented as mean±S.E.M. The study was approved by the Ethical Committee of the Faculty of Medicine University of Osijek (Croatia).

RESULTS: HBO2 treatment per se in spleen induced increase of SOD activity (p=0.040 compared to CTRL). Mice with DSS-induced colitis had increased activity of SOD (p=0.012 compared to CTRL) in the colon, and reduced CAT activity in MLN and spleen (p=0.023 and p=0.032, respectively) compared to CTRL group. HBO2 treatment increased CAT (p=0.020) and GPx (p=0.001) activities in the spleens of the DSS+HBO2 group of mice. Basal H2O2 and ONOO- production in the MLN lymphocytes from the DSS+HBO2 group was increased compared to the CTRL group (p=0.033). PMA stimulation of MLN resulted in increased H2O2 and ONOO- production in CTRL (p=0.031) and DSS+HBO2 (p=0.012) groups. In the spleen, HBO2 increased H2O2 and ONOO- production in CTRL+HBO2 and DSS+HBO2 groups (p=0.004 and p=0.007 compared to the CTRL; and p=0.005 and p=0.009 compared to the DSS group). Their production after PMA-induced activation was decreased in the CTRL+HBO2 group (p=0.018 compared to basal level).

CONCLUSION: Results confirmed that HBO2 exerts an anti-inflammatory effect on DSS-induced colitis in mice, and this effect at least involves antioxidative enzymes activity regulation. Further studies are necessary to determine direct effect of HBO2 treatment on antioxidative enzymes function in DSS-induced colitis.

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MR activation in response to volume depletion and hyperkalemia (Shibata et al., 2013). In this work we examined the mechanisms involved in MR modulation by S843p. To that end we used mouse MR phosphomimetic mutants S839D and S839E (equivalent to S843 in human), or non-phosphorylatable mutant S839A. MR-S839D and S839E are inactive even at high aldosterone Surprisingly, aldosterone was able to induce nuclear translocation of MR-S839D and S839E, although at a slower rate than wild type receptors. Structure modeling of MR LBD and docking experiments show that S839D mutation or S839p produce the same effect, namely a small decrease in steady-state agonist docking energy. Taken together, these results suggest that the effect of S839p cannot be fully explained by decreased aldosterone binding affinity and may imply a defect in transactivation coupling. We demonstrate that uncoupling is due at least in part to decreased interaction with coactivators, such as SRC1. Co-transfection experiments showed that phosphomimicking mutant S839D significantly decreases wild type MR activity but does not affect MR dimerization. Assuming that dimerization follows a binomial distribution, our results are consistent with a dominant negative role of MR-S839D in the dimer. This has an important physiological implication, since even a low amount of MR-S839 phosphorylation would have a large impact on function.

Shibata S et al. (2013). Cell Metab 18, 660-671

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**PCB177**

**Glucocorticoids modulate in vivo brown adipose tissue thermogenesis in humans**

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Introduction: There is increasing evidence that brown adipose tissue (BAT) has an important physiological role beyond that of thermoregulation in newborn infants and rodents (1-3). Adult humans thus have significant amounts of BAT (4-6) and as a highly metabolic tissue with the capacity to oxidise both glucose and lipid, attention has turned to its involvement in the pathogenesis of obesity (7). Central regulation of BAT is through the adrenoreceptor activated cAMP pathway and the liberation of free-fatty acids (FFA), which are acute metabolic substrates for the BAT specific uncoupling protein (UCP1). Rapid activation of UCP1 at birth is mediated in part by the prepartum surge in cortisol (8), but whether excess cortisol administration may potentiate sympathetic nervous system (SNS) induced BAT thermogenesis is unknown. This was the first in vivo study to examine the effects of acute hypercortisolemia on BAT function in healthy human subjects, assessed by thermal imaging.

Methods: Eight healthy male volunteers (age 20 y, weight 75 kg, BMI 23.0 kg·m$^{-2}$) participated in this randomised, double-blind, placebo controlled study. Thermal imaging of the supraclavicular neck region (SCR) was conducted prior to and post a standardised calorie-controlled meal, proceeded by a 14hr constant infusion of hydrocortisone (HC; 0.2 mg·kg$^{-1}$·h$^{-1}$) or saline (S). The following morning, isoprenaline (ISO; 25 ng·kg fat-free mass$^{-1}$·min$^{-1}$) was infused for 1hr. BAT thermogenic activity was measured at baseline and throughout the infusion. Blood samples were drawn throughout and analyzed for cortisol, glucose, triglycerides, and FFA.

Results: HC significantly increased plasma cortisol concentrations (AUC 143±16 vs 235±290 nmol/l, p=0.001) and basal NEFA (AUC 1073±98 µmol/l; p=0.027 compared to control).

At thermoneutrality, acute hypercortisolaemia increased basal SCR (35.92±35.49°C, p=0.004). ISO increased NEFA both under control conditions (AUC 1549±173 µmol/l; p=0.001 compared to basal) and during HC (AUC 2039±194 µmol/l; p=0.001). HC did not have any effect on the magnitude of plasma NEFA increase in response to ISO (AUC 953±155 vs 979±175 µmol/l). Plasma glucose concentrations did not change during ISO infusion. β-adrenergic stimulation resulted in a highly localized increase in SCR temperature (0.68±0.18°C, p=0.001) representative of BAT thermogenic activity, both under control and hypercortisolaemia conditions (0.52±0.14°C, p=0.001).

Conclusions: GCs can modulate human BAT activation by the induction of β-AR-induced lipolysis and provide a novel insight into the potential role of BAT in targeting obesity and cushing’s syndrome disorders.


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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**PCB178**

**Thyroid hormone regulation of mitochondrial development in skeletal muscle of the ovine fetus near term**

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During late gestation, there is a rapid increase in fetal triiodothyronine (T$_3$) bioavailability$^1$, which has been shown previously to correlate with a rise in mitochondrial density in fetal skeletal muscle$^2$. At birth, there is a thyroid hormone-dependent increase in fetal whole body oxygen uptake and meta-
bolic rate. Fatty acid availability rises after birth, and there is a metabolic switch from glucose to predominantly fatty acid metabolism in several tissues at birth. This study examined whether fetal thyroid hormones influence mitochondrial respiration in skeletal muscle during late gestation in preparation for the increased energy demands and altered substrate availability at birth.

Under the Animals (Scientific Procedures) Act 1986, 13 twin-bearing ewes were anaesthetised (2mg/kg alfaxalone i.v. and 2% isoflurane in 5:1 O₂:NO₂ inhaled) at 102-104 days of gestation (d; term ~145d) for fetal thyroidectomy (TX) or sham operation of one of each of the twins. Ewes and fetuses were euthanased (200mg/kg sodium pentobarbitone) at d126-129 (n=7 ewes) or d140-144 (n=6 ewes). Samples of biceps femoris were immediately collected into preservation medium. Muscle fibres were dissected and permeabilised to measure fat (palmitoylcarnitine; 40µM) and carbohydrate (pyruvate; 5mM)-supported, ADP (5mM)-coupled oxygen consumption. β-hydroxyacyl-CoA dehydrogenase (HOAD) activity, an enzyme involved in fatty acid oxidation, was measured spectrophotometrically. Fetal plasma T₃ concentration was measured by radioimmunoassay. Data were analysed by two-way ANOVA with Tukey’s post-hoc test.

TX reduced skeletal muscle oxygen consumption with both pyruvate (fig.a; P<0.01) and palmitoylcarnitine (fig.b; P<0.001) as substrates. These effects were more pronounced near term than earlier in gestation. Rates of oxygen consumption were higher for pyruvate than palmitoylcarnitine in all fetuses, irrespective of treatment or age (P<0.01, all cases). HOAD activity was significantly lower in muscle of TX fetuses than controls at both ages (fig.c; P<0.0001). Muscle taken from fetuses near term had significantly higher palmitoylcarnitine-supported respiration rate and HOAD activity than earlier in gestation, and combining all data, there was a significant positive correlation between fetal T₃ levels and both palmitoylcarnitine-supported oxygen consumption (r=0.603, n=23, P=0.0023) and HOAD activity (r=0.601, n=22, P=0.0031). Overall, the results show that thyroid hormones regulate mitochondrial respiration rates in skeletal muscle during late gestation. There was also an increased capacity for fat oxidation and utilisation in energy production as the fetus nears term, which may be partly dependent on the prepartum rise in T₃.

**Figure.** Means±SEM ad) ADP-coupled mitochondrial oxygen consumption rate and c) HOAD activity in ovine fetal skeletal muscle. *P<0.05, TX vs controls at same age or †P<0.05 d143 vs d127 with same treatment.

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Melatonin's role in skeletal muscle of rats with LPS induced endotoxemia

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Motivation/Problem Statement Melatonin is known as an effective anti-inflammatory agent in various animal models of inflammation and sepsis and functions stimulation of various antioxidant enzymes, contributing to enhance the antioxidant defense and to protective effects on mitochondrial function. In this study we aimed to investigate the effects of multidose melatonin treatment, which was injected before and/or after endotoxemia on biochemical and histologic changes in skeletal muscle and blood glutathione (GSH) level high dose of LPS induced endotoxemia.

Methods/Procedure/Approach: We divided rats into 4 groups, control, lipopolysaccharide (LPS) (20 mg/kg, i.p. single dose), melatonin (10 mg/kg, i.p. three times), and melatonin + LPS. Melatonin was injected i.p. 30 min before and after the 2nd and 4th hours of LPS injection. At the end of the experimental period, blood samples were taken from heart to determine serum concentrations of creatinine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, and blood leukocyte. Blood GSH levels were measured spectrophotometrically using DTNB (5,5-dithio-bis-(2-nitrobenzoic acid) reagent. For hematoxylin and eosin (H&E), the muscle tissue samples were fixed in 10% buffered formalin and embedded in paraffin wax. The slides were evaluated under light microscopy. Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey’s multiple comparison tests. Values of P < 0.05 were regarded as significant.

Results: Serum CK, ALT, AST, and blood leukocyte values were found to be increased in LPS groups as compared with those of other groups (P < 0.05). In the LPS group, glucose levels were observed to be decreased compared to control group (P < 0.01). In the melatonin + LPS group, glucose levels were higher than the LPS group (P < 0.01). Antioxidant status was determined by GSH measurement in the blood. Section of skeletal muscle the sections were then stained with hematoxylin and eosin. The stained sections were visualized and photographed. In the Melatonin + LPS group, blood GSH levels were increased compared with the LPS group (P < 0.01). With H&E staining, we observed weak fibre boundaries and irregular - shaped nuclei in the LPS group. The appearance of muscle fibers in the melatonin + LPS groups was the same as those of the controls.

Conclusion/Implications: Our findings showed melatonin treatment prevented muscle damage by increasing glucose and blood GSH levels in rats with LPS induced endotoxemia. Keywords: glutathione, lipopolysaccharide, melatonin, skeletal muscle

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PCB180

Dynamics of cytokines concentrations in blood serum of larynx squamous carcinoma patients

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The pluripotent role of “cytokines network” in inflammation and tumor dissemination is still the issue of the day. Cytokine families are investigated to find out the reliable markers for the prognosis and monitoring of the course of cancer on the basis of the correlation between their concentrations in the blood serum and stages of cancer disease. The aim of our research was to investigate concentrations of TNF-α, IL-1β, IL-6 (proinflammatory), IL-4, IL-1RA (antiinflammatory) in blood serum of patients with inflammation and different stages of squamous epithelium carcinoma of the larynx and to validate their role as predictors of tumor progression.

Informed consent was given by all patients. Serum samples were collected by centrifuging whole blood at 3000 rpm (1000g) for 10 minutes at 15°C. I set of samples—inflammation (n=22); II set of samples—squamous epithelium carcinoma (n=43), among them 1 group—initial stages of malignant transformation T1N0M0 (n=14), 2 group—tumor progression T2N0M0, T3N0M0 (n=26), 3 group—tumor dissemination T4N0M0, T4N1(2)Mx, T1NxMx (n=21); III set of samples—healthy persons (n=18). Concentrations of interleukins were investigated by commercially available validated kits (ELISA, Germany) by immunoenzyme method. Statistical analysis: STATISTICA 10.0, ANOVA. Inflammation resulted in increase of both pro- and antiinflammatory interleukins: TNF-α-11.35±1.73 pg/ml vs.3.11±1.23 pg/ml control (p<0.01); IL-1β—12.41±2.88 pg/ml vs.4.37±2.91 pg/ml (p<0.01); IL-6—9.99±1.76 pg/ml vs.3.89±0.91 pg/ml (p<0.01); IL-4—17.45±2.45 pg/ml vs.7.04±2.66 pg/ml (p<0.01); IL-1RA—2306±115.3 vs.521.8±36.22 pg/ml (p<0.01). The stage-dependent significant changes of “cytokines network” were revealed in sera of patients with cancer. In 1 group of II set: TNF-α—15.91±1.19 pg/ml (p<0.01); IL-1β—6.34±2.21 pg/ml (p<0.05); IL-6—7.85±1.98 pg/ml (p<0.01); IL-4—11.26±2.02 pg/ml (p<0.01); IL-1RA—604±64.5 (p<0.05). In 2 group of II set: TNF-α—18.38±1.32 pg/ml (p<0.01); IL-1β—13.16±2.66 pg/ml (p<0.01); IL-6—14.42±2.72 pg/ml (p<0.01); IL-4—15.48±1.22 pg/ml (p<0.01); IL-1RA—2016±198.6 (p<0.01). In 3 group of II set: TNF-α—29.10±4.73 pg/ml (p<0.01); IL-1β—17.70±2.12 pg/ml (p<0.01); IL-6—18.73±1.76 pg/ml (p<0.01); IL-4—7.88±2.68 pg/ml (p<0.01 1 and 2 groups);IL-1RA—721.1±101.5 (p<0.05 1 set, p<0.01 2 group).

Therefore, at the initial stages of cancerogenesis TNF-α, IL-1β and IL-6 may be generated by malignant cells as well as the “host’s” immune system against the tumor antigens, and contribute in the tumor proliferation. At the stage of cancer dissemination proinflammatory cytokines reach their maximum with the significant drop of the antiinflammatory IL-4 and IL-1RA that confirm the malfunction of organism immune surveillance. The cooperative examination of pro- and anti-inflammatory cytokines levels in the blood serum may be useful biochemical factors for prognosis of carcinoma metastasis.

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Renal sympathetic nerve activity is markedly increased by the combination of insulin and leptin centrally

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Leptin is a hormone released from adipose tissue and acts in the brain to play an important role in influencing metabolic and cardiovascular function, and it has sympatho-excitatory effects on cardiovascular organs such as the kidney. Insulin is a hormone produced by the pancreas and can also act centrally to increase sympathetic nerve activity to the kidney. The aim of the present study was to determine whether these hormones together can elicit a much greater increase in renal sympathetic nerve activity (RSNA), mean arterial pressure (MAP) and heart rate (HR) than when given alone. Male Sprague-Dawley rats were anaesthetised using isoflurane (2-5%) in O₂. The femoral artery was cannulated for mean arterial pressure (MAP), and heart rate (HR) recording and the femoral vein was cannulated for intravenous administration of urethane to maintain anaesthesia (1.4-1.6 g/kg + top ups of 0.05 ml of a 25% solution, as required). Depth of anaesthesia was monitored by the absence of pedal and corneal reflexes. The renal nerve was carefully dissected. RSNA, mean arterial pressure (MAP) and heart rate (HR) were recorded prior to and for 3 hours following intracerebroventricular (ICV) saline (control, n=5), leptin (7 μg, n=5), insulin (500 mU, n=4) and the combination of both insulin and leptin (leptin was administered 15 minutes after insulin, n=4). Following ICV administration of leptin or insulin alone, RSNA was significantly increased by a maximum of 74% and 62% respectively (P<0.0001 compared to saline). ICV injection of leptin and insulin together significantly increased RSNA by a maximum of 124% compared to either hormone alone (P<0.0001 compared to saline). ICV administration of leptin alone did not significantly increase HR compared to saline. By contrast, insulin alone increased HR significantly compared to saline (P<0.0001). This tachycardic effect of insulin was significantly attenuated when both hormones were administered. MAP was not increased following ICV injection of saline (control), leptin alone, insulin alone and leptin and insulin together and it was not significantly different between groups. Resting levels of MAP and HR prior to the ICV injection of drugs were not significantly different between groups. These findings show that (i) insulin and leptin together enhance RSNA and (ii) leptin appears to attenuate the tachycardic effects of insulin. Therefore, these results suggest that in conditions where both hormones are elevated, such as in obesity, the enhanced effects on RSNA may be contributing to the elevation of RSNA seen in such conditions and this may contribute to the cardiovascular complications that accompany obesity.

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Influence of DSS-induced colitis on kidney injury and ECM change of mice

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Inflammatory bowel diseases (IBDs) are chronic diseases of gastrointestinal tract, characterized by inflammation and mucosal damage. As IBD is known to associate with complex crosstalk between immune response and oxidative stress, and thus the extraintestinal manifestations crossed to other organs are commonly investigated1. Dextran sulfate sodium (DSS)-induced colitis closely approximating to human IBD is a widely used model of intestinal inflammation. Recently DSS-colitis is reported to induce kidney inflammation through IL-62. However, neither renal injury nor renal extracellular matrix (ECM) change after DSS-colitis induction is still unknown. The aim of this study is to investigate the influence of DSS-induced colitis on renal injury and ECM change, and to elucidate the relationship between inflammation and ECM remodeling. ICR male mice (28-30g, n=6) were used as acute colitis animal model by treating with 3.5% (w/v) DSS in drinking water, and those with water were used as control. After 8 day DSS treatment, mice were anesthetized with pentobarbital (60 mg/kg, i.v) and body weight measurement, detection of renal function, histological damages and renal ECM deposition were further conducted. All detected values are represented as means ± S.E.M., compared by unpaired t test. The results showed that 15% of body weight lost as compared to control (p<0.05), and serum BUN level raised up to 40.92 ± 4.08 whereas only 18.57 ± 0.44 for control (p<0.05). H&E staining showed swelling renal tubules and high-level neutrophil/monocyte infiltration in both colon and renal tissues, proving both colitis and renal inflammations were induced by DSS. Western blotting analysis on renal cortex indicated the values of inflammation cytokines of IL-6, TNF-α, and inflammation mediators of COX-2, iNOS were 2.64 ± 0.39, 1.63 ± 0.143, 1.35 ± 0.06, and 1.82 ± 0.24, respectively, as compared to those of control (p<0.05). Moreover, values of protein expression of nephrin and podocalyxin in podocyte, and VE-cadherin in glomerular capillary were 0.52 ± 0.05, 0.48 ± 0.11, and 0.81 ± 0.04, respectively (p<0.05), indicating that renal inflammation may led to dysfunction on podocyte and glomerular capillary. PAS and Masson’s staining showed renal glomerular mesangial matrix expansion, vascular adventitia as well as thickening on glomerular basement membrane and proximal tubules. In addition, ECMS in cortex such as collagens I and V showed increasing values of 1.35 ± 0.05 and 1.37 ± 0.06, respectively (p<0.05), whereas collagen IV and fibronectin showed decreasing values of 0.46 ± 0.016 and 0.44 ± 0.07, respectively (p<0.05). These facts imply that DSS-induced colitis contributes to renal inflammatory injury, leads to podocyte dysfunction, renal basement membrane damage, and affect renal ECM remodeling.

343P
**PCB184**

**Effect of age and endurance training on mitochondrial function in sedentary volunteers**

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Contrary to the opinion of some (Lanza & Nair, 2010), human exercise training studies suggest the decline in mitochondrial function with age is not related to ageing per se, but rather reflects a decrease in muscle mitochondrial content resulting from age-related deconditioning (Broskey et al. 2014). We therefore investigated the impact of age and endurance exercise training on intrinsic mitochondrial function and an index of mitochondrial proliferation (relational mtDNA copy number, mtDNA) in sedentary young and older volunteers.

Ten young (mean±SEM, age 28.0±1.6 yrs and BMI 26.0±2.4 kg·m⁻²) and 10 older (age 70.7±1.6 yrs and BMI 28.5±1.0 kg·m⁻²) sedentary, healthy, volunteers underwent 8 wks of supervised cycle exercise training (30 min at 65% peak power, 3 x wk). Before and after training, peak VO₂ was measured during incremental cycling exercise, and a muscle biopsy was obtained from the Vastus Lateralis. Maximal rates of mitochondrial ATP production (MAPR) were measured bio-luminometrically in freshly isolated mitochondria in the presence of glutamate with succinate or malate (Glut/Succ, Glut/Mal), and normalised to citrate synthase activity to give intrinsic mitochondrial function. Relative mtDNA copy number was assessed by quantifying hydroxymethylbilane synthase gene and NADH:ubiquinone oxidoreductase subunit 1 in isolated genomic and mitochondrial DNA, respectively. Peak VO₂ was greater in young than in older subjects at baseline and increased with training in both groups. There was no difference in intrinsic MAPR or relative mtDNA copy number between groups at baseline. Training increased intrinsic MAPR in young, which was blunted in older subjects (in particular Glut/Mal). Training also increased relative mtDNA copy number in young, but not in older subjects.

Intrinsic mitochondrial function is not impaired in muscle from sedentary, healthy older compared to young. However, the magnitude of increase in intrinsic MAPR after 8 wks of training is blunted in older subjects, and appears to be related at least partly to diminished mitochondrial proliferation with exercise.

### Table 1. Peak VO₂, intrinsic mitochondrial function and relative mtDNA copy number in young and older subjects before and after 8 wks training

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Older</th>
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<tbody>
<tr>
<td></td>
<td>Before training</td>
<td>After training</td>
</tr>
<tr>
<td></td>
<td>Relative</td>
<td>Relative</td>
</tr>
<tr>
<td>Peak VO₂ (µmolO₂ kg⁻¹ min⁻¹)</td>
<td>44.7 ± 1.6</td>
<td>51.4 ± 1.3***</td>
</tr>
<tr>
<td>Glut/Mal µmol ATP mmol acetyl-CoA⁻¹</td>
<td>5.9 ± 0.7**</td>
<td>12.9 ± 1.1***</td>
</tr>
<tr>
<td>Glut/Succ µmol ATP mmol acetyl-CoA⁻¹</td>
<td>8.3 ± 0.7**</td>
<td>15.6 ± 1.1***</td>
</tr>
<tr>
<td>mtDNA copy number</td>
<td>1.0 ± 0.0</td>
<td>1.3 ± 0.1***</td>
</tr>
<tr>
<td>Relative mtDNA copy number</td>
<td>1.0 ± 0.0</td>
<td>1.3 ± 0.1***</td>
</tr>
</tbody>
</table>

Values are mean±SEM. µmol O₂ kg⁻¹ lean body mass⁻¹ min⁻¹, µmol ATP mmol acetyl-CoA⁻¹, relative mtDNA copy number. *, **, ***Significantly different from baseline within group (ANOVA); #, ###Significantly different from the corresponding young group value.


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**PCB185**

**Limited oxidative phosphorylation capacity in white adipocytes is a hallmark of obesity - irrespective of the glucose tolerance status**

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A series of obesity studies speculate on a causal link between altered mitochondrial metabolism in white adipose tissue and the development of obesity related glucose intolerance. We here aimed i) to define the level of impairment in white adipocyte mitochondria in obesity ii) to clarify the relation between white adipocyte mitochondrial impaired function, obesity, and glucose intolerance.

Mice were fed for 6 months a high-fat diet (HFD). We applied a non-targeted proteomic approach to identify the most vulnerable aspect of mitochondrial physiology in this model of obesity and glucose intolerance. Primarily, we found massive reduction in oxidative phosphorylation (OXPHOS) enzymatic equipment, with 40 out of a total of 98 respiratory chain complex subunits significantly downregulated in HFD compared to control mice (n=3, students t-test followed by Benjamini-Hochberg correction, significance level p<0.05).
Next, we assessed OXPHOS in white adipocytes on the functional level. We generated bioenergetic profiles comprising basal, leak and maximal respiration, and calculated cellular respiratory control ratios (maximal / leak). In line with the reduction in OXPHOS enzymes, respiratory control ratios were significantly decreased in white adipocytes from HFD fed mice (table 1) (1). Finally, we investigated white adipocyte mitochondrial bioenergetics in three further murine models of obesity (table 1), characterized by either impaired (Lepr<sup>ob/ob</sup>) or normal oral glucose tolerance (Mc4R<sup>K16/X16</sup>, and mice fed one week control diet subsequent to 6 months HFD). Compared to the 6-months HFD feeding model, we found similar reduction in OXPHOS capacity, most notably both in the absence and the presence of impaired glucose tolerance (table 1) (1).

Altogether, here we describe systematic downregulation of OXPHOS capacity, due to reduction in enzymatic equipment of the respiratory chain, as a consequence of obesity. Decreased mitochondrial respiratory capacity in white adipocytes proved a hallmark of obesity, irrespective of glucose tolerance status. Thus, impaired OXPHOS capacity in white adipocytes alone is not the proximate trigger for the development of systemic glucose intolerance.


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Iodine deficiency (ID), which still affects almost two billion persons worldwide nowadays, has been associated to the incidence of several pathologies. In breast, animal studies showed that ID induces oxidative stress, structural and functional alterations and increases its sensitivity towards carcinogens. A protective role against breast cancer was also attributed to iodide. Besides, ID induces a vascular activation in thyroid, salivary glands and stomach, other iodide uptaking organs. Since angiogenesis is an important step in tumor progression and since the sodium/iodide symporter (NIS) is overexpressed in 80% of breast cancers, we have studied the vascular effects of ID in vivo and in a cancerous (MCF7) and a non-cancerous (MCF12A) breast cell line.

Eight week NMRI mice were fed with iodine deficient diet and perchlorate (NIS inhibitor) containing water during 0 to 10 days, with or without bevacizumab (VEGF inhibitor) injection. Breast blood flow was measured with a laser Doppler in mice anesthetized with ketamine (80 mg/kg, ip) and xylasine (12 mg/kg, ip). Cells were grown in culture medium containing iodine. Medium was then changed for iodine-containing (control) or deficient (ID) medium for 2 to 8 hours. Values are given as mean±SEM, compared by a one-way ANOVA followed by a multiple comparison post hoc tests.

In vivo, increased VEGF expression was observed by immunohistochemistry in the stroma and in epithelial cells from 1 to 2 days of ID (N=8) and a significant VEGF-dependent increase in mammary glands blood flow was observed after one day of ID treatment (134.1±5.1% of control (100±4.1%)). Blood flow then decreased from day 2 to day 10 to values not significantly different from control. The pathway leading to VEGF activation was then studied in cell culture. In MCF7 cells, ID induced a transient increase in ROS. The increased HIF-1α mRNA (qPCR, 146.5±11.8% of the control (100±4%)) after 4 hours. The increased HIF-1α protein and VEGF mRNA were followed by an increase in the expression of hypoxia induced factor 1α (HIF-1α) protein (Western blot, N=5) as well as VEGF mRNA (qPCR, 146.5±11.8% of the control (100±4%)) after 4 hours. The increased HIF-1α protein and VEGF mRNA were inhibited by N-acetylcysteine, a ROS inhibitor, and by rapamycin, a mammalian target of rapamycin (mTOR) inhibitor. However, mTOR activity was not inhibited by N-acetylcysteine. In MCF12A cells, ID induced a transient increase in ROS. A mTOR and ROS-dependent increase in VEGF mRNA (qPCR, 184.3±19.3% of the control (100±4.1)) was observed after 3 hours of ID.

These data indicate that breast cells induce a vascular activation when exposed to ID through a VEGF overexpression, which is usually associated to a bad prognosis in cancers, and via ROS and mTOR signaling, which is a key regulator of cell cycle. This work could thus help understanding the established correlation between ID and breast pathologies, among which cancers.

Iodine deficiency induces a microvascularal response in breast

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PCB187

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PCB188

Alterations to cellular electrophysiological properties following prolonged exposure to a sulfonylurea blocker of ATP-sensitive potassium channels

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ATP-sensitive inward-rectifying K+ (KATP) channels play a vital role in coupling cell metabolic activity to the electrical activity of the plasma membrane (Ashcroft & Rorsman 1990). In cells expressing these channels, which include pancreatic beta cells and neurons, increases in ATP levels reduce the KATP activity triggering membrane depolarization and a range of cellular responses, including release of insulin from beta cells. Conversely, reduced cellular metabolism opens KATP channels, leading to a hyperpolarization of the membrane potential and suppression of electrical activity. One class of drug, the sulfonylureas, which are commonly used as anti-diabetic drugs, akin to ATP close the channel. However, chronic sulfonylurea treatment has been shown to cause progressive long-term insulin secretory failure (Matthews et al. 1998; Remedi & Nichols 2008). Interestingly, patients with mutations that render KATP channels overactive display a higher frequency of secondary treatment failure (Sesti et al. 2006). These studies indicate that activity of KATP channels may play a role in the homeostatic regulation of intrinsic cellular excitability. In this study we have investigated the effect of chronic sulfonylurea treatment on cellular electrophysiological properties in two different KATP expressing cell-lines.

Cultures of the rat insulinoma cell line (INS-1) or the mouse hypothalamic cell line (GT1-7) were pre-incubated with either: vehicle (Cntl) or Gilbenclamide (Glib, 100 nM) for ~48 hrs. Whole-cell patch-clamp recordings were then made at 33 ± 1 °C in the absence of any drug. The pipette solution lacked ATP. To characterize both leak and voltage-gated current components voltage steps of 100 ms duration were applied from a holding potential of -70 mV to between -80 and +60 mV in 10 mV increments.

In INS-1 cells, pre-treatment with glibenclamide significantly depolarised the resting membrane potential (Vrest) (mV: -73.3±5.2 (Cntl, n=17) vs. -58.2±5.1 (Glib, n=18), p = 0.046) and decreased the normalised conductance of the background leak current (nS/pF: 0.8±0.18 (Cntl, n=17) vs. 0.3±0.07 (Glib, n=18), p = 0.02). Conversely, in GT1-7 cells a significant depolarisation in Vrest was not observed after chronic administration of glibenclamide (mV: -91.6±1.29 (Cntl, n=13) vs. -87±2.04 (Glib, n=16), p = 0.08). However, similar to INS-1 cells, there was a reduction in the capacitance-normalized conductance of the leak current (nS/pF: 1.02±0.19 (Cntl, n=13) vs. 0.52±0.07 (Glib, n=16), p = 0.026). Depolarization-activated outward current components that activate positive from ~-40 mV were not changed by glibenclamide pretreatment. These data suggest that a period of chronic block of KATP channels produces lasting changes to intrinsic membrane properties that could affect both cellular physiology and drug efficacy.

Real-time monitoring of intracellular IP3 upon induction by bradykinin

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Inositol 1,4,5-trisphosphate (IP(3)) as a second messenger regulates complicated signaling processes in various physiological events. Development of a high-throughput screening system to monitor temporal changes of IP(3) is essential for screening of new potential therapeutic compounds. A simple, sensitive and rapid method for measuring IP(3) are described based on luciferase fragment assisted complementation strategy, which converts the ligand-induced conformational changes to light. Designed sensor comprising the IP(3)-binding core domain of IP(3)-receptor fused between complementary non-functional fragments of firefly luciferase allows direct detection of IP(3) in presence of luciferin substrate both in cell lysate and in living cells. Based on our design, the screening time was very fast and maximum response was obtained up to 11-fold higher than untreated cells. Moreover, the designed method was able to monitor release of IP(3) upon induction by Bradykinin. The current sensor not only provides a specific IP(3) detector in vitro but also facilitates monitoring of the response of IP(3) in living organisms.


Research council of Tarbiat Modares University for financial support of this work

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Use of quantum dots as a potential method for delivery of growth factors to the developing placenta
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The defective development of the placenta plays a major role in miscarriage, intrauterine growth restriction and pre-eclampsia. In vivo animal studies have demonstrated the importance of insulin like growth factor I (IGF-I) for the development of the placenta and embryo. In humans, IGF-I levels in umbilical cord blood correlate with fetal weight at birth while in vitro, IGF-I regulates turnover of cytrophoblasts, which continuously proliferate ensuring appropriate growth and functioning of the placenta. Therefore manipulation of the IGF-I axis might offer a therapeutic route to improve the performance of the placenta during pregnancy. Quantum Dots (QD), have been used to deliver a range of molecules such as growth factors, antibodies and drugs to various tissues. Using QD conjugated to IGF-I and a placental targeting peptide (iRGD), as a means of selectively delivering IGF-I to the placenta, we investigated the transcellular IGF-I route from the syncytial microvillus membrane (MVM) to the underlying cytrophoblasts and analysed IGF-I turnover in placenta.
First trimester placental explants were pre-incubated for 1h with the clathrin-dependent endocytosis inhibitor CPMZ (1h, 100uM) and subsequently treated with IGF-I (20nM), QD-biotinylated-IGF-I (QD-IGF; IGF 100nM) or iRGD-QD-IGF for 30min-24h. Immunohistochemistry (IHC) and Western blotting (WB) with antibodies to phosphorylated (p)IGFR, pAKT, p70S6K, pGSK, and the endosomal markers clathrin and EEA1, were used to investigate IGF-I mediated signalling and turnover. All IGF conjugates induced phosphorylation of the IGFR after 30min (WB), with iRGD-QD-IGF triggering a higher response as compared to QD-IGF (WB, t-test P<0.05). IHC revealed two stages of IGF-I signalling: incubation with IGF-I for 5 min triggered phosphorylation of IGFR at MVM, followed by translocation of the p70S6K to the apical and basal membranes of the syncytium. Longer incubation (15-30min) generated exclusive activation of IGFR in the cytrophoblasts, and was followed by phosphorylation of AKT and GSK. CPMZ inhibited IGF-I stimulated pAKT and p70S6K, but not pGSK. Following internalisation, QD-IGF co-localised with clathrin but not the early endosomal marker EEA1, and accumulated near the basial syncytiial membrane, whereas iRGD-QD-IGF crossed this membrane and was internalised by cytrophoblasts. QD-IGF is a useful tool to investigate the delivery, turnover and effects of IGF-I on placentical tissues and might be considered as an effective delivery vehicle for growth factors to the placenta. Conjugates of IGF-I and QD are efficient in triggering an IGFR response in the syncytiium and underlying cytrophoblast, followed by the activation of signalling related to mitosis (pAKT and pGSK) and protein synthesis (p70S6K). However, the IGF-I transcytosis route from MVM to cytrophoblast layer requires further investigation.
Effect of tributyltin and omega 3 on erythrocyte fragility in rats

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Tributyltin is an important chemical in industrial production. Especially it is used in manufacturing of vinyl polymers (Batt, 2006) and as antifouling agents on the water contacting surfaces of aquatic vehicles (Boyer, 1989). However they possess various health hazards in food web and to humans. They produce health problems such as endocrine disruption, immune suppression (Krajnc et al, 1984) and oxidative stress (Mitra et al, 2013). In this study effect of tributyltin on erythrocyte fragility, antioxidant parameters and hematological parameters were studied. 28 days of oral administration of tributyltin chloride (5 mg/kg), vehicle for tributyltin (corn oil), omega 3 oil (250 mg/kg) and their concomitant use was conducted on male Wistar-albino rats weighing 200-250 grams (n=7 for each group). Rats were anaesthetised with a mixture of ketamine (50mg kg-1) and xylazine (4 mg kg-1, both i.p.) and blood was withdrawn into EDTA containing tubes. Erythrocyte fragility was measured as level of osmotic hemolysis due to increased distilled water content and read with a spectrophotometer at 546 nm. Hematological parameters (hemoglobin, hematocrit, red blood cell, white blood cell, platelet, lymphocyte, monocyte, neutrophil count, percentages of them and calculations such as mean corpuscular volume) were measured with an automated device for animal studies. Concentration of glutathion in blood was measured (Beutler et al, 1963). Superoxide dismutase was measured according to percent inhibition of formazan dye formation at 505 nm. Glutation peroxidase was assayed by targettigs oxidation of glutathion by cumene hydroperoxide. Malondialdehyde which is an indicator of lipid peroxidation in erythrocytes was measured based on thiobarbituric acid reactivity. Results, which are all given as Mean±Standard Deviation, indicate significant increase in erythrocyte fragility due to vehicle used for dissolving tributyltin at 0.5% concentration (58%) compared to control (36%) and no additive effect of tributyltin was observed on fragility (54%). Tributyltin decreased level of an important antioxidant enzyme glutathion peroxidase (17.24±1.9) compared to control (23.97±2.76) (p<0.05) and concomitant omega 3 administration with tributyltin recovered this untoward result (24.6±5.22). Although insignificant, hematological findings of the study indicate a lymphocyte suppression in quantity in tributyltin group (4.90±1.10) compared to control (6.19±2.16). This effect was also ameliorated with omega 3 use (5.51±1.10). Results of this study suggest that consumption of omega 3 can at least partially recover some untoward physiological effects of tributyltin intake consumed from the same aquatic food source.


Boyer, I.J. (1989). Toxicity of dibutyltin, tributyltin and other organotin compounds to humans and to experimental animals, Toxicology 55, 253–258.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Poster Communications

**PCB194**

Rho GTPase regulation of GLUT4 translocation induced by extracellular ATP in skeletal muscle cells

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Exercise stimulates glucose uptake into skeletal muscle, through the redistribution of GLUT4 from intracellular vesicles to the sarcolemma. The underlying mechanism is still under investigation, and our recent studies show that ATP is an autocrine mediator of this response¹. How ATP would contribute to GLUT4 translocation is unknown. Insulin also stimulates glucose uptake and this involves activation of the Rac1 GTPase and dynamic rearrangement of actin-filaments, in addition to stimulating the canonical Akt pathway and downstream Rab GTPases². Here we investigate if extracellular ATP induces cortical actin remodeling and if this occurs through Rho activation, leading to GLUT4 insertion into the cell surface.

**Material and Methods:** L6 myoblasts and myotubes stably expressing GLUT4myc were used as readout, by stimulation with, ATP (100 µM, 5 min) or insulin (100 nM, 5 min). Actin filaments were visualized by confocal fluorescence microscopy using Alexa488-conjugated phalloidin. Rac and Cdc42 activation was assessed by GELSIA. L6 muscle cells were also pretreated with latrunculin A, or siRNA to Rac1 prior to stimulation as above followed by assessment cell-surface GLUT4myc quantification.

**Results:** Extracellular ATP and insulin each promoted rapid GLUT4 translocation and insertion of GLUT4 into the membrane of L6 GLUT4myc myoblasts. Extracellular ATP rapidly activated GTP-loading onto Rac1 and Cdc42. ATP-induced translocation of GLUT4 was partially blocked by silencing Rac1 or Cdc42. Work in progress is assessing the role of the actin cytoskeleton in the response to ATP.

This work suggests that myotube contraction leads to autocrine secretion of factors including ATP that contribute to GLUT4 mobilization, via an intracellular signalling pathway involving Rac1 and Cdc42.

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**PCB195**

Oxytocin influences food intake and body weight in estrogen-treated rats

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A wealth of data shows that estrogen decreases food intake and body weight in ovariectomized (OVX) rats, but the mechanisms by which these effects occur is not fully understood. One possibility involves the neuropeptide neurotransmitter, oxytocin (OT), which also has been shown to reduce food intake. The OT promoter region contains an estrogen response element (ERE), and when estrogen binds its receptor, this complex translocates to the ERE located within the promoter region of the OT gene. We hypothesized that estrogen acts to decrease food intake by interacting with the OT system. Accordingly, the goal for this study was to investigate interactions between estrogen and OT on food intake and body weight. Female Sprague-Dawley rats (n = 34) were OVX, and an intracerebroventricular cannula was implanted in the lateral ventricle (coordinates from bregma = AP -1.2, ML -1.7). These procedures were performed under 2.5% isoflurane anesthesia (flow rate = 2L/min). Rats were allowed to recover for one week and then treated with 10 µg estradiol benzoate (EB) or oil vehicle (OIl) on days 1 and 2 of a 4 day protocol. On day 4, rats were injected with 0, 0.25, or 0.5µg of OT into the lateral cerebral ventricle. Food intake and body weight was monitored throughout the experiment. Results show that 0.5 µg of OT caused the greatest reduction in food intake and body weight, especially in EB-treated rats. These data show that the combined effects of estrogen and oxytocin decrease food intake and body weight, supporting the idea that estrogen-modulated oxytocin expression may be one pathway by which estrogen is involved in the regulation of food intake and body weight. Further studies will be necessary to determine whether oxytocin mRNA expression and protein production is influenced by EB, as well as whether blockade of OT receptors reverse the inhibitory effects of EB on food intake. All animal protocols were approved by the Oklahoma State University – Center for Health Sciences Institutional Animal Care and Use Committee.

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**PCB196**

Melatonin abolishes cannabinoid-induced spermatotoxicity in rat *in vitro*

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The presence of cannabinoid receptors (CB1 and CB2) in mammalian sperms have been documented (Cobellis et al., 2006). The spermatotoxic effect of Δ⁹-tetrahydrocannabinol (THC), the main psychoactive cannabinoid in marijuana, has been well reported (Park et al., 2004). However, short-term *in vitro* exposure to melatonin was shown to improve aspects of sperm motility (Ortiz et al., 2011). Recently, melatonin was shown to exacerbate marijuana-induced gonadotoxicity in vivo (Alagbonsi et al., 2016). However, the possible cross-talk on
the in vitro modulation of sperm motility and kinetics by cannabinoid and melatonin receptors stimulation is not known and necessitated this study. In vitro experiments on rat sperms were performed in modified Biggers-Whitten-Whittingham (BWW) culture medium (Amoaka et al., 2013). Stock solutions of the drugs used were prepared with dimethyl sulphoxide (DMSO) and diluted to the required concentration with BWW immediately before each experiment such that the final DMSO concentration was 0.2% (vol/vol). Dose-response and time-course modulation were determined by incubating sperms in various concentrations of THC and melatonin (0μM-10mM) for different durations (0 min-60 min). Consequently, the sperms were incubated for 30 min in each of the following: 1) 0.2% DMSO in BWW culture medium (control) (Amoaka et al., 2013), 2) THC (1mM), 3) melatonin (5mM), 4) rimonabant hydrochloride (CB1R antagonist, 1mM)+AM630 (CB2R antagonist, 1mM)+ THC, 5) THC+melatonin, and 4) RIM+AM630+THC+melatonin. The sperm motility and kinetics were recorded using the computer-assisted sperm analyzer. Values are Means±S.E.M of 5 animals’ semen, compared by ANOVA followed by post-hoc least significant difference. The sperm progressive motility was lower in THC (6.08±1.16; p<0.001) but higher in melatonin-treated (52.47±0.75; p<0.001) than control sperm (39.72±0.38), and higher in THC+melatonin (22.75±2.01; p<0.001), RIM+AM630+THC (41.38±0.36; p<0.001), and RIM+AM630+THC+melatonin (50.47±0.38; p<0.001) treated sperm than THC treated sperm. Similarly, the average path, curvilinear, and straight line velocities were lower in THC (5.64±0.82, 6.96±0.74, 2.75±0.23; p<0.001) but higher in melatonin-treated (16.33±0.33, 21.79±1.01, 7.48±0.21; p<0.001, p<0.01, p<0.05 respectively) than control sperm (13.70±0.29, 18.04±0.58, 7.54±0.34), and higher in THC+melatonin (8.10±0.40, 8.90±0.30, 6.12±0.42; p<0.001), RIM+AM630+THC (14.70±0.76, 21.39±0.73, 6.86±0.47; p<0.001), and RIM+AM630+THC+melatonin (15.73±0.38, 21.07±0.55, 6.99±0.06; p<0.001) treated sperm than THC treated sperm. In conclusion, THC reduced sperm motility and kinetics while blockade of cannabinoid receptors abolished these effects. Moreover, melatonin improved these parameters in THC-treated rats either when the receptors were blocked or not. 


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Poster Communications

PCB197

Extended winter fasting drives changes in gut microbiome composition and function in a hibernating mammal

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Bacteria that reside in the vertebrate gut are part of a complex consortium that greatly expands the metabolic capabilities of the host, providing biochemical functions such as conversion of indigestible dietary components into usable forms, and modulation of host development, immunology, physiology and behavior. Hibernation provides a unique platform to understand the interplay among host biology, diet and the commensal microbiota, due the extended absence of dietary substrates that many gut bacteria rely on for metabolic needs. The annual hibernation cycle modifies the gut microbiota of 13-lined ground squirrels: it increases relative abundance of taxa that can degrade host glycans including Akkermansia, a dedicated mucin-degrader, and reduces abundance of many taxa that prefer plant glycans. To determine functional significance of these seasonal changes we gavaged active season squirrels and aroused hibernators with 13C-labeled substrates including inulin, a plant-derived fiber, and mannitol, a simple sugar alcohol, neither of which can be metabolized by mammalian enzymes. Subsequent measurement of 13CO2 in breath is used an index of bacterial degradation of the substrates in vivo. Results suggest that as hibernation progresses the capacity to degrade complex plant-derived glycans, but not simpler sugars, diminishes. Maximal changes in δ13CO2 (in o/o; mean±SE) after 13C-inulin gavage (25 mg/kg) are 160±20 in summer microbiota and -15±2 in late winter (4 months hibernation) (n=3-5 per group). In contrast, after 13C-mannitol gavage (15 mg/kg) maximal responses average 50±10 in summer and 80±38 in hibernators (n=3-4 per group). Antibiotic manipulations of bacterial communities can reveal whether the microbiota affects seasonal cycles in hibernators. Low dose penicillin (Pen, 13.4 mg/L) given to pregnant squirrels during gestation and lactation has modest effects on total bacterial abundance of their pups but substantial effects on community composition, including greatly increased abundance of Akkermansia which persists through the hibernation season. Pen exposure in early life increases adiposity by midsummer and increases gut serotonin content. The results provide novel insights into host-microbiome interactions in natural models of extreme dietary change, with potential implications for treating microbiome alterations in patients undergoing temporary or complete bowel rest due to gastrointestinal disease.

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PCB198

Multivalent Cell specific therapeutics: Limiting side-effects in the treatment of metabolic disorders

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Metabolic Homeostasis is mediated through multi-organ endocrine signaling. Because many systems are involved, therapeutics used to treat these disorders have wide-ranging effects. We propose that ‘drugs’ targeted to specific cell types within the homeostatic system will provide outcomes with limited side-effects. We have shown cell-type specificity can be achieved ifa ligand with multiple binding domains can be developed that binds to a combination of cell surface receptors which distinguish the cell type of interest from other cells. We have synthesized several such bivalent ligands that may target cells within the homeostatic axis; melanocyte stimulating hormone linked to CCK (MSH/CCK) and Glucagon Like Peptide 1 (GLP-1) linked to Glibenclamide (Glb, a Sulfonyl urea receptor antagonist), or Yohimbine (α2 adrenergic receptor antagonist). An apparent $K_d$ of ~5 nM for MSH/CCK, ~10nM for GLP-1/Glb and ~5nM for GLP-1/Yhb were observed when binding was assessed using a high throughput screening assay with live cells expressing the respective complementary receptor pairs. Binding constants for individual ligand domains within the bivalent ligands were 100 nM or greater, based on binding to cells expressing only one of the pair, indicating that these ligands will only bind with high affinity only to cells expressing both complementary receptors. All three bivalent agents maintain signaling capacity, though in each case both the magnitude and sensitivity of 2nd messenger and physiological effects differ, though from the constituent monomers. Using the βTC3 cell line, GLP-1/Glb was found to elicit a half maximal Ca²⁺ response at ~10nM reflecting its $K_m$ but the magnitude of the Ca²⁺ response was 10X less than monomeric Glb. Unlike the Ca²⁺ response, cAMP production was depressed by only ~20% compared to monomeric GLP-1. Although signaling is depressed, glucose stimulated insulin secretion (GSIS) was potentiated to similar levels as elicited by the combined monomers (133 ± 25% vs 110 ± 15%). Like GLP-1/Glb, the maximal potentiation of GSIS by GLP-1/Yhb was equal to that elicited by the combined monomers, however the EC50 was reduced from 10 nM to 1nM for the dimer. The effects of GLP-1/Yhb on glucose disposal during i.a. glucose challenge (1 gm/kg) in conscious male Sprague-Dawley rats was tested. Compared to equal concentrations of monomers, GLP-1/Yhb decreased the peak glucose concentration following an injected glucose load by greater than 30% (320 mg/dl vs 420 mg/dl), and the recovery to baseline from 15 to 5 min. Both findings suggest that GLP-1/Yhb exhibits a strong β-cell specific enhancement of GSIS potentiation. Thus, these bivalent ligands exhibit enhanced affinity, cell type specificity and maintain functional efficacy, and thereby hold promise as cell specific therapeutic agents.


Juvenile Diabetes Research Fnd, American Diabetes Association

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PCB199

Metabolic effect of Gum Arabic (Acacia Senegal) in patients with Type 2 Diabetes Mellitus (T2DM): Randomized, placebo controlled double blind trial. 

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Abstract:

Background: Gum Arabic (acacia Senegal) is a safe dietary fiber indigestible to both humans and animals. Lipid lowering effect of Gum Arabic (GA) was extensively studied in animals; on the other hand a paucity of data regarding the effects of GA on metabolic elements in patients with Type 2 Diabetes Mellitus (T2DM), this study was conducted to determine the effects of regular (GA) ingestion on control of metabolic elements among T2DM. Methods: A two-arm randomised, placebo controlled, double-blind trial was conducted at Academy Charity Teaching Hospital, Khartoum. Total number 91 patients with T2DM completed the study. They were divided to two groups: A test group of 46 patients were received oral GA (30 gm /day) for 3 months and a group of 45 patients were received oral pectin (5 gm/day) as a placebo for the same period of time considering taking their anti-diabetic drugs. Weight, height, waist circumference, blood pressure, triglyceride, cholesterol, LDL, FGB and HDL, were measured before and after intervention. Results: Pre and post intervention results were compared to the control group (mean ± SD) showed significant decrease of FGB (145.61±38.82 mg/dl vs. 197.43±62.09 mg/dl pre- intervention, p<0.05), decrease of systolic and diastolic blood pressure by 7.6% and 6.8% respectively within GA group. Lipid profile was decreased by 5.9% for LDL-Cholesterol, 9.8% for HDL-Cholesterol and 8.5% for Total Cholesterol. Triglycerides significantly decreased (121.22±52.10 mg/dl vs. 136.15±55.74 mg/dl pre-intervention, p<0.05). HDL-Cholesterol showed borderline increase by 19.9% within GA group with insig-
significant increase compared to placebo group. Waist circumference significantly decreased by 1.4% within GA group and decrease of BMI (27.09±5.49 vs. 27.66±5.37 pre-intervention, p<0.05). Also another visceral fat distribution indicator is the visceral adiposity index (VAI) has shown significant decrease (2.43±1.82 vs. 3.17±2.29 pre-intervention, p<0.05). Conclusions: GA decrease risk of diabetes mellitus complications by strict control of metabolic elements in patients with type 2 diabetes mellitus. Key words: Gum Arabic, T2DM, BMI, LDL, HDL, Cholesterol, Triglycerides.

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Neck circumference: a supplemental tool for the diagnosis of metabolic syndrome

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Deposition of fat around the neck is a unique place representing upper body subcutaneous adipose tissue. This study explores usefulness of neck circumference (NC) as supplemental tool for diagnosing metabolic syndrome (MS) while identifying its cutoff values. This was a case-control study conducted on 165 subjects, with and without type 2 diabetes, aged 35 to 65 years. Evaluation was done for MS by measuring anthropometric, clinical and biochemical parameters as according to the criteria proposed by International Diabetes Foundation. Variables in both cases and controls were correlated with NC and cutoff of NC determined for diagnosing MS. The NC correlated best with waist circumference in men (r = 0.721, p=0.000) and body surface area in women (r = 0.747, p=0.000). The area under the curve of NC for MS was 0.760 for men (p<0.001) and 0.631 for women (p<0.05). Optimal NC cut-off points to determine MS were ≥38 cm for men and ≥34 cm for women. The calculated odds ratios (ORs) suggested that patients of MS are more than 12 times likely to have a NC ≥38 cm than in control subjects (OR = 12.44; 95% CI = 4.13–37.41). Similarly, women with NC ≥34 cm, were 3.34 times more likely to develop MS than controls (OR = 3.34; 95% CI = 1.26–8.80).

Neck circumference strongly correlates with adiposity indices and has definite cutoff point (men: ≥38 cm, women: ≥34 cm). It can hence be used as a useful adjunct for clinical screening of MS.

Keywords: Neck circumference, waist circumference, metabolic syndrome, obesity, diabetes mellitus.


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Furanocembranolides improve liver insulin resistance in-vivo and in-vitro

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Background and Aims

There is an urgency to find new treatments for type 2 diabetes mellitus (DM2), a devastating disease worldwide. Insulin resistance is a major hallmark of DM2. This is why current pharmacological therapies to treat DM2 are focused countering insulin resistance. In this work, we aimed to investigate the potential therapeutic use of Leptolide (a member of furanocembranolide’s family) to enhance insulin sensitivity in HepG2 cells and in diet-induced obese mice.

Material and Methods

Six weeks old C57BL/6J male mice were fed regular diet (SD) or high fat-diet (HFD; 60% kcal fat) for 6 weeks. Afterwards, they were treated with daily i.p. injections of Leptolide (100µg/kg) or vehicle for one month receiving the same kind of diet (SD/ HFD). Intraperitoneal glucose tolerance and insulin sensitivity were assessed at the end of the treatment. Plasma insulin, triglycerides (TG) and cholesterol (CHL) were measured after treatment. Insulin signaling (p-PKB/PKB) was measured in liver of HFD/+ Leptolide mice. Insulin resistance was activated in HepG2 cells using 0.2mM palmitate and insulin signaling was studied 24h after 0.1µM Leptolide treatment.

Results

Diet-induced obese mice exhibited improved glucose tolerance and insulin sensitivity. Livers of HFD-mice showed enhanced ~2-fold PKB phosphorylation after insulin injection when treated with Leptolide. In parallel, HFD treated with Leptolide showed ~30% reduced plasma insulin levels and ~12% reduced plasma TG levels. Importantly, these beneficial effects on glucose homeostasis, lipid levels and insulin sensitivity were not accompanied by toxicity. These results nicely correlate with those obtained in insulin resistant HepG2 cells, where Leptolide increased insulin signaling (p-PKB/PKB) by 2-fold.

Conclusion

We have identified Leptolide as a new potential treatment for insulin resistance and DM2.

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Effects of acute and chronic metformin administration on pulmonary artery endothelial function

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Introduction: Type 2 diabetes (T2DM) is a chronic disease characterized by insulin resistance and/or abnormal insulin secre-
tion, which prevalence is increasing in epidemic proportions worldwide. Furthermore, T2DM has been associated with a higher prevalence of cardiovascular disease, and recently it was associated with the risk of developing pulmonary arterial hypertension (PAH). The possibility that endothelial dysfunction causes PAH has been studied with contradictory results (1,2,3). Metformin is a widespread prescribed medicine indicated to treat insulin resistance and has been shown to improve endothelial function in rat diabetic models (4,5).

Aims: Herein we have investigated the effect of acute and chronic metformin treatment in endothelial functionality in control and prediabetes animals.

Methods: Experiments were performed in male Wistar rats aged 4 months. The prediabetes model used was the high-fat (HF) model, which is obtained by submitting the animals to a 60% lipid-rich diet during 3 weeks. This model was compared with age-matched controls that fed a standard diet. Endothelial function was evaluated by monitoring the relaxation effect of ACh over the contraction induced by prostaglandin F2α (PGF2α) or phenylephrine (PE) contraction. To evaluate the effect of chronic metformin on endothelial function, the drug was administered at a concentration of 200mg/kg/day in drinking water for the subsequent 3 weeks after starting the diet protocol, both in control and HF animals. Acute metformin effects were investigated by submitting the pulmonary artery to 3mM of metformin during 20 minutes.

Results: HF diet did not alter endothelial function in the pulmonary artery, as the dose-response curves to ACh over PGF2α and PE contraction were similar between control and HF animals. Chronic metformin treatment showed a tendency to improve endothelial function in control and HF animals, as an intense relaxation in response to ACh over PGF2α was found, although without statistical significance. In contrast, while acute metformin administration did not affect the relaxation to ACh in control animals, it almost blocked completely the relaxation to ACh in the HF animals (ACh 30μM: % of PGF contraction: 74.8±13.1 n=6 vs. 33.7±8.9, n=10 p<0.001; % of PE contraction 93.4±4.5, n=6 vs. 52.0±8.1 n=10 p<0.001).

Conclusions: Prediabetes does not cause significant endothelial damage to the pulmonary circulation. Additionally, chronic metformin treatment improved endothelial function over baseline. Interestingly, acute metformin treatment affected differently the endothelial function in controls and HF animals, suggesting that in HF animals, metformin interfered with the release of nitric oxide from the endothelium. As a whole this data suggest the existence of different mechanisms of action for the acute and chronic metformin effects on endothelial function.


Poster Communications

Is an imbalance between the Cav3-NOS-RyR2 axis cardioprotective or pathological in the pre-diabetic heart?

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Background: Cardiovascular pathologies are a major cause of death in people with diabetes. Impaired excitation-contraction (EC) coupling, involving ryanodine receptor (RyR2), is a hallmark of both type 1/2 diabetes1. We have previously reported an interaction between caveolin-3 (Cav3) and RyR2; however, the functional consequences of this interaction are unknown. Cav3 is a negative regulator of nitric oxide (NO) production via NO synthase (NOS). Significantly, post-translational modification of RyRs, S-nitrosylation, the addition of nitroso group, has been linked to ‘leaky’ channels. We hypothesize that the formation of a Cav3-RyR2 complex has a cardioprotective role regulating NOS activity within cardiomyocytes by maintaining the nitroso-redox state of the receptor a relationship that is perturbed in obesity and diabetes.

Methods: Male Sprague Dawley rats were randomly split into two groups and fed high-fat diet (HFD) (45% fat) ad libitum and control group (10%) for 16 weeks and glucose, insulin and weight were recorded over time. Echocardiography was conducted prior to sacrificing the animals under constant 1.5% Isoflurane and 50% oxygen. All procedures were performed in accordance with Scientific Procedures Act 1986. Protein expression levels analyzed using Western blot. Data were expressed as mean ± SEM and analyzed using unpaired t-test. Recombinant SPRY domain of RyR2 and full-length Cav3 were expressed and purified and microscale thermophoresis (MST) used for interaction studies.

Results: The pre-diabetic (obese) heart exhibits early signs of cardiac dysfunction, with trends towards increased LV mass, fractional shortening and E/A ratio. We have determined a down-regulation of Cav3, nNOS and eNOS in the pre-diabetic heart but no change to RyR2 levels. Although Cav3 expression levels are also down-regulated in STZ hearts nNOS (p<0.008) and eNOS (p=0.011) are up-regulated. Bioinformatics analyses identified multiple putative caveolin binding motifs (CBMs) within the RyR1 and RyR2 primary sequences. Analysis of a 3-D structure of skeletal RyR1 allowed us to identify one of the CBMs within a SPRY domain. Sequence alignment with RyR2 showed this region is conserved. Our preliminary data indicate a putative interaction between recombinant Cav3 and a recombinant SPRY domain using MST.

Conclusions: While both pre-diabetic (obese) and type 1 diabetic models show a down-regulation of Cav3, and no change to RyR2, we determined that the eNOS/nNOS profiles were significantly different. Functional studies are underway to investigate the implications of an imbalance between nNOS/eNOS and Cav3 in terms of RyR2 nitrosylation and calcium release. Biophysical and structural studies are ongoing to investigate

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the putative SPRY-Cav3 interaction, to further understand the implications of Cav3 depletion in the diseased heart.


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PCB204

Hypoglycemic and ameliorative effects of gamma-sitosterol obtained from Ficus exasperata on the pathophysiological complications of type 1 diabetes mellitus in albino rats

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Previous studies have reported that various extracts Ficus exasperata exhibited better hypoglycemic and ameliorative effects than standard antidiabetic drugs and suggested further studies to isolate and characterize the components of the plants responsible for the activities. This study was therefore designed to isolate and conduct preliminary characterization of the components of F. exasperata that are responsible for the hypoglycemic and ameliorative properties on the complication of diabetes mellitus in albino rats. Methanol extract of F. exasperata was partitioned using ethyl acetate and n hexane. Preliminary in vivo study to assess the hypoglycemic potential of the partitions was conducted using male albino rats as model. The ethyl acetate partition (with the best hypoglycemic effect) was further fractionated by column and thin layer chromatography. Fractions obtained were further used for in vivo study. Adult male rats were divided into 4 treatment and one control groups (n=5 rats). The treatment groups were induced by a single intraperitoneal injection of 150mg/kg alloxan monohydrate. The effects of treatment with 300mg/kg of the fractions and 10mg/kg of a standard antidiabetic drug (glibenclamide) on the blood glucose, haematology, liver enzymes, lipid profile and histopathology of some organs were thereafter studied. All treated rats responded positively to treatment with the fraction and hyperglycemia was reversed within 7 days of treatment. Treatment with the fraction induced significantly higher (p<0.05) haemopoetic values and lower hyperlipidemia values than the standard antidiabetic drug. Various degrees of degeneration were observed in the pancreas, kidney, liver and heart of treated and untreated diabetic rats. However, the degrees of degenerations were milder in rats treated with the fraction compared with the rats treated with glibenclamide. The fraction contained 7 compounds and the most prominent compound is gamma-Sitosterol with a percentage of 25.49. Result therefore showed that the fraction is a good candidate drug for the treatment of diabetes mellitus.

Key words: Diabetes mellitus, Ficus exasperata, hypoglycemic, ameliorative, Pathophysiology, histopathology

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PCB205

Insulin action at the rat carotid body is mediated through the activation of Kv1.3 channels

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Background: The carotid bodies (CB) are peripheral chemoreceptors that classically sense arterial O2, CO2 and pH levels (Gonzalez et al., 1994). In addition to hypoxia, in the last decade a role for the CB has a role in the control of energy homeostasis (Koyama et al., 2000; Wehrwein et al., 2010) has emerged. Recently, our group has described that insulin triggers CB activation and that carotid sinus nerve resection prevents the development of insulin resistance, suggesting the involvement of the CB in the regulation of peripheral insulin sensitivity (Ribeiro et al., 2013). However the mechanism(s) and the effectors involved on CB insulin action remains unclear. It is known that Kv1.3 channels are an important player on insulin pathways as they are involved in insulin signaling in central nervous system and periphery (Fadool et al., 2000; Xu et al., 2004). The hypothesis herein tested is that Kv1.3 channels are the effectors of insulin signaling in the CB being the phosphorylation of Kv1.3 channels, one of the potential mechanisms by which insulin modulates CAV activity. Methods: Experiments were performed in CB dissociated cells isolated from Wistar rats. Rats were anaesthetized with sodium pentobarbital (60 mg/kg i.p), tracheostomized and subsequently dissociate of CB cells. The effects of insulin and specific blockers of Kv1.3 channels, Margatoxin (MgTx) (1-10nM) and Shk-Dap22 (100pM) have been studied on voltage-activated K+-currents by whole-cell voltage-clamp recordings. Also, the expression of Kv1.3 channels and its phosphorylation in response to insulin was evaluated by immunocytochemistry and western blot, respectively. Finally, the effect of insulin and MgTx (10nM) on dopamine release from the CB has been evaluated. Results: We have found that insulin promotes a decrease in K+-current in CB type I cells and that MgTx and Shk-Dap22 mimic insulin action. Also, the reduction of the effect of insulin in K+-currents with increasing concentrations of MgTx demonstrates the involvement of Kv1.3 channels in this mechanism. Immunocytochemical studies detected the presence of Kv1.3 channels in CB type I cells and western blot the presence of the protein in the whole CB. When trying to unravel how insulin receptors interact with Kv1.3 channels, we have found that insulin was able to modulate the Kv1.3 channels activity through phosphorylation at residue tyrosine 135. Additionally, MgTx was capable of blocking the dopamine evoked-release by insulin in the whole-CB. Conclusion: We demonstrate for the first time that Kv1.3 channels are functional in CB and those channels mediate insulin action
in the CB. Modulation of their activity may serve as a novel therapeutic target for insulin resistance.

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PCB206

Partial inhibition of insulin secretion in prediabetes animal models improves peripheral insulin action and restores sympathetic activity

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Insulin resistance and disturbed insulin secretion are the main pathogenic events involved in prediabetes. Hyperinsulinemia has been traditionally looked at as a compensatory phenomenon, in response to defective peripheral insulin action, intimately linked to sympathetic overactivity. Considerable evidence suggest that increased insulin secretion, and not insulin resistance, is the primary abnormality in prediabetes (Juan et al. 1999; Koopmans et al. 1997). Herein, we studied the effect of pharmacological partial blockade of insulin production on insulin action and on sympathetic nervous system activity. Two groups of male Wistar rats were used: the high-fat diet group (HF, 60% lipid-rich diet for 4 weeks) and the control group. After 3 weeks of diet sexopotentotin (STZ, 25mg/kg, i.p.); a drug that decreases endogenous insulin secretion; was randomly administered to half the elements of each group and, afterwards, the animals were maintained on the respective diets for another week. Insulin action was evaluated at baseline, immediately after STZ administration and one week post-STZ administration, through an insulin tolerance test (100μu/kg i.v.). Oral glucose tolerance was determined two days after insulin action (2g/kg bw, per os). After completing 4 weeks of diet, rats were anaesthetized with pentobarbital (60mg/kg) and blood pressure and heart rate were recorded to evaluate autonomic nervous system, using classical spectral analysis of heart rate variability in HF rats (sympathovagal balance was 0.55%glucose/min; p<0.001, ±0.15%glucose/min, (n=8). STZ did not alter insulin action in control animals, but it restored the hypoglycemic response to an exogenous insulin bolus in HF rats (KITT control=4.71±0.55%glucose/min; p<0.001, n=8). STZ did not alter insulin action in control animals, but it restored the hypoglycemic response to an exogenous insulin bolus in HF rats (KITT HF post-STZ=4.90±0.15%glucose/min, n=16). STZ did not modify fasting plasma glucose, glucose tolerance or mean arterial pressure in any of the groups tested; however it significantly decreased circulating insulin levels (from 1.3±0.14ug/l in HF group to 0.60±0.12 ug/l in HF post-STZ group; p<0.05) and the sympathetic component of heart rate variability in HF rats (sympathovagal balance was 7.39 in HF group and decreased to 1.15 in HF post-STZ group). Our results indicate that indicate that hyperinsulinism plays a pathophysiological role in the development of decreased insulin action and that prevention of the early diabetes hyperinsulinemic response, through pharmacological blockade of insulin secretion, restores peripheral insulin action and decreases sympathetic nervous system activation in animal models.


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PCB207

Combined effect of whole-body vibration and parathyroid hormone on bone structure and material properties of ovariectomized mice

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Estrogen deficiency in postmenopausal osteoporosis alters estrogen receptor expressions, thereby elevating bone mechano-responsiveness. Parathyroid hormone (PTH), the first-approved bone anabolic drug, also sensitizes bone cells to mechanical stimuli and lowers the bone-modelling threshold of mechanical strain. Thus, the concurrent use of PTH with mechanical stimuli may be effective in preventing postmenopausal bone loss. We evaluated the combined bone-anabolic effect of PTH and low-intensity whole body vibration (WBV), which could be easily available for treating elderly osteoporotic patients. Female C57BL/6j mice were bilaterally ovariectomized (n=66) at 9 weeks of age and divided into six groups (n=11 each): the control group (C) and the groups treated with PTH (P), sine-wave WBV (s-W) at an acceleration of 0.3 g and 45 Hz for 20 min/day, noise-like WBV (n-W) at a root mean squared acceleration of 0.3g and frequency components of 45 to 100 Hz, s-W combined with PTH (s-W/P), and n-W combined with PTH (n-W/P). Two weeks later, the P, s-W/P, and n-W/P groups were subcutaneously given human PTH (1–34) at a dose of 30 μg/kg/day; the s-W, s-W/P, n-W, and n-W/P groups were exposed to sine-wave or noise-like WBV for 20 min/day. In the combined treatment, PTH was administered 30 min before each WBV session. After 18-day treatment, all mice were euthanized by pentobarbital overdose (i.p.) and the left tibiae were harvested. The proximal metaphyseal region was μCT-scanned, and a volume extending a distance of 1.5 mm distal to the growth plate was reconstructed for structure analysis (9-mm voxel resolution). In addition, its cortical bone cross-section was analyzed by Fourier transform infrared microspectroscopy and nanoindentation testing. Single application of PTH or sine-wave WBV had no effect on bone.
structure and material properties of cortical bone. In contrast, noise-like WBV and the combination of PTH with either noise-like or sine-wave WBV increased the volume fraction of trabecular bone (Fig. 1, left). Furthermore, the concurrent use increased the trabecular bone connectivity and the hardness of cortical bone (Fig. 1, right); the latter would be attributed to the elevation of mineral maturity. When combined with PTH, noise-like WBV also increased cortical bone and trabecular bone thickness. These results suggest the therapeutic potential of combining WBV with PTH for treating postmenopausal osteoporosis. Noise-like vibration may be more bone-anabolic than stationary vibration.

Fig. 1 Effects of PTH and WBV on trabecular bone volume and cortical bone hardness in the proximal tibia of OVX mice. There were no significant PTH-WBV interactions, as determined by the two-way ANOVA. C: control mice; P: mice treated with PTH; s-W: mice treated with sine-wave WBV; n-W: mice treated with noise-like WBV; s-W/P: mice treated with both PTH and sine-wave WBV; n-W/P: mice treated with both PTH and noise-like WBV. *P<0.05, **P<0.01, #P<0.005 versus C (Bonferroni post hoc tests). Bars represent mean ± S.D.

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PCB208

Sural nerve activity, as non-invasively assessed using a point of care device, in individuals with and without type 2 diabetes

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Neuropathy, dysfunction of the nerves, is a known complication of diabetes. In clinical practice neuropathy is assessed by determining patients’ ability to detect pressure using monofilaments. However, monofilaments are an insensitive assessment for neuropathy. The quick, non-invasive assessment of sural nerve activity using the novel, point of care device, NC-stat DPN-check, has been proposed to detect early evidence of neuropathy. Little is known about the variability of sural nerve activity (conductance and amplitude) in healthy individuals and individuals with type 2 diabetes but no clinical evidence of neuropathy. This study aims to examine (1) intra-participant variability of sural nerve conductance velocity and amplitude in healthy individuals and (2) whether these parameters are altered by age or diabetes.

Study 1: 5 healthy individuals were recruited (age range: 20-29yrs). Sural nerve conductance and amplitude was assessed using the DPN-Check device on 5 separate occasions for each participant. Coefficient of variation (CV) was calculated (standard deviation/mean x 100) to assess intra-participant reproducibility. Study 2: 3 groups were recruited: young healthy group (n = 19, age range: 20-36yrs, 8 males); older healthy group (n = 20, age range= 48-75yrs, 10 M) and a type 2 diabetes (T2DM) group with no clinical evidence of neuropathy (n = 25, age range = 56-81yrs, 21 M). Each participant underwent the assessment of sural nerve conductance and amplitude by DPN-Check.

Results: Study 1: Intra-participant CVs ranged from 4.0-6.9% and 26.0-39.4% for the assessment of sural nerve conductance and amplitude, respectively. Study 2: Sural nerve conductance was lower in individuals with T2DM compared to age-matched controls (T2DM group mean (SD): 43.5 (3.4); older healthy group: 47.1 (3.4), p = 0.003, Mann-Whitney U test), but was not altered by age (young healthy group: 58.4 (3.4)). Similarly, sural nerve amplitude was significantly reduced by diabetes (p = 0.012) but not age (T2DM group: 7.1 (5.7); older healthy group: 11.7 (7.3); young healthy group: 11.0 (3.7)). The non-invasive assessment of sural nerve conductance by DPN-Check demonstrates good intra-participant reproducibility in healthy individuals. The assessment of sural nerve amplitude was more variable in these individuals. Diabetes but not age per se was associated with a reduction in sural nerve conductance and amplitude prior to the development of clinical symptoms of neuropathy.

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PCB209

High fat diet increases basal carotid sinus nerve activity and the responsiveness to hypoxia

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We have recently described that animal models of diet-induced metabolic syndrome develop an overactivation of the carotid body (CB) (1). This CB overactivation results in an increase in sympathetic nervous system activity and in a reduction in insulin sensitivity and hypertension (1). These pathological features were prevented by chronic carotid sinus nerve (CSN) resection, meaning that the CB is primordial in controlling peripheral insulin sensitivity and that CB dysfunction is involved in the genesis of these disturbances. In the present work, we have investigated if the high-fat diet alters the chemosensory activity of the CSN in basal conditions and in response to CB classical stimuli- hypoxia and hypercapnia. Two groups of male Wistar rats (12-15 weeks) were used. The control (CTL, n=12) group fed a sham diet and the high-fat (HF, n=9) group fed a 60% lipid-rich diet during 21 days. After this period, insulin sensitivity was evaluated by an insulin tolerance test (ITT) and expressed by K_{ITT}. For the recording of CSN activity, CB-CSN was dissected and transferred to a recording chamber superfused with Tyrode bicarbonate equilibrated with normoxia (20%O_2+5%CO_2). Chemoreceptor activity was identified (spontaneous generation of action potentials at regular intervals) and confirmed by its response to hypoxia (0%O_2). Protocols consisted in the perfusion of CB-CSN with solutions equilibrated with hypoxias of 2 intensities (0% or 5%O_2+5%CO_2) and hypercapnia (20%O_2+10%CO_2). All animals were killed by an intracardiac overdose of pentobarbital sodium (60 mg/kg i.p.).
HF diet significantly decreased insulin sensitivity to 1.54±0.30% glucose/min (p<0.001) (KITT CTL=4.44±0.33%glucose/min). HF diet increased basal CSN frequency of discharge by 124.14% (CTL=1.74±0.38 Hz p<0.05). HF diet did not change the CSN chemosensory response to intense hypoxia as it showed a similar area under of the curve (AUC) for the frequency comparing to controls. However, CSN chemosensory response to moderate hypoxia was higher in the HF than in the control group, as the AUC of the frequency curve increased by 86.19% (p<0.05). Interestingly, the latency of the response to intense and moderate hypoxia was significantly decreased by 42.26% (p<0.05) and by 49.59% (p<0.01), respectively in the HF group suggesting a higher responsiveness to hypoxia. Moreover, in the HF group the time to reach the maximal activity (time to peak) was significantly decreased in response to intense hypoxia (CTL=193.82±10.49 sec vs HF=140.70±19.39 sec, p<0.05), without being altered in moderate hypoxia. HF diet did not alter the responses of CSN to hypercapnia.

In conclusion, the HF diet increases the basal frequency of the CSN activity and the response to moderate hypoxia as well as the responsiveness to hypoxia.


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**PCB210**

**Maternal obesity during pregnancy alters microRNAs that regulate circadian clock genes in the fetal mouse heart**


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Introduction: Maternal obesity and diabetes are increasingly prevalent during pregnancy and may have long-term consequences on the offspring’s health. Currently, metformin (MET) is prescribed to treat gestational diabetes but its impact on the developing fetus is unknown. In mice, maternal obesity during pregnancy results in altered blood pressure and heart size in the adult offspring. It remains to be determined whether such changes have their origins during fetal development and what effects maternal MET has on fetal hearts. MicroRNAs (miRNA) are implicated in cardiac differentiation and remodelling and affect the susceptibility to cardiovascular diseases. We therefore investigated whether high fat (HF) diet-induced maternal obesity with or without MET alters miRNA expression and expression of the target mRNAs within the fetal heart, which may underline the postnatal cardiovascular phenotype.

Methods: Female C57BL6 mice were fed either a HF (45% kcal fat) or control (C, 7% kcal fat) diet six weeks prior to conception and during pregnancy, with half of HF and C dams given MET in drinking water (250mg/kg bodyweight/day) through-out pregnancy. This generated four dam groups (n=4 per group): C, C+MET, HF and HF+MET. On day 16 of pregnancy dams were killed and fetal hearts taken for analysis. MicroRNA expression was measured using small RNA sequencing (Illumina miRNA-Seq, Expression Analysis, USA). Differential expression analysis identified both up and down-regulated miRNAs (p<0.05) and pathway analysis was carried out using DIANA miRPath. Expression levels of the circadian clock genes Clock, Bmal1, Per2 and Cry2 were measured by quantitative real-time PCR. Data were analysed using two-way ANOVA.

Results: 33 miRNAs were expressed differentially in hearts of fetuses from C vs HF dams, while 48 miRNAs were expressed differentially in hearts of fetuses from C vs C+MET dams. On the other hand, 71 miRNAs were expressed differentially in hearts of fetuses from C vs HF+MET dams. Pathway analysis identified circadian rhythm as a potential microRNA regulated gene pathway that is altered by both maternal obesity and metformin. Maternal obesity reduced fetal heart mRNA expression of the clock genes Per2 and Cry2 (p<0.05) vs those from lean mothers.

Conclusion: This study identified miRNAs that alter the circadian rhythm pathway in fetal hearts in response to maternal obesity. The circadian genes Per2 and Cry2 are targets of these microRNAs. This suggests that maternal obesity during fetal development could alter the expression of miRNAs and their target genes in fetal heart, and this may underlie the associated cardiovascular dysfunction in postnatal life. This work is supported by the BBSRC & Diabetes UK.

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**PCB211**

**The metabolomic response to exercise in moderate hypoxia**

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Variations in oxygen availability are known to disrupt homeostatic control, and it is known that exercise in hypoxia increases cellular metabolic permutations. However, to date, the metabolic response to exercise in hypoxia remains unknown. To examine this, 24 male participants completed one hour of exercise at a workload corresponding to 75% of pre-determined VO2peak in hypoxia (FIO2 = 0.16%), and a control repeated in normoxia (FIO2 = 0.21%), while pre- and post-exercise and 3 hour post-exercise metabolites where analysed using a LC ESI-qTOF-MS untargeted metabolomics approach in serum samples. Exercise performed in hypoxia and in normoxia independently increased metabolism as shown by a change in twenty-two metabolites associated with lipid metabolism (p<0.05, pre vs. post-exercise), though hypoxia per se did not induce a greater metabolic change when compared to normoxia (p>0.05). Recovery from exercise in hypoxia independently decreased seventeen metabolites associated with lipid metabolism (p<0.05, post vs. 3 hrs post-exercise), compared with twenty-two metabolites in normoxia (p<0.05, post vs. 3 hrs post-exercise). Twenty-six metabolites were identified as sensitive responders to exercise and recovery (pooled hypoxia and normoxia pre vs. recovery, p<0.05). These include metabolites associated with purine metabolism (adenine, adenosine and hypoxanthine), the amino acid phenylalanine, and several acylcarnitine molecules. This work demonstrates that exercise as an independent factor can activate pathways associated with lipid, protein and purine nucleotide metabolism. We conclude that exercise in...
hypoxia can activate metabolic pathways aligned to purine metabolism, but this effect is not selectively different from moderate exercise in normoxia. Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB212

Effect of Yoga on HOMA-IR and atherogenic lipid profile parameters of Mets syndrome in early postmenopausal women

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Background- Postmenopause comes with its constellation of adverse health events, metabolic syndrome being one of the leading factors of morbidity and mortality. Yoga has been described as having beneficial effect on HOMA-IR and lipid profile in various populations. Methods and Materials-67 women within five years of menopause between 45 and 60 years of age attending menopause clinic of Smt. Sucheta Kripilani Hospital fulfilling inclusion and exclusion criteria and consenting were enrolled for study with permission of Institutional Ethical Committe. Subjects were divided into 37 cases and 30 controls on the basis of their willingness to learn and practice Yoga. The cases received intervention in the form of Integrated Yoga for 12 weeks under a trained Yoga instructor from Naturopathy and Lifestyle Intervention Centre, Lady Hardinge Medical College. Fasting blood samples were collected from the subjects for serum insulin, blood glucose and atherogenic lipid profile at baseline and 3 months post intervention. HOMA-IR was calculated as per U.S. formula. Statistical analysis was done using Graph Pad Prism Version 6 software. Values are means ± S.E.M., compared by paired t-test. Statistical significance was set up at p<0.05. Result- In the control group, a significant increase in Fastig S. Insulin (7.94±1.03 pre vs 9.65±1.09 post, p=0.0066) and HOMA-IR (2.17±0.31 pre vs 2.62±0.37 post, p=0.0212) was observed at the end of 3 months. Fastig S. Insulin (6.52±0.49 pre vs 7.41±0.52 post, p=0.1681) and HOMA-IR (1.56±0.11 pre vs 1.76±0.12 post, p=0.1801) in the Yoga group showed a non-significant trend towards increase. Fasting blood sugar in the Yoga group (97.95±2.52 pre vs 97.51±2.76 post, p=0.8414) and Non-Yoga (106.9±5.13 pre vs 105.7±4.70 post, p=0.8119) group did not show any significant changes. The atherogenic lipid profile in the Yoga Group [Total Cholesterol/HDL (4.25±0.19 pre vs 4.08±0.147 post, p=0.7247), Serum Tri-glycerides/HDL (2.87±0.22 pre vs 2.82±0.26 post, p=0.2482), LDL/HDL (2.68±0.16 pre vs 2.5±0.12 post, p=0.5071), Non-HDL (141.3±5.603 pre vs 141.1±5.31 post, p=0.9804) and A.I.P (0.42±0.029 pre vs 0.39±0.037 post, p=0.8712)] and Non-Yoga Group [Total cholesterol/HDL (4.46±0.18 pre vs 4.52±0.18 post, p=0.9018), Triglycerides/HDL (3.56±0.28 pre vs 3.62±0.34 post, p=0.6190), LDL/HDL (2.73±0.15 pre vs 2.81±0.144 post, p=0.9508), Non-HDL (144.4±6.265 pre vs 151.8±5.58 post, p=0.1413) and A.I.P (0.51±0.04 pre vs 0.52±0.03 post, p=0.8120) showed no significant changes post intervention. Conclusion- Three months duration of Yoga practice attenuated the tendency for rise in insulin resistance in the case group and hence may be used as a strategy to delay or prevent the onset of insulin resistance associated with early postmenopause. Longer studies on larger sample size is needed to elucidate the effect of Yoga on atherogenic lipid profile in our population.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB213

Type I skeletal myofibre density mediates the interactive effects of maternal and post-weaning high-fat diet on muscle contraction force in adult mouse offspring

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Obesity impairs skeletal muscle strength. However, most myofibres are formed prenatally and their density is reduced by prenatal undernutrition (1) and high-fat (HF) feeding (2). In mice, maternal HF feeding during pregnancy and lactation minimized the reduction in isometric contraction force in adult female offspring soleus muscle (m) caused by a post-weaning HF diet (3). Here, we determined if this interactive effect was mediated by altered soleus m. structure.

Female C57BL/6j mice were fed a control (C: 7 kcal fat) or HF (45 kcal fat) diet 6 wks pre-mating and throughout pregnancy and lactation (PRE). Offspring were weaned onto the C or HF diet (POST), creating 4 diet groups: C/C, C/HF, HF/C, HF/HF (n=7-8 per group). Female 30 wk offspring soleus m. peak force of isometric contraction was measured in response to several electrical stimulation frequencies. 10 μm mid-belly sections of frozen muscle were cut. Fluorescent secondary antibodies (AlexaFluor) were used against primary antibodies (DSHB, Iowa) for type I (BA-F8), type IIA (SC-71), type IIb (BF-F3) and type IIX (H61) myofibres. Average myofibre density and cross sectional area (CSA) were determined in 5 fields of view. Data are mean±SEM and were analysed by 2-way ANOVA.

Across all offspring groups, higher type I myofibre density (R2 0.185, B 64.11, p<0.05), and lower type I (R2 0.197, B -9.47, p<0.05) and IIA (R2 0.234, B 14.34, p<0.01) CSA, were associated with higher peak force contraction. Across all groups, greater myofibre density was associated with smaller CSA in type I (R2 0.353, B 0.85, p<0.001) and IIA (R2 0.256, B 8.01, p<0.01) myofibres. Type I myofibre density was increased with PRE HF alone (fibres/mm2: C/C, 209.7±15.0; C/HF, 258.7±13.1, p<0.05). Conversely, type I myofibre density was decreased (fibres/mm2: C/C, 209.7±15.0; C/HF, 153.6±18.9, p<0.05) and
CA was increased (µm²; C/C, 1640±130.9; C/HF, 2089±109.1, p<0.05) with POST HF alone. Type IIA myofibre density tended to be increased in POST HF animals (fibres/mm²; C/C+HF/C, 256.7±11.6; C/HF+HF/HF, 288.9±11.2, p=0.057), and CSA was higher with POST HF alone (µm²; C/C, 1395±85.9; C/ HF, 1728±116.9, p<0.05). Across all myofibre types, density was lower in C/HF (fibres/mm²; C/C, 533.8±256.7; C/HF, 1728±116.9, p<0.05). Our data suggest that reduced type I myofibre density contributes to lower peak force in the C/HF offspring soleus muscle (3). Increased type I myofibre density in HF/C animals suggests a mechanism by which peak force contraction is maintained better in the HF/HF group. Overall, these findings reinforce the potential importance of prenatal nutrition in determining muscle health in an obese adult.


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**PCB214**

**Protective effect of 1,25 dihydroxyvitamin D3 on HCl/ethanol-induced gastric injury in rats**

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Beyond its role in the calcium and phosphate homeostasis, calcitriol - the physiologically active form of 1,25 dihydroxyvitamin D3 - is known as an important modulator of cellular proliferation, differentiation, inflammation, and immune systems. (1) The aim of the present study was to determine if vitamin D3 has a protective effect against tissue injury in a rat model of HCl/ethanol-induced gastric ulcer. Sprague-Dawley rats of both sexes (250-300 g; n=8 per group) were fasted for 24 hours. A gastric injury was induced by acidified ethanol solution (0.3 M HCl/60% ethanol) per os (0.2 ml, 2 ml per os). Ulcer groups were treated with 1,25 dihydroxyvitamin D3 (VitD; 0.25 mg/kg; intraperitoneally) for 14 days alone or along with the non-selective nitric oxide synthase inhibitor L-NAME (20 mg/kg; intraperitoneally). ATP-sensitive K⁺ channel blocker glibenclamide (10 mg/kg; orally) or the non-selective cyclooxygenase inhibitor indomethacin (Indo; 10 mg/kg; subcutaneously). On day-15, the rats underwent ulcer induction by HCl/ethanol and decapitated 60 min later. The stomachs were examined macroscopically. Stomachs and trunk blood were sampled for biochemical assays. All procedures were approved by the Marmara University, Animal Care and Use Committee. Values are means±SEM, compared using ANOVA and Student’s t-tests. Gastric macroscopic lesion score of the untreated ulcer group (33.13±5.09) was decreased by pretreatment with VitD (19.00±4.34; p<0.05) and this effect was augmented by L-NAME (0.11±0.05; p<0.01) and attenuated by Indo (45.33±6.04; p<0.01) given along with VitD. Ulcer group revealed increased gastric malondialdehyde (MDA) (15.20±1.64 nmol/g; p<0.001), reduced endogenous antioxidant glutathione (GSH) levels (0.69±0.09 mmol/g; p<0.05) and increased myeloperoxidase (MPO) activity (20.96±0.86 U/g; p<0.001) in comparison to control. The changes in these parameters were effectively suppressed by VitD (7.63±0.31 mmol/g; p<0.01, 1.33±0.16 mmol/g; p<0.01 and 8.82±0.41; p<0.001 U/g, respectively). Indo reversed the effect of VitD on MDA, GSH and MPO levels (p<0.05-p<0.001) whilst NEM had a slighter effect on gastric MDA and MPO levels achieved by VitD (p<0.05-p<0.001). Gastric levels of the endogenous antioxidants superoxide dismutase (SOD) and catalase did not show statistically significant changes in the untreated ulcer group in comparison to control and VitD did not seem to change these parameters. In conclusion, pretreatment with VitD protected the gastric tissue against oxidant injury in a rat model of HCl/ethanol-induced gastric injury via mechanisms possibly involving the cyclooxygenase system.


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**PCB215**

**Depot resolved quantification in clinical FDG-PET/CT scans reveals a higher than assumed brown fat mass**

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Brown adipose tissue (BAT) provides non-shivering thermogenesis. In humans, active BAT can be visualized by its 18F-fluoro-deoxyglucose (FDG) uptake as detected by positron emission tomography (PET) combined with computer tomography (CT). The retrospective analysis of clinical scans is a valuable source to identify anthropometric parameters that influence BAT mass and activity and thus the potential efficacy of envisioned drugs targeting this tissue to treat metabolic disease. We analyzed 2854 FDG-PET/CT scans from 1644 patients. We identified 98 scans from 81 patients with active BAT (+). We quantified the volume of active BAT depots (mean values in ml ±SD (n): total BAT 162±183 (98), cervical 40±37 (53), supraclavicular 66±68 (71), paravertebral 51±53 (69), mediastinal 43±40 (51), subphrenic 21±21 (29)). Since only active BAT is detectable by FDG uptake, these numbers underestimate the total amount of BAT. Considering only 32 scans of the highest activity as categorized by a visual scoring strategy, we determined a mean total BAT volume of 308±208 ml. In 30 BAT+ patients with three or more repeated scans we calculated a much higher mean probability to re-detect active BAT (52±25%) as compared to the overall prevalence of 4.9%. We analyzed parameters that characterize BAT+ subjects by comparing them to the overall cohort and to an age- and sex-matched control group. We observed a higher proportion of young (p<0.001) and female (p<0.001) patients in comparison to the total cohort and, in the case-control design, a clear annual pattern of less BAT+ scans during summer and
an according difference in mean outdoor temperature during scans (p=0.03).

We also analyzed parameters that influence the extent of activation within the BAT+ group by calculating a BAT activity index (BFI) based on volume and intensity of individual BAT depots. By a linear regression model we detected higher total BFI in younger patients (p<0.009), while sex, BMI, height, mass, outdoor temperature and blood parameters did not affect total or depot specific BAT activity. Surprisingly, renal clearance as estimated from mass, age and creatinine was a significant predictor of BFI on the total (p=0.005) as well as on the level of several individual depots.

In summary, we detected an amount of active BAT higher than previously reported. BAT-positive patients represent a group with higher than usual probability to activate BAT during a scan. This characteristic was found more often in young, female subjects and was amplified by cold weather. Estimated renal clearance correlated with the extent of activated BAT in a given scan, probably as a secondary reporter of sympathetic tone. These data imply an efficacy of drugs targeting BAT to treat metabolic disease that is at the same time higher and subject to a larger individual variation than previously assumed.

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Bicarbonate intake promotes M1 to M2 macrophage polarization and limits renal tubulointerstitial damage in Dahl salt-sensitive rats

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We have recently reported that NaHCO3 supplementation (drinking water) limits the development of tubular casts and fibrosis in the kidneys of high salt fed Dahl salt-sensitive (SS) rats, independent of lowering systemic arterial pressure. M1 or classically activated macrophages are the first line of defense against infection and release inflammatory cytokines and damage tissue. Conversely, anti-inflammatory macrophages, also called M2 or alternatively activated macrophages, release anti-inflammatory cytokines and promote tissue repair after injury. Much recent evidence implicates macrophage polarization in the progression of numerous disease states including acute kidney injury. However, the role of macrophage polarization in progressive renal injury models such as the Dahl SS rat has not yet been investigated. In the current study we investigated whether NaHCO3 supplementation alters macrophage polarization in Dahl SS rats.

11 week old male Dahl SS rats were either treated with vehicle (0.1M NaCl; n=10) or NaHCO3 (0.1M; n=11) in drinking water for the length of the study. At the beginning of the study, rats were maintained on a 0.4% NaCl diet (low salt, LS: DyetsAIN76A) for 4 days before being switched to an 8% NaCl diet (high-salt, HS) for 14 days. On day 14 of HS, rats were anesthetized with isoflurane (inhalation 2-5%), arterial blood gas measurements taken and both arterial blood (n=5 each group), or the spleen (n=5 each group) and the left kidney excised for flow cytometry analysis. Rats were then humanely euthanized by pneumothorax without recovery from anesthesia. Data were analyzed by ANOVA.

Our data indicate that NaHCO3 treatment promoted a robust shift in the renal macrophage phenotype toward M2 macrophages (vehicle M1:0.26±0.03, M2:0.12±0.02 vs bicarbonate M1:0.12±0.02, M2:0.20±0.03 % of total kidney cells respectively, pTREATMENT =0.0002) and that this was associated with reduced tubulointerstitial injury without causing systemic alkalosis in HS fed rats. This shift in macrophage polarization was evident even in Dahl rats treated with NaHCO3 for 3 days while fed LS (vehicle M1:0.30±0.06, M2:0.20±0.05 vs bicarbonate M1:0.16±0.04, M2:0.40±0.02 % of total kidney cells respectively, pTREATMENT =0.007) and occurred prior to the advent of differences in kidney injury in this group. Furthermore, examination of splenic tissue revealed a similar effect of NaHCO3 to promote a predominantly M2 phenotype (vehicle M1:8.6±0.5, M2:6.4±0.5; bicarbonate M1:5.8±0.8, M2:8.3±0.5 % of total splenic cells respectively, pTREATMENT =0.0005).

These data indicate that NaHCO3 may be a relatively safe and effective way to modulate macrophage phenotype in vivo and that a shift in macrophage polarization toward regulatory M2 macrophages may underlie some of the protective effects of NaHCO3 treatment on renal function.

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Sex-specific and oestrous-cycle dependent effects of oestrogen on electrolyte balance in mouse colonic epithelium

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The large intestine plays a major role in the regulation of whole-body fluid and salt homeostasis. Every day 1–1.5 l of fluid enters the colon with only 0.1–0.2 l being lost in the faeces under normal conditions. The balance between Na+ absorption and Cl− secretion underpin intestinal fluid movement1. Oestrogen, (17β-estradiol, E2), produces a female sex-specific, anti-secretory effect in rat and human distal colon2. This response involves the inhibition of KCNQ1:KCNE3 potassium channel activity via PKCδ modulation3. The aim of this work was to determine the molecular mechanisms underlying E2 modulation of colonic electrolyte homeostasis using genetically modified mouse models. Experiments were carried out on wild-type (WT) and genetically modified IK-1-KO and PKCΔ to promote a predominantly M2 phenotype (vehicle M1:0.30±0.06, M2:0.20±0.05 vs bicarbonate M1:0.16±0.04, M2:0.40±0.02 % of total kidney cells respectively, pTREATMENT =0.007) and occurred prior to the advent of differences in kidney injury in this group. Furthermore, examination of splenic tissue revealed a similar effect of NaHCO3 to promote a predominantly M2 phenotype (vehicle M1:8.6±0.5, M2:6.4±0.5; bicarbonate M1:5.8±0.8, M2:8.3±0.5 % of total splenic cells respectively, pTREATMENT =0.0005).

These data indicate that NaHCO3 may be a relatively safe and effective way to modulate macrophage phenotype in vivo and that a shift in macrophage polarization toward regulatory M2 macrophages may underlie some of the protective effects of NaHCO3 treatment on renal function.

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PCB216

PCB217
Serum level of calcium, inorganic phosphate and alkaline phosphatase in pre and post menopausal women in Owerri, south eastern Nigeria

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Oestrogen deficiency is a major pathological factor in osteoporosis in postmenopausal women (1). Postmenopausal women are at greater risk of developing several forms of bone diseases especially osteoporosis. Osteoporosis is characterized by low bone mass and micro-architectural deterioration of bone tissues with susceptibility to fractures (2). Serum levels of calcium, inorganic phosphate and alkaline phosphatase are good markers to ascertain bone integrity. There is a lack of adequate biochemical data and reference values for the management of age and sex related diseases in the developing world. This study was carried out with a view to establish a baseline normative reference values for the management of osteoporosis in postmenopausal women (1). Postmenopausal women were at higher risk of developing several forms of bone diseases especially osteoporosis. Osteoporosis is characterized by low bone mass and micro-architectural deterioration of bone tissues with susceptibility to fractures (2). Serum levels of calcium, inorganic phosphate and alkaline phosphatase are good markers to ascertain bone integrity. There is a lack of adequate biochemical data and reference values for the management of age and sex related diseases in the developing world. This study was carried out with a view to establish a baseline normative reference values for the management of osteoporosis in postmenopausal women.

We wish to acknowledge the assistance of the entire staff of the Department of Human Physiology of Abia State University, Uturu and encouragement by Dr C.D. Ubani, Director of Medical Services, Abia State University Uturu Medical Centre towards the success of this study.

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largely on morphometric measures, metabolic markers, liver pathology, fibrosis, inflammation, and gut microbial profile. As expected, 20 months of HFD feedings augmented body weight compared to LFD-fed mice (p<0.05). This increase in body weight was in part due to the significant expansion of epididymal, retroperitoneal, and mesentery fat (p<0.05). In addition, HFD mice showed marked hepatosplenomegaly, a common indicator of chronic liver disease. HFD mice experienced significantly elevated concentrations of fasting glucose and insulin (p<0.05). These results were consistent with the HOMA index in which HFD mice exhibited insulin resistance (p<0.05). HFD-fed mice had severe steatosis; ballooning degeneration, lobular and portal inflammation, and pericellular fibrosis were evident. Oil red O staining exhibited macrovesicular accumulation of triglycerides in the hepatocytes of HFD mice. With regards to the microbiome, the HFD group showed a different microbial community structure compared to the LFD group; HFD-fed mice showed lower richness but higher evenness compared to the LFD-fed mice. At the phylum level, the HFD group had higher relative abundance of Firmicutes and lower abundance of Bacteroidetes compared to the LFD group. Our data indicates that chronic HFD consumption results in significant NAFLD that is related to changes in the gut microbiome profile; we report a significant increase in liver steatosis, ballooning degeneration and inflammation in association with an altered gut microbiome profile following prolonged HFD feedings in mice.

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PCB220

The effect of brotizolam on ghrelin levels in mice
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Ghrelin is one of the modulatory hormones of food intake which is mainly secreted from gastric fundus and pylorus (1). Benzodiazepines are intuitively considered as suppressors of the ghrelin release in humans (2, 3), although they are intuitively known as appetite stimulators in animals (4). Even though, controversial reports are noticeable in the medical literature (5). In the present study, we investigated the change of circulating ghrelin levels in mice, in which brotizolam, a benzodiazepine that is legislatively approved against insomnia in several countries, was administered. To this aim, two groups of adult male Balb/c mice (n = 8 for each) were intraperitoneally received either saline or brotizolam (2 µg/kg). Thirty minutes after the administration, blood samples were collected and serum ghrelin levels were measured by using an enzyme-linked immunoassay kit (Elabscience Biotech., China). The data were analyzed with Student's t-test and a p-value below 0.05 was considered to be statistically significant. As compared to saline-injected controls, circulating ghrelin levels were found to be significantly higher in brotizolam-administered animals (p < 0.001). Therefore, the present study demonstrated that brotizolam can enhance the levels of circulating ghrelin. This effect may be arisen from a direct stimulation on ghrelin secreting cells or a blockage of the negative feedback of ghrelin on its receptors in the hypothalamus.


Authors have no conflict(s) of interest to disclose.

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PCB221

Regulation and distribution of intracellular pH defines the role of a dynamic microenvironment in cancer cells
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The transition in cellular metabolism supporting cancer cell proliferation generates an acidic extracellular pH (pH\textsubscript{e}) environment to provide survival advantage over non-malignant cells(1). The intrinsic intracellular buffering capacity (pH\textsubscript{i}) dictates the intracellular pH (pH\textsubscript{i}) response to acidic pH\textsubscript{e}(2). Additionally, pH\textsubscript{i} regulatory mechanisms establish the pH\textsubscript{i} homeostasis influencing cellular processes contributing to oncogenesis(3). Hypoxia exacerbates acidosis exerting selection pressure that promote and sustain oncogenesis(4). We hypothesized that altered acidic microenvironment influences pH\textsubscript{i} regulation in ovarian cancer cells (SKOV3). We investigated the change in buffering capacity of the cancer cells and correlated the pH recovery rate to attain steady state pH\textsubscript{i} in response to an altered pH\textsubscript{e}. The pH\textsubscript{i} change was mapped to understand the proton dynamics of pH\textsubscript{i} regulation in altered pH\textsubscript{e}. Cells were cultured at 6.3-6.5 or 7.2-7.4 pH\textsubscript{i} in parallel with either normal conditions (0.21 atm) or at 0.029 atm PO\textsubscript{2} mimicking in vivo tumour environment. The measured pH\textsubscript{i} distribution was mapped across the cSNARF(pH-sensing dye) loaded cells, using wide-field microscope in live cell setup. The acid dumping capacity of the cell was assessed by acutely loading them with acid and monitoring the subsequent pH\textsubscript{i} recovery towards initial levels in respective pH\textsubscript{e}. The pH\textsubscript{i} recovery data points were fitted through the two limbs of a segmented linear regression curve. The differences between the slopes separated by breakpoints described an abrupt change in pH\textsubscript{i}
recovery in altered pH_i. The β_i of cells (N>7) drifted from 0.2 to 0.6 (mM/pH) when switching from physiologically pH_i (7.4) to 6.3 at atmospheric PO_2. The pH_h recovery was achieved by multiple acid extruding mechanisms. A pulse of 20mM NH_4^+ produced gradual pH_i recovery of 0.3 & 0.5 (pH/minutes) for pH_i 7.4 & 6.3 respectively. The recovery slowed to 0.08pH/minutes and 0.09pH/minutes after 18,16 minute breakpoints for pH_i 7.4 and 6.3 respectively. Interestingly the slopes of linear regression lines during initial 2 minutes of pH_i recovery were significantly (p<0.005) different. The centre of the cells reported a predominant pH_i of 6.99 for cells in 6.3 pH_e and 7.35 in 7.4 pH_e at 0.21 atm PO_2. The pH_i distribution manifested differentially, contributing to unique steady state pH_i in altered pH_e. The extent of β_i of ovarian cancer cells was dependent on altered microenvironmental proton dynamics.

Our results highlighted that cancer cells adapt to changes in their microenvironment favouring unique pathophysiological processes through regulation of pH_i. It is important to understand how proton dynamics influence transformation of non-malignant cells, to develop potential therapeutic strategies. Further investigations will reveal new avenues to meet the challenge of targeting tumour metabolism.


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**PCB222**

**Melatonin ameliorates inflammation in kidney and liver of the rats with type 2 diabetes mellitus**

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Inflammation is one of the main causes of complications of Type 2 diabetes mellitus. There are many studies related to diabetic microvascular complications with inflammation (Lim et al, 2012). Melatonin is a hormone released from pineal gland with circadian rhythm. It is known that melatonin has anticancerogenic and antiapoptotic effects (Sainz et al, 2003). Melatonin is also related to inflammation (Chen et al, 2015). Human studies show that insulin secretion is related to circadian rhythm in diabetic patients had altered diurnal melatonin secretion (Peschke et al, 2006).

Inflammation that occurs by different mechanisms causes a progress in diabetic microvascular complications. The inhibition of inflammatory cytokines would cause to gain extra benefits in treatment of the complications. In this study, we have chosen melatonin because of its both anti-inflammatory and antioxidant effects. In this study, we figured out the effect of melatonin on inflammation in kidney and liver tissues of rats with Type 2 diabetes mellitus. 2.5 months old Sprague Dawley male rats were used for this experiment. Type 2 diabetes was induced with nicotinamide (NA 100mg/kg) and streptozotocin (STZ 50mg/kg). We had 3 experimental groups consisted of 10 rats each; control group, type 2 diabetes mellitus groups and type 2 diabetes mellitus group treated with melatonin (500 µg/day) during 4 weeks. Xylazine (10mg/kg) and Ketamin 100 mg/kg were used for anaesthetics. After tissues were taken, rats were decapitated.

We studied inflammation markers, IL-1beta, TNF-alpha, IL-6 and NFκB protein levels both with Western Blot and immunohistochemistry in the liver and also we evaluated stated markers in kidney with immunohistochemistry. Protein bands of western blot studies were monitored with BCIP/NBT and protein densitometric analysis were performed with Image J programme. We found out that all inflammation marker levels were increased significantly in type 2 diabetes group than the control group (p<0.05). Our data showed that inflammation markers of melatonin treated groups decreased significantly when compared to type 2 diabetes group (p<0.05) both in kidney and in liver tissues.

Type 2 diabetes is a metabolic disease affects all the tissues and the systems in the body through its complications. Liver and kidney are one of the tissues most affected of the disease due to their roles in the body. Earlier increased levels of inflammation markers were reported in diabetes (Donath et al, 2016). Inflammation isblamed for the microvascular complications of diabetes. Melatonin is a hormone well known by its anticancerogenic and antiapoptotic effects. Now in this study we observed that melatonin decreased inflammation in kidney and liver tissue of diabetic rats. Melatonin might be an important agent to prevent microvascular complications by decreasing inflammation in type 2 diabetes mellitus.


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**PCB223**

**Suppressors of cytokine signalling 2 influences the effects of 17β-Estradiol in vivo**

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17β-Estradiol (E2) has been shown to induce SOCS2 which in turn negatively regulates GH-STAT5 signaling (1) in liver. This could be clinically relevant because STAT5 is of particular importance in the regulation of endocrine, metabolic, and gender-differentiated actions of GH in liver (2). In this work,
we have evaluated the influence of SOCS2 on E2-regulated somatic growth and hepatic transcriptome. The influence of SOCS2 on E2-regulated somatotropic-liver axis was shown by E2 did not prevent body weight gain and longitudinal growth. At end point, significant differences, in comparison with vehicle-treated mice, in body and femur lengths remained in E2-treated WT but not in E2-treated SOCS2-/- mice. Notably, E2 increased hepatic IGF-I mRNA levels in both genotypes without changes in circulating IGF-I. In WT mice, E2 induced hepatic mRNA expression levels of SOCS2, CIS, and SOCS1 whereas they were not induced in SOCS2-/- mice. However, E2 induced a female pattern of genes in liver and the absence of SOCS2 was enough by itself to feminize male liver. SOCS2-/- mice had increased in WT mice whereas cell cycle and proliferation immune response in the absence of SOCS2 whereas it was extensive re-programming of liver physiology by short-term E2 treatment and the influence of SOCS2-/- on E2's effects 678 genes were induced and repressed by E2, respectively, in SOCS2-/- but not in WT. Overall, these results reveal an

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Effect of feeding cycle on excitability of rat hippocampal neurons – the role of voltage-gated Na+ channels biophysics

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Recent research has been performed to understand how if variations of metabolism may condition brain activity. Specifically, studies on hippocampus have gained relevance owing to its primary importance in cognitive processes. In this regard, in our laboratory, we found that feeding cycle

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Ghrelin sensitises colonic myenteric neurons to the neurostimulatory effects of glucagon-like peptide 1

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Background Ghrelin is an orexigenic hormone derived from the stomach that is at peak levels prior to ingestion of food. Following meal ingestion ghrelin concentration drops back down to basal levels immediately. Subsequently the incretin hormone glucagon-like peptide-1 (GLP-1) is secreted by intestinal L-cells in response to nutrients. GLP-1 is thought to act

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via its receptor to inhibit gastric acid secretion and motility. Ghrelin directly stimulates L-cell to secrete GLP-1 and has been shown to prime L-cells for nutrient-induced GLP-1 release. The aims of the study are to investigate the effects of ghrelin on GLP-1-mediated neuronal regulation of gut motility. Methods Cross-sections of Sprague Dawley distal colon and longitudinal muscle myenteric plexus tissue preparations were fixed permeabilised and blocked prior to immunofluorescent labelling of ghrelin and GLP-1 receptors. Colonic motility was assessed in organ baths. Results Both ghrelin and GLP-1 receptors were detected in the smooth muscle and myenteric nerve plexus of the distal colon with significant co-localisation. In longitudinal muscle myenteric neuron tissue preparations, both GLP and ghrelin receptors were localised to the cell membrane where they were expressed in a punctate pattern. Myenteric neurons regulate colonic motility and consistent with this function, ghrelin increased circular muscle contractility (p<0.001) as measured by an increase in area under the curve. GLP-1 had a smaller effect (p>0.05) and application of GLP-1 after prior stimulation with ghrelin potentiated the GLP-1 response by comparison to the control. In longitudinal muscle, ghrelin (p<0.05) and GLP-1 (P<0.05) increased contractile activity. However, the effect of GLP-1 was not further potentiated in this tissue by prior exposure to ghrelin. Consistent with a differential effect in each of the muscle layers, ghrelin enhanced the amplitude of the carbachol-evoked contractions in circular (p<0.05) but not longitudinal (p>0.05) muscle.

Discussion GLP-1 and ghrelin receptors are highly co-localised in myenteric neurons of the distal colon, indicating possible crosstalk in relation to neuronal regulation of smooth muscle contractile function. Indeed, ghrelin increased contractile activity in both circular and longitudinal muscle. However, in circular muscle while GLP-1 did stimulate a contractile response, this was enhanced by prior exposure to ghrelin. This was not observed in longitudinal muscle. These findings suggest that ghrelin may sensitise myenteric neurons to the neurostimulatory effects of GLP-1.

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Antibody mediated targeting of FGFR1c increases glucose uptake into white and brown adipose tissue

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Current estimates indicate that 8% of adults are diabetic world-wide, a large proportion of which live in the developing world. Existing therapies are unable to provide durable and robust change in metabolic status, thus there remains an imperative to identify novel therapeutic agents able to effect meaningful metabolic change in this patient population. Antibody-mediated targeting of fibroblast growth factor receptor 1 (FGFR1) has been shown to ameliorate hyperglycaemia and protect from diet and genetically induced obesity in rodents. Specifically, we have previously reported that administration of a monoclonal antibody (IMC-H7) which selectively targets the FGFR1c isoform, acts centrally to lower caloric intake and ultimately reduce body weight in an natural animal model of obesity, the Siberian hamster. However, it is currently unknown which tissue(s) contribute to these effects following administration of these monoclonal antibodies. Thus, to further elucidate the mechanistic underpinning of the glucose lowering effects associated with targeting the FGFR1c, we employed whole body positron emission computed tomography (PET/CT) scanning with a glucose tracer (18F-flurodeoxyglucose; 18F-FDG). Given the emphasis on FGF action in adipose in recent publications, we paid particular attention to uptake in white (WAT) and brown (BAT) adipose tissue. Male mice (n=16) received a single subcutaneous injection of vehicle (saline, n=8) or IMC-H7 (3mg/kg, n=8). Group 1 (n=4 per treatment) were transferred to metabolic cages 24 hours post treatment for whole-body metabolic analysis for 48 hours. Group 2 (n=4 per treatment) were assessed for tissue specific glucose uptake via PET/CT. When compared to vehicle controls, mice treated with IMC-H7 exhibited a reduction in body weight (+0.83 ± 0.13 vs -2.97 ± 0.29 g, p < 0.0001) and food intake (4.35 ± 0.44 vs 2.76 ± 0.37 g, p < 0.001). However, there was no effect of treatment on energy expenditure (94.78 ± 10.73 vs 97.50 ± 4.09 kJ/kg/hr). Interestingly, PET/CT imaging showed treatment with IMC-H7 significantly increased 18F-FDG uptake in BAT (4.95 ± 0.45 vs 8.24 ± 0.47 SUV, p < 0.01) and WAT (4.16 ± 0.55 vs 6.74 ± 0.79 SUV, p < 0.05), as well as the brain (3.61 ± 0.09 vs 5.24 ± 0.44 SUV, p < 0.01). Whilst 18F-FDG uptake in the quadriceps were significantly reduced in response to treatment with IMC-H7 (2.47 ± 0.12 vs 1.71 ± 0.20 SUV, p < 0.05). This effect, however, was compensated for by the increased glucose uptake in BAT and WAT, as blood glucose was significantly reduced after treatment with IMC-H7 (14.3 ± 0.8 vs 7.5 ± 1.7 mmol/l, p < 0.001). Taken together, these data suggest that antibody-mediated targeting of FGFR1c exerts its powerful glucose-lowering efficacy primarily due to increased glucose uptake in WAT and BAT.

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Alloxan-induced diabetic and insulin resistant effects on ovulation and phases of estrous cycle in virgin female Sprague-Dawley rats

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Sexual disorders have been extensively studied in diabetic men and diabetes in pregnant women has been associated with increase chances of miscarriage and, increased risk of birth defects. Possible changes in the sexual function of diabetic women have only recently received attention. The aim of this study is to investigate the effects of alloxan-induced diabetes and insulin resistant diabetes on estrous cycle and ovulation in virgin female rats.

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Virgin female Sprague-Dawley rats showing two consecutive estrous cycles of the same length were randomly divided into 3 groups (n=60). Group 1 (n=20): Control group; fed on normal rat chow. Group 2 (n=20): Alloxan-diabetic group: at the 4th week received a single intravenous injection of Alloxan monohydrate, 40 mg/kg body weight into the lateral tail vein (Ilanoye et al., 2013). Hyperglycaemia was confirmed 72 hours later (glucose oxidase method by Hugget and Nixon, 1957). Group 3 (n=20): Insulin resistant group were fed ad libitum on a special diet containing 25% fructose mixed with 75% normal rat chow (w/w) for 6 weeks and hyperglycaemia confirmed at the 6th week (Arikawe and Olatunji-Bello, 2004). All animals had free access to drinking water throughout the duration of the study and weekly body weight was recorded. Estrous cycles were monitored by colpocytological examination (vaginal smears) daily for 14 consecutive days. (Marcondes, et al., 2002). Five animals from each group were sacrificed by cervical dislocation on proestrus, estrous, metestrus and diestrus phase and blood collected through cardiac puncture for hormonal analysis (LH, FSH, Estradiol, Progesterone). On the morning of estrus phase, the oviducts were excised, viewed under the microscope and ova found were counted. Results showed a significant reduction (p<0.05) in the frequency of estrus phase in diabetic groups (Alloxan group: 0.6±0.2, insulin group: 1.6±0.2) compared with control (1.3±0.2). Number of ova released was reduced significantly in the diabetic group (control 5.4±0.9, Alloxan group: 1.4±0.4 and insulin group: 1.8±0.4) while atretic follicles were significantly increased (p<0.05) in the diabetic groups (Alloxan: 6.0±0.5; insulin: 3.2±0.4) compared with the control (0.4±0.1). LH, FSH, Estradiol, and Progesterone were significantly reduced at estrous and metestrous phases in all the groups. Estrous level was significantly reduced (p<0.05) in the diabetic groups during proestrus and estrus phases (proestrus: control 86.0±0.5, Alloxan 39.2±0.2 insulin group 56.0±0.3; diestrus control 85.0±0.4, Alloxan 57.0±0.3 insulin group 60.0±0.2) ng/ml. This study show that hyperglycemia/diabetes alters the anterior pituitary hormones causing a “disordered estrous” cycle and reducing the number of ova released. Iranloye Bolanle O, Oludare Gabriel O, Morakinyo Ayodele O, Esuame Naomi A, Ekeh Lucy C. (2013) t. Journal of Human Reproductive Sciences Volume 6 Issue 4, 267-272


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Modulatory effects of guava (Psidium guajava) extract on Adriamycin induced toxicities in Wistar rats

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Adriamycin (ADR), though, a drug of choice in cancer therapy, its use is associated with acute and chronic complications, which are capable of exacerbating the conditions of the patients. Thus, efforts are being directed at evaluating several compounds, antioxidants and natural products that are capable of ameliorating these complications whilst still retaining the potency and efficacy of Adriamycin as an anti cancer agent. The modulatory and ameliorative effects of methanol guava leaf extract (MGLE) (Psidium guajava Linn) against Adriamycin/Doxorubicin toxicity was evaluated in Wistar rats.

Thirty Male rats were divided into six groups A – F of five rats each, Group A fed normal saline, B- single acute dose of ADR, C- 500mg/kg guava extract alone, D to F - ADR in combination with 125, 250 and 500mg/kg body weight of guava leaf extracts,(MGLE) respectively for seven days after which blood samples were collected from all the animals. Liver and kidney damage markers were assessed in serum, liver histopathology, and micronucleated polychromatic erythrocytes (mPCEs) from bone marrow were assessed, and data represented as mean ± SE. Adriamycin resulted in considerable liver and kidney damage as seen in the form of elevated liver enzymes, total protein as well as urea and creatinine levels. It also resulted in significant (P<0.05) increases in the total triglyceride and cholesterol levels. The use of the extract alone on the other hand showed hepatoprotective and nephroprotective properties of guava leaf extract in that the above parameters were significantly lower than those of the untreated control. Hepatic histopathology indicated no visible lesion in the control group, necrotic hepatocytes observed in ADR, no visible lesion seen in the MGLE treated group and mild mononuclear cellular infiltration in ADR +MGLE treated groups. ADR induced significant mPCEs formation in rat bone marrow (P < 0.05), a dose dependent decrease (P < 0.05) in mPCEs in ADR + MGLE rats compared with ADR was observed. The MGLE exhibited hepatoprotective and anticlastogenic potentials. Our findings suggest that MGLE may mitigate the effect of ADR-induced toxicities.

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Asparaginase-induced pancreatitis via protease-activated receptor depends on calcium and ATP

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L-Asparaginase is an essential element in the successful treatment of Acute Lymphoblastic Leukaemia (ALL), the most common type of cancer to affect children. Unfortunately, Asparaginase treatment results in acute pancreatitis (AAP) in about 5 – 10% of cases forcing to discontinue Asparaginase treatment. Our main approach for studies of physiological/pathophysiological signalling in pancreatic cells was the monitoring of Ca2+ concentrations in the cytoplasm and organelles. Pancreatic acinar cells were freshly isolated from mice. Animals were sacrificed according to the Animal Scientific Procedures Act, 1986 and approved by the Ethical Review Committee of Cardiff University. After digestion the pancreas was digested using collagenase-containing solution (200 U/ml, Worthington, UK). For measurements of [Ca2+]i,
isolated pancreatic acinar cells were loaded with Fluo-4-AM (5 µM). Necrotic cell death was assessed with propidium iodide uptake. Data are presented as mean ± SEM, statistical significance and P values calculated using t-test or ANOVA with P<0.05 considered significant. Monitoring Ca2+ concentrations in the cytoplasm, we have shown for the first time that intracellular Ca2+ release followed by Ca2+ entry and resulting in [Ca2+]i plateau is the main type of Ca2+ response to Asparaginase. The primary intracellular Ca2+ release was largely dependent on IP3 and NAADP signalling mechanisms. The IP3 blocker caffeine (20 mM) markedly inhibited the Asparaginase-induced [Ca2+]i elevations in the absence of external Ca2+ (n=8). The PLC inhibitor has blocked the Asparaginase-induced [Ca2+]i elevation (n=11). ACh (1 µM) was applied at the end of each experiment but did not elicit any change in [Ca2+]i. Ryanodine (100 µM) markedly inhibited the Asparaginase-induced Ca2+ signals (n=13). Ned-19 (100 µM) has also prevented the Asparaginase-induced [Ca2+]i elevation (n=8). The Asparaginase-induced Ca2+ signals were practically eliminated by the protease-activated receptor 2 (PAR2) inhibitor FSLLLRY-NH2 (10 µM) (n=32). The toxic Ca2+ signals caused significant necrosis (17.4%, n>250 in each independent series of experiments). Inhibition of calcium signals as well as PAR2, or pyruvate supplementation significantly blocked Asparaginase-induced necrosis. Understanding AAP pathogenesis could lead to development of effective therapies for this complication, potentially reducing toxicity and allowing re-exposure to continued treatment with Asparaginase.

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Depressive-like behavior is reduced by alpha-lipoic acid in lipopolysaccharide-treated mice

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Depression is a common mental disorder characterized by long lasting or recurrent low mood and disturbed cognition. It is associated with confused appetite and sleeping pattern, and leads to increased morbidity and mortality. The oxidative stress plays important role in development of anxiety and depression. Hence, strong antioxidants that target oxidative stress may be promising for protection against anxiety and depression. Alpha lipoic acid (ALA) has been shown to improve depression-associated oxidative stress parameters in a mouse depression model. However, few knowledge has emerged as to the antidepressant activity of ALA yet. In the present study, the efficiency of ALA in lipopolysaccharide-induced depression-like behavior was investigated. All experimental procedures have been approved by Selcuk University Animal Care and Ethics Committee. The animal model of depression was created in Swiss albino mice (n = 30) by a single intraperitoneal injection of lipopolysaccharide (LPS) in a dose of 0.83 mg/kg (3). Solely physiologic saline was administered to the control group (n = 10). The animals that received LPS were equally divided into three groups as LPS (no treatment was given), positive drug control (pre-treated with 10 mg/kg, i.p. fluoxetine for 7 days), and ALA (pre-treated with 100 mg/kg, i.p. ALA for 7 days). Depression-like behaviors were assessed 24 hours after the LPS injection. All behavioral tests were carried out by the same researcher, and scored by another researcher who was blind to the groups. The data were analyzed by using one-way ANOVA post hoc Tukey’s test, and presented as means ± standard deviations. The statistical significance was considered as p < 0.05. The animals in the LPS group displayed increased immobility in both forced swimming (p < 0.001) and tail suspension tests (p < 0.001). Furthermore, those animals showed less sucrose preference (p < 0.01). There was no statistical significance between groups for spontaneous locomotor activity. According to the results, it can be suggested that LPS provokes depression-like behavior in mice. Once daily pre-treatment with ALA or fluoxetine for 7 consecutive days prevented LPS-induced abnormal behavior. ALA and fluoxetine shortened the immobility duration in forced swimming and tail suspension tests (both p < 0.001). Therewithal, only LPS group was statistically different than other groups in regard to the sucrose consumption (for both LPS vs ALA and LPS vs fluoxetine p < 0.01). The findings of the present study demonstrated that alpha-lipoic acid may be useful for alleviating anhedonic and depression-like behaviors in mice. Further studies should be performed to elucidate precise mechanisms of the mentioned features of alpha-lipoic acid.

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PTZ groups entered in shorter times, indicating memory dysfunction. NES (3 µg/kg/day) increased the delay in entrance (p<0.05). Seizure-induced elevations in MDA, NO, and CL levels were reduced in NES- (0.3 µg/kg), and PH+NES-treated groups, while all doses of NES, but not PH, elevated depleted GSH level (p<0.001). BDNF levels were lower in NES-treated PTZ group, but the differences were not significant. Neuronal cell degeneration in cerebral cortex, hippocampal CA3 and dentate gyrus of saline-treated PTZ group (p<0.001) was reduced in NES- (0.3 µg/kg), PH- and PH+NES-treated rats. Increased TUNEL-positive (apoptotic) cells were determined in the cortices of PTZ-administered rats compared to control rats, but no significant difference was observed among treatments. Increased GFAP immunolabelling determined in the hippocampal CA3 fields and cortices of PTZ-administered group was reduced in NES- (0.3 µg/kg)-treated and PH+NES-treated groups (p<0.05). Nesfatin-1 appears to have a synergistic effect with phenytoin, potentiating its anti-seizure effect along with an additional neuroprotection on seizure-induced oxidative brain injury.


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PCB234
Investigating signatures of hypersynchronous neural activity produced by diverse convulsant molecules in 4 days post fertilisation zebrafish larvae
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Epilepsy is a chronic disorder of the brain characterised by recurrent seizures. Around 1-2% of the world’s population are affected, with many suffers exhibiting or developing resistance to drug therapy. Animal models have played a significant role in shaping our understanding of the neural mechanisms that underpin epilepsy; however current acute seizure models have a range of limitations. The physiological complexity and ready availability of larval zebrafish affords an ideal vertebrate model for high throughput screening of seizurogenic compounds. 4 days post fertilisation (dpf) zebrafish have a complex nervous system (~10^5 neurons) and seizure-like behaviours can be elicited by a range of convulsant molecules. Here we investigate the utility of a lower vertebrate, the zebrafish (Danio rerio), to explore patterns of neural activity (~0.4 Hz, n=3) typical of a GABAergic modulation.

PCB235
The acute effects of the hypoxia mimetic protocatechuic acid ethyl ester on synaptic transmission and plasticity in the rat hippocampus
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A reduction in blood flow in the brain results in a number of disturbances including the development of a hypoxic condition. During hypoxia a number of physiological changes occur within neurons including the stabilisation of hypoxia-inducible factors. The activity of these proteins is regulated by O2, Fe2+, 2-OG & ascorbate-dependant hydroxylases which contain proyl-4-hydroxylase domains (PHDs). PHD inhibitors have been widely used and shown to have a preconditioning and protective effect against a later and more severe hypoxic insult. However very little research has been carried out on their putative actions on synaptic transmission and plasticity (1,2). In this study we have investigated the acute effects of the PHD inhibitor and hypoxia mimetic, protocatechuic acid ethyl ester (EDHB) on baseline transmission and long-term potentiation (LTP) in isolated rat (Wistar) hippocampus. Excitatory post-synaptic potentials were elicited by stimulation of the medial perforant (mDG) or Schaffer collateral pathway. LTP was induced by high frequency stimulation consisting of 3 trains of 1s duration every 20s at 100Hz. Responses were analysed using WCP software (J Dempster, Strathclyde). Baseline recordings were elicited at 40% of the maximal response and normalised to 100%. Values are means±S.E.M., compared by ANOVA. We report for the first time, an acute, concentration-dependent inhibitory effect of EDHB on synaptic transmission which was seen in the mDG (100µM, 98.0±3.9%, N=5; 100µM 70.5±4.5%, N=9, P<0.001) but not in the CA1 region (100µM 98.3±3.2%, N=7). The effect of EDHB in the mDG was significantly reversed by prior application of APV (25µM, 86.3±2.8% N=6, P<0.01) and picrotoxin (100µM, 83.7±2.7% N=8, P<0.01). There were no changes in the ratio of paired responses (50ms interval) after EDHB application suggesting a post-synaptic mechanism of action. EDHB at higher concen-
trations (100μM), was found to inhibit LTP in both the mDG and CA1 regions (111.7±5.6%, vs control 152.1±10.4%, N=5, P<0.05, and 110.0±4.4% vs 143.2±8.2% control, N=5, P<0.01, respectively). Application of exogenous iron (100μM) did not reverse EDHBs inhibitory effect on baseline transmission or LTP, suggesting a HIF-independent mechanism of action. The inhibitory effects of EDHB in both regions were reversible following washout. These results highlight a novel modulatory role for the PHD inhibitor EDHB in hippocampal synaptic transmission and plasticity. The effects are unlikely to be mediated post-synaptically as is observed in hypoxia, where O2 levels are decreased in brain tissue and adenosine receptors are activated. A novel post-synaptic mechanism of action may be involved possibly involving NMDA and GABA receptor activation.


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**Is deep brain stimulation of the ventrolateral periaqueductal gray effective at decreasing blood pressure in conscious spontaneously hypertensive rats?**

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Our recent clinical findings have revealed that deep brain stimulation of the ventrolateral periaqueductal gray (vPAG) decreases arterial pressure in patients with drug- and device-resistant hypertension ((1) and unpublished). However, the mechanism(s) underlying this effect is(are) not fully understood. Studies in anaesthetised, normotensive animals suggest that acute activation of the vPAG induces hypotension and sympatho-inhibition (2,3). Therefore, in this study, we sought to determine the blood pressure response to electrical stimulation of the vPAG in a conscious rat model of essential hypertension, the spontaneously hypertensive rat (SHR).

(SH rats (male, 250-350g, n = 16) were anaesthetised (ketamine 60mg/kg and medetomidine 250μg/kg) and implanted with radio-transmitters to measure blood pressure. Rats recovered over 7-10 days and were re-anaesthetised (sodium pentobarbital 50mg/kg) and using stereotaxic coordinates a bipolar electrode was placed chronically into the PAC at ventral and dorsal sites (verified histologically post hoc; 0.8-1.0mm lateral to midline, 7.2-7.6mm caudal to bregma and 5.4-6.4mm ventral to the dura). Electrical stimulation (2-6V, 20-40Hz, 0.2ms pulse width) of the PAC was applied while recording blood pressure. The electrode was cemented at the site of maximal depressor response (present in 8 of 16 rats) and the rats recovered for 6-8 days before chronic PAC stimulation in conscious animals. Values are mean ± SEM.

Electrical stimulation at histologically confirmed sites within the vPAG decreased arterial pressure (-19±4 mmHg, n = 8; P<0.05, student’s t test) when rats were anaesthetised with sodium pentobarbital but did not decrease arterial pressure or heart rate in conscious, freely-moving rats. In 2 SHRs that had depressor responses under anaesthesia displayed a pressor effect in the conscious state. The hypotensive action of the vPAG under anaesthesia has been attributed to inhibition of sympathetic preganglionic neurons (4). We therefore used an in situ decerebrate preparation (5) of juvenile SHRs (5 weeks old) to investigate whether activation of vPAG neurons produced sympatho-inhibition below the threshold at which a blood pressure response could be observed. No measureable sympathetic responses were evoked from the vPAG using either electrical stimulation (100μA, 20Hz, 0.2ms) or excitatory amino acid microinjections (D,L-Homocysteic acid, 1mM, 60nl). In contrast, sympatho-excitatory responses were evoked by both stimuli in the dorsal PAG.

We conclude that the vPAG is not a reliable antihypertensive locus in the SHR in which to model human deep brain stimulation. Whether the dorsal PAG in the SHR has utility as a model system for understanding intractable postural hypotension remains to be seen.


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**PCB237**

**Potential differentiation of rat neonatal spermatogonia to motor neuron like cells**

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Embryonic stem cell (ESC) therapy is an exciting way to treat neurodegenerative disease and central nervous system (CNS) injury. However, many ethical and immunological problems surround the use of embryonic stem cells. Finding an alternative source of stem cells is therefore pertinent. In the current study, spermatogonia stem cells (SSCs) were used to generate mature motor neurons. SSCs were extracted from neonatal Albini Vistar rat testes and cultured in DMEM/F12 medium for 3 weeks. Characterization of SSC derived ESC-like cells were confirmed by RT-qPCR, Immunostaining, alkaline phosphatase activity and their ability to form embryoid bodies (EBs). The EBs were induced by retinoic acid (RA) and Sonic hedgehog (Shh), and trypsinized to obtain single induced cells. The sin-
gle cells were cultured in neural medium for 18 days. Characterization of neural precursors and mature motor neurons was confirmed by RT-qPCR analysis at the 7th day (early stage) and 18th day (late stage), respectively. Gene expression levels of Olig2, Pax6, Isl1, Isl2 and HB9 were analyzed by RT-qPCR. The expression levels of motor neuron progenitor markers, Olig2 (t_6=2.96; P<0.05) and Pax6 (t_6=2.55; P<0.05) in the early stage were significantly higher than those in the late stage. Furthermore, the expression levels of motor neuron indicators Isl1 (t_6=7.04; P<0.001), Isl2 (t_6=6.32; P<0.001) and HB9 (t_6=4.92; P<0.01) in the late stage were significantly higher than those in the early stage (As shown in Figure 1). A decrease in Olig2 and Pax6 expression levels was paralleled by an increase in HB9, Isl1 and Isl2 levels during cell culture in neural medium, suggesting cells may be differentiating from neuron progenitor cells to mature motor neuron like cells. Immunocytochemical analysis has shown that the early stage neural precursors were positive for nestin (61.4%) and Sox2 (49.9%) and a small fraction of cells expressed β-Tubulin III (4.8%). Upon further differentiation at late stage, multipolar neurons were detected that expressed β-Tubulin III (32%) and MAP2 (25.8%) markers. Moreover, the expression levels of Olig2 and PAX6 were significantly lower while HB9, Isl1 and Isl2 expression levels were higher at the late stage when compared to the early stage. These results show that SSCs have the potential to differentiate to motor neurons and express markers specific for mature motor neurons. However, the functional ability of these cells remains to be evaluated in future studies. Differences were considered significant at P<0.05. Data are presented as mean ± S.E.M.


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PCB238

Cancer chemotherapeutics in early life alter spinal nociceptive processing in the adult

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Survivability of paediatric cancers has tripled since the 1970s, however treatment comes with severe side effects[GH1]. Cisplatin is a front line treatment for solid state tumours yet its main dose-limiting effect is chemotherapy induced peripheral neuropathy (CIPN) that negatively effects quality of life for decades[GH2]. This can be especially damaging in children, where early life insults can fundamentally alter the development of spinal nociceptive pathways. This study aimed to determine the extent of changes in developing spinal nociceptive pathways following chemotherapeutic treatment in early life.

All animal use procedures were specifically licenced by the UK Home Office. Sprague Dawley rats pups of both sexes were given 1mg/kg cisplatin (CIS) (0.5mg/ml) (n=3) or vehicle (VEH) (n=4) via intraperitoneal (IP) injections for 5 days from postnatal day 7 to 11. On postnatal day 45 animals were perfusion fixed using 4% paraformaldehyde. Spinal cords were removed and immunohistochemistry performed on 40µm sections of the lumbar enlargement. Antibodies for neuronal and glial targets involved in nociceptive pathways used were: neurones (NeuN), Microglia (IBA1), astrocytes (GFAP), peptidergic neurones (CGRP), non-peptidergic neurones (IB4), peptidergic nerve growth factor receptors (TrkA), peptidergic interneurones (vGLUT2) and neurofilament 200 (NF200). The dorsal horn (DH) was imaged on a confocal microscope and regions of interest (ROI) in laminae I-V analysed using ImageJ and Graphpad Prism. Comparisons between groups (CIS vs VEH) were made using Students t-Test. There were no significant differences between CIS and VEH groups in NeuN (CIS=17.8±0.6, VEH=16.8±0.7, p=0.3) CGRP (CIS=43.7±3.1, VEH=42.9±2.6, p=0.3) staining. However, there were significant increases in TrkA (CIS=93.54±41.8, VEH=59.2±2.8, p=0.0001), vGLUT2 (CIS=43.8±0.2, VEH=26.7±0.1, p=0.0001) and IB4 (CIS=27.9±1.2, VEH=23.6±0.9, p=0.0001) staining, as well as NF200 (CIS=36.3±0.7, VEH=18.1±0.3, p=0.0001) staining, suggestive of neuronal sprouting.

Over the entire DH IBA1 did not show any significant changes (CIS=3.2±0.1, VEH=2.9±0.1, p=0.1). However, in laminae V specifically, there was an increase in IBA1 in CIS treated rats (CIS=2.7±0.2, VEH=2.2±0.1, p=0.02). There was a significant decrease in GFAP in CIS animals (CIS=6.6±0.1, VEH=9.2±0.1, p=0.0001).
Cancer chemotherapeutic treatment in early life significantly alters the structure and function of spinal nociceptive networks in later life. These changes explain the altered sensory and pain perceptual changes experienced by cancer survivors and indicate alterations in other central nervous system structures.


We would like to thank the help given from members from V. Chapman and G. Hathway’s research group.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**PCB239**

**Effects of long term low calorie diet started from adolescent period on learning and memory; an evaluation in terms of inflammatory markers and oxidative stress**

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The beneficial effects of calorie restriction (CR) without malnutrition on lifespan and brain functions are explained by reduction of oxidative and inflammatory markers. Interestingly, it is recently believed that proinflammatory cytokines such as TNF (Tumor necrosis factor)-alpha play an essential role in learning and memory by mediating LTP (long term potentiation), which is critical for memory, related signal proteins. Nutrition in adolescent period is known to be important for diseases affecting cognitive functions in adulthood. Earlier we found that mild low calorie diet (LCD) applied in adolescence had positive effects on cognitive functions in adulthood. In this study we aimed to investigate how long term LCD (begins in adolescence and ongoes in adulthood) affects cognitive functions in different memory tasks and we also aimed to evaluate inflammatory and oxidative marker levels in prefrontal cortex (PFC; important in behavioral inhibition in past functions in different memory tasks and we also aimed to affect) and hippocampus (HC; important in spatial memory). 28 days old female rats were used. Two groups were formed: 8 weeks standard diet (SD), 8 weeks LCD. After the related diet, hippocampus-dependent Morris Water Maze (MWM) task and then PA task were performed. After the behavioral tests, rats were decapitated and brains were removed. TNF-alpha, CRP (C-reactive protein), TAS (total antioxidant status), TOX (total oxidant status) and CRP levels were determined by ELISA in HC and PFC. Results were compared between groups by Mann-Whitney test. LCD caused insignificant improvement on spatial memory in MWM task but significant increase in thigmotactic swimming which is an anxiety indicator (p<0.05). In PA task, LCD reduced the step-through latency in 1st hour (amnesic effect) but not in 24th hour and 3rd day. There was not a significant difference between groups in terms of CRP levels in HC and PFC. TNF-alpha level in PFC of LCD group was significantly lower than SD group (p<0.05) but there was not a significant difference in HC. TAS levels in both HC and PFC were increased significantly in LCD group (p<0.05). TOS levels were decreased significantly in LCD group in both HC and PFC (p<0.01 and p<0.05 respectively). Long-term LCD had different effects on learning memory function depending on the task type. Our original results suggested that negative effect of LCD to nonspatial memory may be related to reduced TNF-alpha levels in PFC and to the long term LCD. Future studies in nonobese humans should focus on the effects of prolonged CR on cognition.

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**PCB241**

**The effects of two weeks administration of ALDH2 and ATP modulators on thermo-nociception in mice**

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**Background.** Mitochondrial dysfunction is associated with different types of pain (1). The most frequent pain syndromes associated with mitochondrial dysfunction, the headache, the neuropathic pain, the muscle pain or the abdominal pain are consequent of mitochondrial diseases or to drug-induced mitochondrial disturbances.

**Aim of Investigation:** to evaluate the effects of 2 weeks administration of mitochondrial ALDH2 modulator (nitroglycerine-NTG and disulfiram-DSF) and ATP modulator (cobalt chloride-CoCl2, riboflavin-RBF and methylene blue-MB) on the thermo-nociception nociception assessed by the hot plate test-HPT and tail flick test-TFT

**Methods:** For each substance, 8 male BALB/c mice (23+2 g) were evaluated for thermo-nociception before and repeated one respectively two weeks after daily drug administration. NTG (10 mg/kg), CoCl2 (25 mg/kg), RBF (50 mg/kg) and MB (5 mg/kg) were injected intraperitoneally while DSF (100 mg/kg) was administered by gavage. The group receiving saline served as control the cut-off was set at 12s for TFT and at 15s for HPT. The results are presented as percentage change from baseline (PCB), calculated for each mouse according to the formula (treated-baseline)*100/baseline. Statistic changes were tested using ANOVA and paired t-tests.

**Results:** In saline group, the PCB for the TFT was 3.97% after one week to 9.95% after two weeks. These changes were not statistic significant. In the treated group, PCB value was similar; no significant statistic changes were recorded for this test. The thermo-nociception evaluated by the HPT in the control group decreases in mean with 4.17% after one week and 9.95% after two weeks. These changes were not statistic significant. Depending on the task type. Our original results suggested that negative effect of LCD to nonspatial memory may be related to reduced TNF-alpha levels in PFC and to the long term LCD. Future studies in nonobese humans should focus on the effects of prolonged CR on cognition.

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genases and adenosine triphosphate activity induce changes in thermo-nociception. The effect is time dependent and the spinal (TFT) or supraspinal (HPT) integrated thermo-nociception is different interrupted by these drugs. In our study, after 2 weeks of administration, the ATP modulators produced the most important changes in pain perception while the ALDH2 modulators have a less significant effect.


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UPREGULATION OF M-CHANNELS CONSTRAINS HUNTINGTON’S DISEASE PATHOLOGY

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Huntington’s Disease (HD) is a human neurodegenerative disorder which leads to progressive and severe disability, characterised by motor, cognitive and behavioural dysfunctions. It is known that HD is a monogenic disease resulting in a CAG trinucleotide repeat expansion (CAG repeats) in exon 1 of the gene huntingtin. Current treatments are palliative, and no disease modifying therapies are currently available. Thus, HD remains untreatable and fatal.

We have established a unique cellular model by generating striatal neurons from human induced pluripotent stem cells (hiPSC) generated from HD patients. In this study, we have employed three HD lines which express different CAG expansion lengths in their huntingtin gene - HD33, HD60 and HD109 - and generated neural progenitors using a patterning protocol described in Telezhkin et al. (2015). We have recently observed that molecular and functional expression of M-type current (carried by Kv7.2/7.3 channel subunits) are dynamically regulated during striatal neuronal differentiation in vitro and in vitro and during differentiation of hiSPC in vitro, thus determining excitability. Furthermore, neurons generated from HD hiPSC neurons demonstrate defective excitability parameters (Telezhkin, Yarova, Allen and Kemp, unpublished) and M-type current is defective in an HD mouse model (Cao Y et al., PNAS, 2015). Thus, we have tested the hypothesis that chronic pharmacological upregulation of M-type current will result in functional rescue of impaired function seen in HD patient, hiPSC-derived striatal neurons during three week differentiations from neural precursors. In HD33 neurons, M-current activation by flupirtine only slightly shifted Vm from -42.4 ± 1.9 mV (n = 10) to -44.7 ± 2.0 mV (n = 10), whereas M-current inhibition with XE991 significantly depolarized Vm to -30.9 ± 2.4 mV (n = 10, p < 0.01). Similarly, in HD60 neurons, flupirtine shifted Vm only modestly from -45.0 ± 2.6 mV (n = 12) to -48.1 ± 2.2 mV (n = 14), whilst XE991 significantly depolarized Vm to -36.5 ± 1.5 mV (n = 12), p < 0.01). In HD109 neurons, flupirtine treatment resulted in insignificant shift of Vm from -38.1 ± 1.7 mV (n = 26) to -39.3 ± 1.4 mV (n = 23) and XE991 produced significant change in Vm -33.1 ± 1.7 mV (n = 25, p < 0.05). Values are means ± SEM, statistics is done with unpaired t-Test. Such alterations in Vm broadly mapped onto parallel changes in excitability.

Thus, upregulation of M-channels, via pharmacological manipulation, partially rescues the depressed excitability seen during differentiation of HD hiPSC-derived neurons. This is especially exciting because M-current activator flupirtine is already in clinical practice for the treatment of another central neuronal disorder, meaning that these drugs could be repurposed to enhance striatal function in early diagnosed HD patients HD, perhaps delaying the progression of this fatal neurodegenerative condition.


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LEVETIRACETAM CAUSES HYPERALGESIA IN ABSENCE EPILEPTIC WAG/RIJ RATS

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Pain is an unpleasant sensory experience which involves detection of potential or actual harms to functional integrity of the organism, and transmission of these neuronal signals to central nervous system and perception with relation to experience, and thereby significantly contributes to homeostasis. WAG/Rij rats are established absence epilepsy animal model in which age-dependent absence epilepsy develops. The aim of this study was to assess possible effect of LEV on pain sensitivity in WAG/Rij rats. The effects of levetiracetam were evaluated in adult WAG/Rij epileptic rats (male, 250-350g, n=11) by “thermal plantar (heat-induced)” test. Different doses of LEV (60 mg/kg and 120 mg/kg, both i.p.) were tested in the same group of rats by 1 week apart. Effect on acute pain was evaluated at interictal period (confirmed by simultaneous electroencephalography through epidurally implanted electrodes) at 10, 30 and 60 minutes after LEV administration and pain threshold responses were compared to their basal pain threshold values. The determined pain threshold values were analyzed by Friedman repeated measures analysis of variances (ANOVA) followed by a pair wise comparison using a Conover test on the ranked data. P<0.05 value was accepted statistically significant. The protocol of this study was approved by the local Ethic Committee. Administration of LEV caused dose-dependent increase in pain sensitivity in thermal plantar test. Low dose of (60 mg/kg) LEV did not cause any significant change in thermal stimulated pain latencies which was 3±1.0 sec before and 2.5±0.5 sec, 2.4±0.6 sec and 2.3±0.9 sec 10, 30 and 60 minutes after 60 mg/kg LEV (p>0.05 for all) (n=11, p>0.05 for all) administration.
120 mg/kg LEV significantly reduced thermal stimulated pain latencies which were 2.9±0.6 sec before and 2.1±0.4 sec (p<0.05), 2±0.4 sec (p<0.05), 30 and 60 minutes after (n=11 for all). Results from this study demonstrate that LEV caused a pro-algesic effect in these epileptic rats. This is contradictory to effects of LEV on healthy rats and we have no clear evidence for underlying mechanism for this proalgesic effect. It was evident that the higher dose used (120 mg/kg) caused a significant decrease in seizures the WAG Rij experienced, and the cessation of seizures by LEV might have caused alteration in endogenous pain modulation in these animals and proalgesia was resulted. This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK Project #214S206).

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PCB244

Intermittent hypobaric hypoxia preconditioning protects against acute severe hypoxic damage in brain

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Acute severe hypoxia (SH) causes an increase in oxidative stress and apoptosis in the brain. In contrast, intermittent hypobaric hypoxia (IHH) can increase brain antioxidant capacity and result in neuroprotection, as we previously reported (Costa et al., 2013). Thus, the present work uses IHH as preconditioning against damage potentially induced by SH. Adult rats were divided into four groups: 1) controls; 2) SH group, subjected to 6 h of acute hypoxia at 7% oxygen; 3) IHH group, exposed to 380 mmHg (equivalent to an altitude of 4000 m) in a hypobaric chamber, 4 h/day for 8 days; and 4) combined IHH-SH group, subjected to acute SH (7% oxygen) for 6 h after the last IHH exposure. Animals were anesthetized with isoflurane inhalation and then sacrificed. The brains were extracted and compared to controls. The study was approved and authorized by the Institutional Committee of Animal Care and Research of the University of Barcelona. The experimental protocol follows the European Community guidelines.

SH induced oxidative stress in the brain, as indicated by increased levels of oxidized proteins, lipid peroxidation, inducible nitric oxide synthase (iNOS) expression and nitric oxide metabolites. This acute hypoxic also resulted in glutathione depletion and increased glutathione peroxidase. As for the apoptosis parameters studied, SH increased cytochrome c in the brain, and the activity of caspase 3 in the brain cortex and hippocampus.

The IHH preconditioning protocol induced the expression of HIF-1 without causing oxidative stress or apoptosis, and induced expression of neuroprotective proteins such as EPO and VEGF. The IHH reduced nitric oxide levels by 28%, the content of oxidized proteins by 30% and lipid peroxidation values by 48%. It also better preserved the ratio of oxidized/ reduced glutathione in brain tissue.

Our study thereby demonstrates that IHH is a useful way to prepare the brain to tolerate the effects of SH better, maintaining antioxidant activity and mitochondrial function, and promoting the expression of neuroprotective factors.


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PCB245

Oxytocin single neurones. A spiking and secretion mathematical model and the role of the AHP

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Magnocellular oxytocin (OT) neurones in the supraoptic nucleus of the hypothalamus project to the posterior pituitary, where they secrete the hormone into the blood stream. OT is essential for breastfeeding and important for parturition. In these cases spike activity is organised into intense bursts[1]. OT has also anorexigenic effects and is involved in osmotic regulation, showing then a continuous increase in spike activity rather than a bursting pattern[1]. In OT cells, spikes are generated in response to afferent inputs that produce fluctuations in membrane potential. That spontaneous spiking activity can be closely matched by models which assume that the afferent input arrives randomly[2]. When spikes propagate to the axon terminals in the posterior pituitary, they trigger exocytosis of hormone containing vesicles. However, OT secretion is a non-linear function of firing rate: the same number of spikes provokes much more secretion if they are close together[3]. It is this feature that makes bursts such an important feature of the milk-ejection reflex. Because of this non-linearity, variance in firing rate that results from the randomly fluctuating synaptic inputs would be expected to be amplified to produce a more variable secretion. Thus there seems to be a conflict: the non-linearity of stimulus-secretion coupling that makes bursts so effective at releasing large pulses of OT during reflex milk ejection will also make secretion variable in response to a “steady” input. Here we present a mathematical model that replicates the behaviour of spike production and axononal secretion in single OT neurones. The secretion model, based on an existing vasopressin secretion model[4], was matched to in vitro[3][5] experimental data, and accurately reproduces the non-linearity of stimulus-secretion coupling in the OT terminals. The spiking model accurately reproduces the spiking behaviour of OT cells in a wide range of experimental circumstances. Combining the spiking and secretion models allows us to study the responses of the OT system to a fixed transient challenge, mimicking the excitatory response to systemic injection of the gut peptide cholecystokinin (CCK), and in particular, it allows us to study how the response magnitude is affected by factors that affect the basal firing rate of OT neurones. We show that a key feature of the electrophysiological phenotype of OT neurones – their expression of a slow afterhyperpolarisation (AHP) - is a critically important determinant of the variability of the plasma OT concentration that results from secretion. The AHP moderates the variability of spike activity in OT neurones, with a resulting substantial impact on the variability of secretion. The AHP “smooths” the mean firing rate of OT cells over a time scale of a few seconds, avoiding extreme excursions that would result in large fluctuations in secretion.

Vasopressin V1a receptors and cushing reflex in rats

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Introduction: Increased intracranial pressure (ICP) results in compression of brain structures and decreased cerebral blood flow [1]. The diminished cerebral perfusion is compensated for by Cushing reflex, which maintains cerebral perfusion pressure (CPP) via elevation of arterial blood pressure [2]. Arginine vasopressin (AVP) is a potent vasopressor neurohormone with vasocostrictive and central effects mediated by vasopressin type 1a receptor (V1a) [3]. AVP is also involved in the pathogenesis of brain oedema resulting in high ICP [4]. Clinical reports point to beneficial effects of blockade of vasopressin receptors in treatment of brain oedema [5], however, there is a limited evidence on the role of vasopressin in the Cushing response, which maintains CPP.

Aim: In the present study we investigate the role of brain and peripheral vasopressin V1a receptors in Cushing reflex. Methods: We implanted adult male Sprague-Dawley rats with arterial and venous catheters for recording of blood pressure and administration of drugs, and with two steel cannulae into the lateral cerebral ventricles (LCV) for intrabrain infusions and measurement of ICP. We recorded mean arterial blood pressure (MABP), heart rate, ICP and CPP. After measurements at rest, saline (10 µL/30sec) or V1a receptor antagonist (d(CH2)51,Tyr(Me)2,Arg8)-Vasopressin; 500 ng/10 µl/30 µl/min was infused into LCV and after 5 min ICP was gradually increased by LCV infusion of 0.9% NaCl at the rate of 60 µl/min till obtaining ICP of 100 mm Hg. In another group of rats we intravenously administered saline (100 µl/30 sec) or V1aR antagonist (5 µg/100 µl/30 sec). All procedures and measurements were performed under anaesthesia (1.5 g/kg b.w., i.p.). We also collected blood samples before elevation of ICP and at the peak increase in ICP. Using enzyme-linked immunosorbent assays we measured serum copeptin, fragment of proAVP stoichiometrically released with AVP, and serum norepinephrine. Results: Increase of ICP resulted in a significant increase of serum copeptin and serum norepinephrine (p<0.05). Neither intravenous nor intracerebroventricular administration of V1a receptor antagonist changed MABP before the elevation of ICP. Acute increase in ICP led to increase in MABP and resulted in a similar decrease in CPP in all groups. Neither peripheral nor central blockade of V1a receptors affected CPP and hemodynamic parameters during ICP increase.

Conclusions: Our results show that Cushing response to acute increase in ICP effectively limits decrease in CPP, regardless of blockade of central or peripheral V1a receptors. Lack of the effect of vasopressin receptor antagonists on Cushing reflex may prove beneficial for mainaining cerebral perfusion in patients with increased ICP.

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PCB246

The role of lifetime and current physical activity on cortisol in older adults

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Normal ageing is associated with increases in cellular and tissue atrophy in the cortices of the brain, which is associated with age-related cognitive decline (1). Age-related cognitive decline is influenced by changes in cortisol secretion caused by dysregulation of the Hypothalamus-Pituitary-Adrenal (HPA) axis (2). Therefore, understanding factors that impact HPA axis regulation may help in determining mechanisms to prevent or delay the progression of cognitive impairment. Physical activity reduces both age-related cognitive decline and cognitive decline associated with neurodegenerative diseases, although the exact biological mechanisms are currently unknown. This study examined the relationship between HPA axis function, measured by morning levels of cortisol, and physical activity in 198 (49% male) healthy older adults (66.95 ± 6.34 years) who volunteered for the Brain in Motion Study. Over 18 months, pre-intervention, intervention, and post intervention measures of morning levels of free blood cortisol were collected.
and analyzed using ELISA assays. Participants’ lifetime and past year physical activity were assessed pre-intervention with the Lifetime Physical Activity interview-administered questionnaire (3) and were reported in Metabolic Equivalents of Task per year. The 6-month aerobic exercise intervention was a three day a week, supervised program, where intensity of the aerobic exercise was determined individually based on heart rate reserve (4). A series of hierarchical linear regression analyses were conducted to determine the impact of lifetime and past year physical activity on the measures of morning cortisol. Past year physical activity was a significant predictor of morning blood cortisol levels at the 6-month baseline ($\beta = .176, p = .026$), with trends noted at the initial baseline ($\beta = .129, p = .098$) and at the post intervention ($\beta = .132, p = .088$). Past year physical activity did not predict change in morning blood cortisol levels from the pre- to post-exercise intervention ($p > 0.05$). Furthermore, lifetime physical activity was not found to be a predictor of morning blood cortisol levels taken at any point in the study nor of change in cortisol levels from pre-to post exercise intervention ($p > 0.05$). These results suggest that past year physical activity is associated with greater HPA axis function, as higher morning levels of cortisol are indicative of a healthier HPA axis (5). These results add to previous evidence that current physical activity is associated with HPA axis health and suggest recent physical activity, not activity levels earlier in an individual’s life, impact HPA axis function in older adulthood.


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PCB248

Transient receptor potential channel V4 ligands differentially modulate dynamic and static responses of stretch-evoked firing in ex vivo rat muscle spindles

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Mammalian muscle spindle primary sensory terminals are exquisitely sensitive to both static length and change of length. How the nerve terminals transduce these mechanical stimuli is still unclear. While piezo2 knock out (1) clearly profoundly affects spindle stretch-evoked firing, the sensory terminals contain a large range of other ion channels whose functions need to be explored. The vanilloid-type TRPs (TRPVs) are of particular interest to mechansensation (2), particularly TRPV4, which is expressed in hair cells of rodents (3) and zebrafish (4). We have therefore examined a putative mechanosensory role for TRPV4 in rat muscle spindles. Fourth deep lumbrical nerve-muscle preparations were dissected from adult male Sprague-Dawley rats (246g-389g) killed by anaesthetic overdose (150-200mg/kg phenopento-barbital, i.p.), then kept in artificial cerebrospinal fluid saturated with 95% O2/5% CO2 at room temperature. Stretch-evoked afferent discharges were recorded during trapezoidal (1 sec ramp - 3 sec hold – 1 sec release) and saw-tooth (25 @ 0.2Hz, 50 @ 1Hz & 50 @ 5Hz) 1mm changes in muscle length (±10% total length) from an electromechanical puller. Total action potential (AP) counts were made during movement (dynamic) and hold (static) phases in drug-free and rising concentrations of ligands (1hr in each). Data were analysed by 1-way repeated measures ANOVA, with Holm-Sidak-corrected post-test comparison between means and a significance threshold of P<0.05 for ‘n’ muscles.

The broad spectrum TRP channel blocker 2-aminobutylic bozotabolished static-phase firing (121.9±28.2 APs/sec pre-drug) at 100 µM (P<0.001, n=3), but increasing rates of stretch (dynamic firing) were progressively more resistant to inhibition (0.2Hz: pre-drug 341.7±51.2 vs 100 µM 0.7±0.3 APs/cycle; P<0.001, 5Hz: pre-drug 13.7±3.1 vs 100 µM 3.7±2.1 APs/cycle; P<0.05). Lower concentrations (1 & 10 µM) had no significant effect on any firing counts. Ligands selective for TRPV4 were then tested. RN-1734 (TRPV4 antagonist, n=4) inhibited static-phase firing at 3µM (pre-drug 134.9±7.1 APs/sec vs 89.1±6.0 APs/sec; P<0.05), but 10µM was needed to inhibit dynamic ramp, 0.2Hz and 1Hz responses (each P<0.05), while 30µM was needed to reduce 5Hz-evoked firing (16.2±1.9 vs 5.7±2.2 APs/cycle; P<0.05). Interestingly, the potent TRPV4 agonist GSK1016790A did not affect firing up to 200nM (n=7), but it blocked 10µM RN-1734’s inhibition of trapezoid-induced dynamic firing (P=0.44, n=4). Immunofluorescence provided further support for TRPV4 expression on spindle primary sensory nerve terminals.

These data suggest the putative mechanotransduction channel TRPV4 is present on spindle sensory nerve terminals, where blockade preferentially inhibits static and slow dynamic firing. Woo SH et al. (2015). Piezo2 is the principal mechanotransduction channel for proprioception. Nat Neurosci 18, 1756-1762.

We thank Dr Xuming Zhang for helpful discussion and gift of GSK1016790A.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**PCB249**

**Inhibition of fibrillation of α-synuclein, a Parkinson’s disease-related protein by TiO2 nanoparticles**

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Protein fibrillation is implicated in the pathogenesis of several neurodegenerative diseases like Parkinson’s disease (PD). α-Synuclein (αS) is natively unfolded protein which is involved in pathogenesis of PD. Fibril formation occurs by nucleation dependent kinetics, wherein formation of a critical nucleus is the determining step, after which fibrillation proceeds rapidly. Nanomaterials have been widely used in great quantities which makes many people more frequently exposed to fabricated nanoparticles (NPs). When NPs are introduced in a living organism they may interact with a variety of different cellular components with yet largely unknown pathological consequences.

NPs have enormous surface areas that can access the brain and some of them are found to enhance the rate of protein fibrillation by decreasing the lag time for nucleation. In our study, we surveyed the effects of three different NPs on αS fibrillation.

αS protein expression and purification were performed and fibril formation was induced at pH 7.4, 37°C, and vigorous shaking in the absence or presence of three types of NPs (TiO2, SiO2, and SnO2). The enhancement of the fluorescence emission of Thioflavin T (ThT) was used to monitor the appearance and growth of fibrils. The adsorption of αS monomers on the surface of NPs, was investigated by tyrosine fluorescence emission measurements. Also transmission electron microscopy (TEM) analysis was performed to explore the ultrastructure of αS aggregates. It was found that TiO2-NPs enhanced αS fibril formation even at concentration of 5 µg/ml while the two other NPs showed no significant effect on the kinetics of fibrillation. Intrinsic tyrosine emission measurements confirmed that the TiO2-NPs interact with αS fibrillation products. It is suggested that TiO2-NPs may enhance the nucleation of αS protein that leads to protein fibrils formation. TEM results are highly correlated with the results of the ThT fluorescence assay.

Conclusion: The fibrillization process of αS protein is affected by the presence of TiO2-NPs. This finding implicates the potential neurotoxicity of TiO2-NPs, which may be considered as a probable risk for PD.


Colvin VL. The potential environmental impact of engineered nanomaterials. Nature biotechnology. 2003;21(10):1166-70

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**PCB250**

**The dynamic cardiovascular response to ischemic stroke: Temporal relationships between blood pressure, intracranial pressure and cerebral oxygenation**

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Over 80% of ischemic stroke patients show an abrupt increase in blood pressure (BP) in the hours and days following stroke; yet whether post-stroke hypertension is beneficial or harmful remains controversial, and the underlying physiological basis is unclear. To investigate the dynamic cardiovascular response to stroke Wistar rats (n=6, 393±34g) were placed under isoflurane anesthesia (~3%), and instrumented with radio telemetry to record BP, ICP and brain tissue oxygen (P02) in the stroke penumbra. After a 1-week recovery period, an ischemic stroke was induced via middle cerebral artery occlusion on Day 0; recordings continued for 10 further days. Immediately following stroke, penumbra P02 decreased to less than half baseline levels during occlusion, but increased back to baseline levels with reperfusion. BP increased rapidly from baseline (108±9mmHg) to a peak of +44±7mmHg above baseline, before recovering by Day 10 (+4±3mmHg). In contrast, ICP increased more gradually from baseline (5±1mmHg), to a peak of +25±8mmHg on Day 3, and recovering to +4±2mmHg above baseline at Day 10. This is consistent with the formation of cerebral oedema. Calculated as the difference between BP and ICP, cerebral perfusion pressure (CPP) increased 24-48 hours after stroke (+27±11mmHg) and decreased by -13±7mmHg during Days 3-4, subsequently returning to baseline.

These findings suggest that the increase in BP immediately after ischemic stroke is temporally matched to the fall in brain tissue P02. However, the observed delayed rise in ICP does not elicit a matched increase in BP, thus CPP falls, before recovering to baseline. These results indicate that cerebral ischemia may be the primary initial trigger for post-stroke hypertension.

Funded by the Health Research Council of New Zealand

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Investigating relative genetic and environmental contributions to subjective perceptions of the colour of “the dress” in a classic twin study

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Human colour vision is enabled by the existence within cone photoreceptors of photopigments with differing spectral sensitivities. However, subjective perceptions of colour are the consequence of several layers of retinal and higher cortical processing, and objects may be perceived to have the same or different colours based on intrinsic and extrinsic properties such as type of illumination, and prior experience. The widely reported phenomenon of starkly differing colour perceptions between subjects viewing a particular photograph of a dress raises intriguing questions and avenues for exploring visual neurophysiology (Schlaffke et al., 2015; Winkley et al., 2015). Are the differences determined by prior environmental experience in terms of how subjects have learned to name colours in different contexts or is there a genetic component to how the dress is perceived? The twin study design permits a relative quantification of the importance of genetic and environmental factors by examining concordance within monozygotic (MZ) and dizygotic (DZ) twin pairs. As part of a wider twin study (which had local ethics committee approval), participants were recruited from the TwinsUK cohort, which comprises adult twins, who are mostly female and of European ancestry. Participants were shown a standard image of the dress, and asked to name the colours, and then were forced to make a choice between the two common alternatives “white and gold” (WG) or “blue and black” (BB). Case-wise concordance was calculated for MZ and DZ pairs as 2C/(2C+D) where C is the number of pairs concordant and D the number of pairs discordant for seeing the dress as blue and black (which is the less common choice). One hundred and seventy-three participants were recruited: of these 118 chose WG, and 55 chose BB. After exclusion of unpaired twins or pairs whose zygosity was unknown, responses were included from 128 twins (43 MZ and 21 DZ pairs). Concordances were 0.67 and 0.18 for MZ and DZ pairs respectively. The findings of a markedly higher concordance in MZ pairs suggests that genetic factors are important in determining the colours perceived. These genetic factors are likely to be multiple, and future studies exploring the contribution of variants in retinal photopigments or proteins involved in higher neuronal processing would be informative.


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Poster Communications

The effect of mild calorie restriction limited to adolescence on nitric oxide synthase isoforms at hippocampal neurogenic niche in adulthood

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Calorie restriction (CR), without malnutrition, has beneficial effects on longevity and brain function including reducing the incidence of age-related neurodegenerative diseases and improving memory function in rodents. The physiological mechanisms and mediators underlying these effects are poorly understood. One process implicated in regulating learning/memory is adult hippocampal neurogenesis (AHN). The effects of external factors such as diet exposed during adolescence (metabolic and cognitive changes) emerge in adulthood. We earlier found that 15% low calorie diet (LCD) applied in the adolescence to improve AHN and spatial memory, increase brain derived neurotrophic factor (BDNF) in hippocampus in the adulthood. Nitric oxide is a critical mediator in learning/memory and neurogenesis (both directly due to progenitor cells-vascular density in neurogenic niche and by positive feedback with BDNF). In this study, we aimed to investigate the effects of %15 LCD applied in adolescence on nitric oxide in hippocampus (particularly involves in memory and neurogenesis) in adulthood. 28 days old Sprague Dawley female rats were separated into 4 groups: 1) 4 weeks standard diet (SD4) 2) 4 weeks LCD (LCD4) 3) 8 weeks standard diet (SD8) 4) 4 weeks LCD+4 weeks standard diet (LCD+SD4). After the related diets, the brains were removed. Nitric oxide synthase (NOS) isoforms (nNOS-neuronal NOS, iNOS-inducible NOS, eNOS-endothelial NOS) were investigated immunohistochemically in dentate gyrus (DG) of hippocampus (where adult neurogenesis occurs). Immunohistochemical scores of the groups were compared by One-way ANOVA repeated test. nNOS increased in LCD4 when compared with SD4 (p<0.01) and increased in LCD4+SD4 when compared with SD8 (p<0.05). iNOS score of LCD4 was increased according to SD4 and score of LCD4+SD4 also increased when compared with SD8 (p<0.001 and p<0.01 respectively). The most intense immunohistochemical staining reaction among NOS isoforms was belonging to eNOS. eNOS score of LCD4 was higher than SD4 (p<0.05) and eNOS score of LCD4+SD4 was higher than SD8 (p<0.001) in DG. These are the first results concerning changes in nitric oxide synthase in hippocampus related to mild LCD in adolescence. Our findings suggest that eNOS may mediate (possibly with adaptation related to the production of eNOS) the beneficial effects of 15 % LCD in adolescence on enhanced hippocampal neurogenesis and memory in adulthood.

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Renal failure is associated with increased sympathetic nerve activity and hypertension. High levels of inflammatory cytokines are found in the diseased kidney including tumour necrosis factor-α (TNF-α). This study investigated the effect of the immunosuppressive agent tacrolimus, a macrolide antibiotic, that can block the transcription of TNF-α, on the high-pressure baroreflex control of renal sympathetic nerve activity (RSNA) in rats with renal failure. Male Wistar rats (275-350g, n=30) were divided into renal failure and control groups. Renal failure was induced using cisplatin (5mg/kg, I.P.) while the control group received a similar volume of saline (0.9% NaCl) 7 days prior to the acute experiment. Renal failure or control rats received tacrolimus (0.25mg/kg/day, I.P.) for 7 days starting on the day of cisplatin or saline administration. A further group of control rats (n=6) received an intra-renal infusion of TNF-α (2ug/kg/h) during the acute study. Following anaesthesia (1ml 16.5:250mg/ml chloralose/urethane I.P.), canulae were inserted into the right femoral artery, to measure mean arterial pressure (MAP) and heart rate (HR), and vein for saline (50µl/min) and supplemental anaesthetic infusion. Flank incisions were used to expose the right kidney, to allow cannula insertion 4.5 mm into the cortex for intra-renal infusions, and left kidney, to permit placing the renal sympathetic nerves onto recording electrodes. High-pressure RSNA baroreflex gain curves were generated using I.V. injections of phenylephrine and sodium nitroprusside (50µg/kg/min) to increase and decrease blood pressure, respectively. RSNA was calculated as a percentage of baseline values. Data are expressed as means ± s.e.m. and compared using student’s t-test or ANOVA where relevant. P<0.05 indicated significance. In the renal failure group (MAP: 98±6mmHg; HR: 391±10 beats/min; RSNA: 1.95±0.52 μV.s), the maximum baroreflex gain (sensitivity) was lower by 57% (P<0.05) compared to the control (MAP: 91±3mmHg; HR: 382±12 beats/min; RSNA: 2.28±0.56 μV.s). Treatment with tacrolimus restored the maximum gain to near normal levels in the renal failure group but had no effect on the maximum gain in controls. Intra-renal infusion of TNF-α in control rats decreased maximum baroreflex gain by 34% (P<0.05) compared to the intra-renal vehicle controls. These findings demonstrate that blockade of the inflammatory process with tacrolimus in renal failure normalises the high pressure baroreflex regulation of RSNA. Moreover, intra-renal TNF-α, blunts the high-pressure baroreflex of RSNA in normal rats. The dysregulation of the RSNA baroreflex control in renal failure is dependent on an intact renal innervation and suggests that proinflammatory cytokines can importantly modulate afferent renal nerve activity.

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PCB255

The bacterial metabolite indole signals to the CNS using L-cells to activate vagal nerve activity

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Introduction: Dysbiosis of the microbiome is associated with functional bowel disorders such as IBS and dyspepsia and CNS diseases such as depression, anxiety, parkinsonism and autism, but how the microbiota in the external environment of the gut signal to the brain is unclear. This is important in both health and disease and is likely to occur with a healthy intact epithelial layer. Thus, a mechanism must exist for the microbiota to communicate with the CNS. In this study, we have investigated one bacterial product, indole, a breakdown product of tryptophan, which interacts with enteroendocrine L-cells to stimulate the secretion of GLP-1. This study aimed to investigate L-cells act as a signalling cell, transmitting microbial signals to the internal milieu and on to the CNS.

Methods: A novel dissection technique facilitated the recording of extracellular nerve activity in mesenteric and celiac plexi and vagus nerves following mucosal stimulation of the distal colon adult male Sprague Dawley rats. Nerve activity was recorded using a bipolar electrode and the signal amplified, recorded and analysed using Chart 7. Additional calcium imaging recordings were carried out in colonic submucosal plexus preparations loaded with the ratiometric calcium indicator, Fura 2-AM. Immunofluorescence images of GLP-1 receptor expression in colonic submucosal neurons were recorded using a confocal microscope.

Results: When the mucosa was exposed to indole, increased GLP-1R immunostaining was evident in the submucosal ganglia. Although direct application of indole had no effect on intracellular calcium in submucosal neuronal, mucosal application of indole potentiated GLP-1-evoked calcium responses (P<0.05, n=3). Excitingly, exposure of the distal colonic mucosa to indole stimulated vagal nerve activity (n=3, P<0.05), a response that was attenuated by a GLP-1 receptor antagonist.

Conclusion: Our findings provide the first tangible evidence that was attenuated by a GLP-1 receptor antagonist. (P<0.05, n=3). Excitingly, exposure of the distal colonic mucosa to indole, a breakdown product of tryptophan, which interacts with enteroendocrine L-cells to activate vagal nerve activity

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PCB256

MEDI1814, a high-affinity antibody directed to the C-terminus of Aβx-42 is able to rapidly prevent or reverse synaptic plasticity impairment in the rat hippocampus in vivo

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Amyloid-β protein (Aβ) plays a pivotal role in the pathogenesis of Alzheimer disease. N-terminus and mid-region directed anti-Aβ antibodies have been shown to have potential therapeutic efficacy based on their ability to reduce Aβ oligomer-mediated disruption of synaptic plasticity. It is not clear if antibodies directed to the C-terminus have the same efficacy. Here we examined the ability of a high affinity monoclonal antibody directed to the C-terminus of Aβx-42 to prevent or reverse the inhibition of long-term potentiation (LTP) by synthetic Aβ aggregates in the hippocampus of urethane (1.5 g/kg i.p.)-anaesthetized rats (male Lister Hooded, 250-350 g). First, we evaluated the effects of acute intracerebroventricular co-injection of MEDI1814 (0.3 mg per rat) with soluble synthetic Aβ1-42 enriched in protofibrils (480 pmol). Whereas i.c.v. administration of MEDI1814 fully abrogated the acute inhibition of LTP by Aβ (123.1 ± 4.1 vs 110 ± 2.1% in Aβ+ vehicle group, n=6-7, p<0.05, one-way ANOVA followed by Holm-Sidak post-hoc tests), co-injection of a relatively low affinity Aβ antibody, (MEDI8490, Kd ~10nM), was inactive (106.9 ± 1.5%, n=6, p>0.05 compared with Aβ+vehicle; p<0.01 compared with the Aβ+high affinity antibody group). Next, we examined if systemic passive immunization could prevent or reverse the persistent inhibition of LTP measured in vivo 7 days after a single i.c.v. injection of protofibril-enriched Aβ1-42. The Aβ (585 pmol) was administered under recovery anaesthesia (ketamine, 80 mg/kg and xylazine, 8 mg/kg, both i.p.). Pretreatment with MEDI1814 systemically via cardiac puncture (3 mg/kg, i.c.) on day 1, fifteen minutes prior to Aβ injection, prevented the disruption of LTP measured 7 days later under urethane anaesthesia (124.4 ± 5.1 vs 108.9 ± 2.7% in animals treated with antibody vehicle prior to Aβ, n=5-6, p<0.05), whereas the same dose of MEDI8490 did not prevent the Aβ-mediated LTP impairment (106.5 ± 2.1%, n=5, p>0.05 compared with vehicle+Aβ group; p<0.05 compared with animals given MEDI1814 prior to Aβ). Similarly, when a single injection of MEDI1814 (1.5 mg/kg, i.c.) was given on day 7, under urethane anaesthesia, it abrogated the delayed persistent impairment of hippocampal LTP caused by Aβ (123.5 ± 2.9 vs 107.2 ± 5.5% in Aβ+vehicle group, n=7 in both groups, p<0.001). In contrast, post-Aβ treatment with MEDI8490 (1.5 mg/kg, i.c.) was ineffective (109 ± 1.4%, n=6, p>0.05 compared with Aβ+vehicle; p<0.01 compared with Aβ+MEDI1814-injected animals). In conclusion, intracerebral or systemic administration of a high affinity monoclonal antibody directed to the C-terminus of Aβx-42 is able to rapidly prevent or reverse synaptic plasticity impairment in the rat hippocampus caused by Aβ1-42 aggregates.

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Intracerebral injection of conditioned medium from certain human iPSC-derived neurons blocks hippocampal LTP in the live rat

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Induced pluripotent stem cells (iPSCs) from genetic forms of Alzheimer’s disease (AD) are important tools to study molecular aspects of the disease. We previously found that neurons generated from familial AD iPSCs generate increased amounts of longer, toxic amyloid β-protein (Aβ) peptides1. We predicted therefore that iPSC-derived neurons may release synaptic toxins to cause synaptic dysfunction, which is widely accepted as the main cellular mechanism of early AD. Here we determined if such factors impair hippocampal long-term potentiation (LTP) in the live rat. Adult (250-350 g) male Wistar rats were used in all experiments. Prior to the surgery, animals were anesthetized with urethane (1.5-1.6 g/kg, i.p.). Cortical neurons were produced from iPSCs derived from fibroblasts of individuals with APP duplication, or mutation in PSEN1 (Intron 4). A stainless-steel cannula (22 gauge, 0.7 mm outer diameter) was implanted above the right lateral ventricle. Intracerebroventricular (i.c.v.) injection of vehicle or conditioned medium (CM) was made via an internal cannula. Field excitatory postsynaptic potentials (EPSPs) were recorded from the stratum radiatum in the CA1 area of the right hippocampus in response to stimulation of the ipsilateral Schaffer collateral-commissural pathway. LTP was induced using 200 Hz high frequency stimulation (HFS) consisting of one set of ten trains of twenty pulses (inter-train interval of 2 s). Test EPSPs were triggered a 50% maximum during the HFS. The magnitude of LTP is expressed as the percentage of pre-HFS baseline EPSP amplitude (= s.e.m.) (*P*< 0.05), compared by paired t and one-way ANOVA-Tukey. Acute injection of CM from APP duplication 30 min pre HFS inhibited LTP at 3h post-HFS (102.8 ± 2.6%, n = 6, P > 0.05 compared with Pre; P < 0.05 compared with non-demented control). Similarly, acute injection of CM from PSEN1 neurons also inhibited LTP at 3h post-HFS (108.2 ± 3.7%, n=6, P < 0.05 compared with Pre; P < 0.05 compared with non-demented control). In contrast, i.c.v. injection of CM from non-demented control neurons did not significantly affect LTP (121.9 ± 2.6%, n = 7, P < 0.05 compared with Pre; P > 0.05 compared with 126.1 ± 3.8% in the vehicle control). Interestingly, CM from PSEN1 neurons that had been immunodepleted of Aβ failed to inhibit LTP (129.7 ± 2.1%, n=6, P < 0.05 compared with Pre and P > 0.05 compared with vehicle control group). Our data indicate that cortical neurons derived from individuals with certain genetic forms of AD release soluble agents that impair LTP that is mediated by Aβ peptides. Moore S, Evans LD, Andersson T, Portelius E, Smith J, Dias TB, Saurat N, McClade A, Kirwan P, Blennow K, Hardy J, Zetterberg H, Livesey FJ. Cell Rep. 2015; 11(5):689-96.

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Identified regions of TREK and TRESK two pore domain potassium channels critical for inhibition by sipatrigine and lamotrigine

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Two pore domain potassium (K2P) channels are responsible for background currents that regulate membrane potential and neuronal excitability. Compounds which alter the activity of these channels are predicted to have therapeutic potential in treating CNS disorders. The TRED family of K2P channels (TREK1, TREK2 and TRAAK) have been shown to play an active role in neuroprotection, schizophrenia, depression and pain, whilst TREK1, with high expression in sensory neurons, has a role in nociception (1). Sipatrigine, a neuroprotective agent and a derivative of the anticonvulsant, lamotrigine, is a known antagonist of TREK channels, whilst lamotrigine is thought to primarily inhibit TRESK channels (2, 3). The aim of this study is to clarify differences in the inhibition of these channels by sipatrigine and lamotrigine and investigate the mechanism of inhibition.

Currents through wild-type (WT) and mutated human K2P channels transiently expressed in tsA-201 cells were measured using whole-cell patch-clamp electrophysiology in the presence and absence of sipatrigine (100 µM) and lamotrigine (100 µM). Sipatrigine was a potent inhibitor of TREK1 channels (87 ± 2%, mean ± S.E.M., n=19), whilst lamotrigine inhibited TREK1 channels by 30±6% (n=6). TREK2 channels were similarly inhibited by sipatrigine (73 ± 3%, n=10), whilst lamotrigine had little inhibitory effect (13 ± 3%, n=10). Lamotrigine only inhibited TRESK channels by 34 ± 5% (n=8), a similar inhibition to that seen for TREK1. More surprisingly, sipatrigine was found to potently inhibit TREK channels by 73 ± 3% (n=17).

The recent crystal structure of TREK2 bound to a molecule of the anti-depressant, fluoxetine, has revealed several amino acids important for binding, including leucine (L) at position 320 (4). Mutation of the analogous site on TREK1 (L289A) showed a significantly reduced inhibition of 56 ± 7% (n=9) by sipatrigine, however, inhibition by lamotrigine was unaltered (31 ± 9%, n=6). Homology models for TRESK channels have identified two key cavity-facing residues, F145 and F352 required for the effectiveness of certain channel blocking compounds (5). The double mutation of TRESK (F145A, F352A) substantially reduced inhibition by both sipatrigine (10 ± 2%, n=9) and lamotrigine (5 ± 2%, n=11).

Our findings show that lamotrigine does indeed inhibit TRESK channels however the compound also inhibits TREK channels. Furthermore, sipatrigine is a potent inhibitor of both TREK and TRESK channels. Mutations of TREK1 and TRESK have demonstrated sites on these channels important for the inhibitory actions of both sipatrigine and lamotrigine. For TREK1 channels, we hypothesise that L289 is important for sipatrigine binding but not for lamotrigine binding. For TREK channels, F156 and F364 are important residues for inhibition by both compounds.

Combined ketamine (100 mg kg\(^{-1}\), i.m.) and xylazine (10 mg kg\(^{-1}\), i.m.) was used for anaesthesia. Seven days after in vivo gene transfer, rats were recorded both under baseline conditions and during exposure to acute air-jet stress. Blood pressure (BP), heart rate (HR) and their short-term variabilities (BPV and HRV) as well as spontaneous baroreflex sensitivity (BRS) were evaluated using spectral analysis and the sequence method, respectively. Values are expressed as mean±SEM. One-way ANOVA for repeated measures followed by Bonferroni post hoc test was used to assess differences between groups. Differences were taken as significant at \(p<0.05\).

Both under baseline and stressful conditions, overexpression of PKI\(_\alpha\) in the PVN of Wistar rats (n=6) induced significant decrease in mean values of systolic BP (BASELINE: 96±5 mmHg vs. 116±4 mmHg for control Wistars; STRESS: 120±1 mmHg vs. 136±2 mmHg for control Wistars, \(p<0.05\) respectively), compared to control Wistar rats (n=6). Moreover, overexpression of PKI\(_\alpha\) in the PVN of Wistar rats increased BRS (3.9±1 ms mmHg\(^{-1}\) vs. 1.9±0.09 ms mmHg\(^{-1}\) for control Wistars, \(p<0.05\)), reduced total systolic BPV (2.29±0.22 mmHg\(^2\) vs. 3.26±0.3 mmHg\(^2\) for control Wistars, \(p<0.05\)) due to decrease in low frequency of systolic BP (0.38±0.07 mmHg\(^2\) vs. 0.97±0.27 mmHg\(^2\) for control Wistars, \(p<0.05\)) and induced decrease in LF/HF-HR ratio (0.18±0.09 vs. 1.21±0.28 for control Wistars, \(p<0.05\)) at rest. Overexpression of PKI\(_\alpha\) in the PVN of SHRs (n=6) did not induce any significant changes in BP, HR and their variabilities compared to non-transfected SHRs (n=6). These data suggest that cAMP-dependent protein kinase signaling in the PVN may be important in modulation of cardiovascular autonomic activity and baroreflex function.

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**PCB261**

Overexpression of protein kinase inhibitor alpha in the paraventricular nucleus of the hypothalamus reduces blood pressure and blood pressure variability in Wistar rats

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The paraventricular nucleus of the hypothalamus (PVN) is an important integrative site in autonomic and neuroendocrine control of cardiovascular system. Its role in the regulation of sympathetic outflow depends upon an interplay of at least 30 identified inhibitory and excitatory neurotransmitters. A number of those neurotransmitters produce their effects by changing the activity of neuronal cAMP-dependent protein kinase. We hypothesize that, by selectively blocking the CAMP-dependent protein kinase, we can modulate PVN neuronal activity involved in autonomic cardiovascular control. All experimental procedures in this study conformed to 86/609/EEC. Experiments were performed in conscious male normotensive Wistar rats and spontaneously hypertensive (SHR) rats, equipped with radiotelemetric device for registration of cardiovascular parameters and bilaterally microinjected into the PVN with adenovirus to overexpress selective CAMP-dependent protein kinase inhibitor-protein kinase inhibitor alpha (PKI\(_\alpha\)) or eGFP (control). Surgical procedures were performed under combined ketamine (100 mg kg\(^{-1}\), i.m.) and xylazine (10 mg kg\(^{-1}\), i.m.) anesthesia. Seven days after in vivo gene transfer
were determined twice weekly. On day 28, rats were sacrificed with an overdose of pentobarbital (100 mg i.p.). MIA/VEGF14 rats showed a significant reduction in weight bearing asymmetry compared to MIA/PBS rats on days 18, 25 and 28. The percentage of weight borne on ipsilateral hindpaw in the MIA/VEGF14 rats vs. MIA/PBS group was 46.48 ± 1.68 vs. 41.16 ± 2.15% on day 18 (2 way ANOVA with post-hoc Tukey’s tests, p<0.05). Treatment of MIA rats with VEGF-A165b on days 0-14 also (p<0.001).

Treatment of MIA rats with VEGF-A165b on days 0-14 also returned mechanical von Frey (vF) withdrawal threshold levels to control levels. Mechanical withdrawal threshold in MIA/VEGF14 group vs. naïve/PBS group was 9.71 ± 1.14 vs. 9.63 ± 1.57 g on day 25 and 8.46 ± 1.16 vs. 9.94 ± 2.30 g at the end of the study. (Data expressed as mean ± SEM). Weight bearing and vF thresholds in MIA/VEGF28 rats were not different from those of MIA/PBS rats.

These results indicate that VEGF-A165b has an anti-nociceptive effect in the MIA model of OA in rat when given early, but not later, in development of the arthritis.


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PCB263

Secretions from the placenta alter neuronal development after hypoxic insult during pregnancy

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Psychological disorders such as autism, schizophrenia and ADD are thought to originate partially due to insults to the foetus during pregnancy. Events such as hypoxia reoxygenation during pregnancy have been shown to increase the risk of psychological disorders in later life. While the placenta normally acts as a protective barrier between the mother and the foetus we have found during a hypoxic insult it releases damaging molecules into the foetal circulation. We modelled obstetric complication and exposed a placental trophoblast barrier model or ex vivo placental explants to variable oxygen levels. We collected tissue culture media from below the barrier and analysed its contents. We found that the placental barrier secreted increased levels of glutamate (4x increase) when exposed to hypoxia and hypoxia/reoxygenation. Previous experiments demonstrated that this media when directly exposed to E18 rat neurone cultures or injected directly into P4 rat brains was able to alter neurones, astrocytes and NMDA receptors in vitro and in vivo.

To investigate if these changes were maintained without direct exposure experiments performed in collaboration with Professor Davidge’s group in the University of Alberta were performed using their established model of Maternal Hypoxia. In this model pregnant rats (n=3) were placed in 11% Oxygen on GD15 for 6 days then the brains of their offspring were examined at P30 (four per litter). These brains demonstrated a reduction in Parvalbumin positive cells (353+/−96 vs 304+/−64 cells per FOV +/-S.D p < 0.05 One way ANOVA), increased TH+ process (7839+/−252 vs 10745+/−236 µm per FOV +/-S.D p < 0.001 One way ANOVA), loss of dendrite length (5995+/−950 vs 3708+/−584 µm per FOV +/-S.D p < 0.001 One way ANOVA) and reduced detection of GluN1 (2000+/−694 vs 5995+/−950 < 0.001 One way ANOVA).

Our hypothesis is that factors including glutamate, released from the placenta during hypoxia might enter the foetal circulation and cause changes in the developing brain. These changes are similar to the types of changes seen in post-mortem brains of patients with Schizophrenia and can be prevented by treatment of the placenta with MitoQ bound to nanoparticles.

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PCB264

Activation of the receptor for advanced glycation end products mediates sensitization of primary afferents in diabetic neuropathy

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Painful neuropathy is a serious diabetic complication that affects up to 20% of diabetic patients. Transient receptor potential vanilloid 1 (TRPV1) sensitization has been implicated in the development of diabetic pain and is ameliorated by vascular endothelial growth factor-A(VEGF-A)165b treatment. We hypothesized that the activation of the receptor for advanced glycation end products (RAGE) by high mobility group box-1 (HMGB1) sensitizes neuronal TRPV1, and this would be prevented with VEGF-A165b.

Diabetes was induced in adult female Sprague Dawley rats (250-350g) with streptozotocin (STZ, 50mg/kg, i.p) and maintained for 3 weeks. Diabetic rats were insulin treated (LinShin, 1/3 slow release pellet, implanted under 2% isoflurane in O2 anesthesia), and treated bi-weekly with recombinant human VEGF-A165b (20ng/g, i.p, n=4) or vehicle (PBS, i.p, n=4). At 3 weeks, animals were killed with an overdose of sodium pentobarbital (60mg/kg, i.p), plantar skin was dissected, protein extracted, and Western blots performed for HMGB1 and actin.
Dorsal root ganglion (DRG) neurons were isolated from naïve adult male Wistar rats, plated onto 96 well plates for measurement of intracellular calcium changes in response to TRPV1 (1µM in 0.1% DMSO) in a high throughput Fluo-4 based assay. DRG neurons were treated for 24h before assay with HMGB1 (RAGE agonist, 10nM), HMGB1+FPSZM1 (RAGE antagonist, 10nM), HMGB1+rhVEGF-A165b (2.5nM), and HMGB1+BIM1 (PKC inhibitor, 1µM). Calcium responses were calculated as AUC for each assay.

HMGB1 expression in the skin was significantly increased in STZ injected rats compared to naïve (STZ + vehicle 5.4±1.8 fold change vs. naïve 1±0.3 fold change, n=4, 1 way ANOVA + post hoc Bonferroni p<0.05). There was no effect of VEGF-A165b treatment on HMGB1 expression. HMGB1 alone increased capsaicin-evoked TRPV1 activity in DRG neurons and treatment with all inhibitors reduced the response (Table 1).

HMGB1 induces TRPV1 sensitization in DRG neurons, through activation of RAGE and PKC, VEGF-A165b had no effect in vivo on expression of HMGB1, but prevented the HMGB1-RAGE mediated TRPV1 sensitization in vitro. HMGB1 expression is increased in the peripheral skin in diabetic rats. These data suggest peripheral sensory neuronal RAGE activation may contribute to peripheral neuronal sensitization in diabetes through modulation of TRPV1.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Vehicle</th>
<th>Inhibitor</th>
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</thead>
<tbody>
<tr>
<td>HMGB1</td>
<td>16.6±6.3</td>
<td>97.7±10.5</td>
</tr>
<tr>
<td>+PSZM1</td>
<td>32.8±7.7</td>
<td>90.4±11.1</td>
</tr>
<tr>
<td>+VEGF-A165b</td>
<td>138.3±19.1</td>
<td>135.3±21.0</td>
</tr>
<tr>
<td>+VEGF-A165b+PKC</td>
<td>52.6±6.5</td>
<td>72.1±4.5</td>
</tr>
</tbody>
</table>

Data are mean ± SEM, 1 way ANOVA+ post hoc Bonferroni, *p<0.05, ***p<0.001

University of Nottingham and Diabetes UK.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

**PCB265**

Effect of prolonged atorvastatin administration on behaviour of rats

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Statins, the cholesterol lowering drugs, are one of the most prescribed medicaments at all. Despite this fact, great variety of side effects - including cognitive, behavioural and motoric changes - is discussed in many studies. However, the variability of results of former studies is enormous and thus, the problem remains unsolved. The aim of this project is to study: 1) behaviour of rats in a wide battery of tests, 2) correlations between the changes of SERT activity, levels of cholesterol and behaviour of rats, 3) effect of dose or length of drug administration.

For this study we used atorvastatin in different doses and lengths of administration. Wistar rat males (n=48) were divided into 6 groups (n=8 for each) – 2 control groups, 4 groups with statin administration. Statin was administered in long (34 days) or short (21 days) administration protocol; in dose of 20 mg/kg, 10 mg/kg or in mixed design. Statins were administered to the rats orally in jelly mixture once a day. Behavioural tests were made before and after administration what allows us to compare the changes in performance of animals.

We used behavioural tests to test impulsivity, anxiety and depression-like behaviour (OF, EPM, FST), cognitive tests (MWM, allotrophic active place avoidance test) and locomotor tests (beam walking, rotarod). At the end of the experiment we collected samples for biochemical analysis.

Analysis of results showed no significant effect of application on performance in MWM. No significant motoric impairment was found. We found a significant effect of long administration of low dose of atorvastatin on performance in reversal learning in AAPA (p<0.01). There was also trend in performance in OF test and EPM after the administration of high dose of atorvastatin.

In conclusion, we found out that prolonged administration of atorvastatin in rats may cause some slight behavioural and cognitive changes. One the other hand, in most of the test the effect was not significant. However, we found marked inter-individual differences in animals and also significant effect of used cohorts (differing in age). The question is whether these effects may mask the effects of statins. Together with ambiguous results of previous experiments it highlights the need for additional experiments, what may better explain potential behavioural and cognitive changes.

This research was supported by PRVOUK P34 of Charles University in Prague, GAUK 392015, GAUK 1508414, IGA MZ CR NT/1448, GACR P304/12/G069 and P303/12/1464, by the project “National Institute of Mental Health (NIMH-CZ)”, grant number ED2.1.00/03.0078, and the European Regional Development Fund, and by the project “Sustainability for the National Institute of Mental Health”, grant number LO1611, with a financial support from the Ministry of Education, Youth and Sports.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

**PCB266**

Molecular characterisation of atrial volume receptors in the atria of the rat: Is ENaC involved?

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Background:

Cardiovascular disease killed 12.9 million people in 2010 [1]. A characteristic of cardiovascular disease is excessive sympathetic tone. Many investigators have studied this but few have explored parasympathetic sensory input to the brain from atrial volume receptors (AVRs). The AVR reflex arc is crucial in maintaining normal plasma volume. Fibres from these receptors convey information about central venous volume via the vagus nerve to the medulla and hypothalamus influencing vasopressin expression and sympathetic activity. Type A volume receptors fire during systole and type B during diastole [2].
The epithelial sodium channel (ENaC) is a major contributor to muscle spindle mechanosensory transduction in rat skeletal muscle [3]. In our study, we hypothesize that AVRs utilize ENaC for mechanotransduction of atrial stretch and that inhibition of ENaC using amiloride will inhibit these afferent signals. To test this, we administered amiloride to an in-vitro rat heart-vagal nerve preparation whilst recording afferent vagal action potentials.

Methods:
Adult male Wistar rats (body mass: 128-190g) were euthanized by cervical dislocation while under 5% isoflurane anesthesia. Mediastinal tissue was rapidly extracted and superfused with oxygenated Tyrode's solution (pH7.4). The great veins were tied and a double-lumen catheter inserted into the right atrium via the tricuspid valve. The right atrium was pressurized under isovolumetric conditions and electrophysiological recordings were taken from the vagal nerves until cardiac units were isolated. Thereafter, 100µM amiloride hydrochloride was added to the tissue bath while vagal recordings continued. Recording and analysis was carried out using Spike2 (CED, UK). All experiments were conducted in accordance with local ethical committee approval and relevant licensing from regulatory authority. Data were analyzed with a paired Student’s test.

Results:
Twelve cardiac units were analyzed with phase histograms (one pressure peak to the next represents 0 to 360°). Five systolic (mean 48°, SD 49°) and 7 diastolic units (288°, SD 16°) were observed. In the pharmacological studies, mean cardiac interval was 368ms (SEM 54.43, n=4) and post 100µM amiloride was 356ms (SEM 43.29, n=4; p=0.74). Mean atrial pressure was 1.07 mmHg (SEM 0.25, n=4) and post 100µM amiloride was 1.07 mmHg (SEM 0.19, n=4; p=1.0). Cardiac vagal recordings were obtained in two of these studies. Amiloride had no effect in one study and reduced vagal firing rate in the other (7.9 Hz to 4.4 Hz).

Conclusions:
The rat atrium possesses both type A and type B AVRs. At 100µM, amiloride has no indirect effects on atrial contraction or rate of beating. This vagal-atrium preparation will permit a resolution of the role of ENaC in atrial mechanotransduction.


We would like to thank the British Heart Foundation and UCD School of Medicine for their support.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Effects of circadian disruption and social isolation in mice on neurogenesis and behaviour

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The internal circadian clock, orchestrated by the master clock in the hypothalamic suprachiasmatic nucleus, drives oscillations in physiology and behaviour. Constant light exposure that is an inherent feature of modern urban life also interferes with circadian rhythms affecting 99% of the population in the US and Europe, resulting in increased risk for various disorders including mood disorders. We hypothesise that socially-isolated animals will be more vulnerable to the deleterious effects of circadian disruption due to the absence of entrainment to the social signals provided by cage-mates. The aim of this study is to investigate the effects of chronic photoperiodic disruption on social motivation and social memory in adult mice, and assess the potential effects of social isolation on these behavioural outputs and on hippocampal neurogenesis, which is often diminished in those suffering from mood disorders.

Adult mice (n=32) were exposed to circadian disruption in the form of dim light at night (12D:12L at 5 lux) or control conditions (12:12 light dark cycle) for a period of 10 weeks. Experimental and control groups were further allocated to a socially housed group (4 per cage) or singly-housed group for the duration of experiments. Locomotor activity, sociability and social memory were assessed using a three-chamber social test. Mice were sacrificed and their brains processed for neurochemistry to provide a measure of neurogenesis.

Socially-isolated mice were more active (p<0.005), whereas circadian disruption resulted in a decrease in distance moved relative to control groups in this test (p<0.01). Although socially-isolated mice exhibited reduced social motivation relative to group-housed mice (p<0.05), dim light at night had no effect on social motivation relative to controls. However, both the circadian disruption and the social isolation induced a deficit in social memory relative to control mice (p<0.05). In the brain, circadian disruption induced a decrease in neurogenesis in the hippocampus as indicated by a reduced expression of doublecortin levels (p<0.05). Doublecortin expression was also marginally lower in the animals that were singly-housed in comparison to those housed in groups (p=0.05). These results support an effect of dim light at night on circadian rhythms, social memory, and on neurogenesis, mechanisms that might mediate previously reported effects of urbanisation and shift-work on behaviour in humans. Group housing might be a useful intervention to attenuate these effects, possibly by amplification of timing cues through social entrainment.

Poster Communications

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Calcium dysregulation in cultured hippocampal neurones from young rodent models of Alzheimer’s disease (3xTg-AD mouse & TgF344-AD rat)

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Research strongly supports a key role for calcium dysregulation in Alzheimer’s disease (AD) onset and progression. This dysregulation may initiate the over production of AD-linked ‘toxic’ metabolites such as amyloid beta (Aβ) and subsequently establish a degenerative feed-forward cycle between the two entities. In this study, we have examined the role that the endoplasmic reticulum (ER) plays in both maintaining calcium homeostasis and in mediating intracellular signalling processes, with a specific emphasis on how these functions may be disrupted in AD.

Cultured hippocampal neurons were prepared from control and transgenic 3xTg-AD and TgF344-AD rats between 3-6 days old. The magnitude of intracellular somatic calcium signals was determined by area under the curve analysis, following loading with the calcium sensitive dye, fluo-2 AM (150µM). Experiments were carried out at room temperature with neurons continuously perfused (2ml/min) with a standard saline solution (HBSS) containing TTX (1µM). A particular calcium loading protocol was adopted which involved pre-load of the ER with Ca2+ (using a brief depolarising stimulus; an extracellular application of 15mM K+) followed by application of a specific group 1 metabotropic receptor agonist (1-mGluR), (S)-3,5- dihydroxyphenylglycine (DHPG; 50µM). Such conditions are thought to crudely mimic ‘synaptic activity’ and have been previously shown to elicit so called ‘supralinear’ or enhanced calcium responses in rat hippocampal neurons5. Data, unless otherwise stated, were analysed using Wilcoxon matched-pairs signed rank test and are expressed as mean ± S.E.M.

In control neurons, from both murine models, 1-mGluR activation combined with the loading stimulus, evoked enhanced somatic Ca2+ signals relative to 1-mGluR activation alone (mouse model, 679 ± 128 %, P = 0.0013, n = 47; rat model, 6948 ± 1821 %, P < 0.0001, n = 46). In contrast, we did not observe enhanced responses in neurons derived from TgF344-AD rats (P = 0.6084, n = 28) and, further, responses were significantly reduced in neurons derived from 3xTg AD mice (79 ± 14 %, P = 0.0006, n = 36). Notably, we observed significantly larger responses to 1-mGluR activation alone, in transgenic neurons of both species compared with control neurons (mouse model, P = 0.0013, n = 36; rat model, P = 0.0044, n = 28; unpaired t test), suggesting a pathological increase in ER calcium levels.

The fact that such stark alterations in calcium homeostasis and signalling have been observed in neurons from rodent models of AD at such a young age (<6 days) suggests that calcium dysregulation may occur at a much earlier stage in the disease progression than previously thought, long before any significant elevations in Aβ concentration or cognitive deficits become apparent.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Micro RNA 134 knockdown delays epileptiform activity in ex vivo brain slices

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Micro RNAs (miRs) are short non-coding RNA sequences with a role in regulating gene expression. MiR 134 influences spine density (1) and volume (2) and is upregulated in epilepsy (1). MiR 134 knockdown protects against seizures (1), though the underlying mechanisms are still not clear. We explored the effects of miR 134 on neuronal excitability and ex vivo epileptiform activity using an acute brain slice model of epilepsy. Adult male Sprague Dawley rats (200-300g) were treated with an antagonir to miR 134 or vehicle control, delivered via intracerebroventricular injection, carried out under anaesthesia with inhalational isofluorane (~2.5% in O2). Ex vivo brain slices were prepared 2-4 days later. Epileptiform activity was induced by bath perfusion with 9 mM K+ and recorded with extracellular micropipettes in hippocampal CA1. Intrinsinc neuronal properties were probed using patch clamp recordings in baseline K+ conditions. MiR 134 knockdown via antagonir injection delayed the onset of epileptiform in ex vivo slices by 182 s relative to control (n = 9 control slices; 11 treated slices; Mann Whitney U test p = 0.002). MiR 134 knockdown had a tendency to increase action potential (AP) rising slope in single neurons, but this did not survive multiple comparisons (control: 179 ± 82 mV/ms, n = 6 neurons; treated: 251 ± 82 mV/ms, n = 7 neurons; independent samples t test p = 0.043, α=0.025). We did not observe any other effects of the antagonir on intrinsic neuronal properties including: input resistance, resting membrane potential, AP amplitude, AP half width, maximum firing rate, miniature EPSC (mEPSC) amplitude, or mEPSC frequency. Thus, although miR 134 is protective against epileptiform activity in ex vivo brain slices, this is not associated with any robust changes to intrinsic excitability in CA1 neurons. In fact, the only change that approaches significance is a counterintuitive increase in action potential rising slope. We hypothesise that the delay to activity onset is associated with a synaptic change, as suggested previously (1), rather than the intrinsic neuronal properties tested thus far in our recordings. Additional work will probe synaptic strength in treated slices to test this. However, our data so far suggest that miR 134 alters seizure thresholds by relatively specific effects on neurons. A better understanding of the impact of miR 134 on neuronal excitability and connectivity will enhance its position as a potential novel therapy in epilepsy.


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Poster Communications

Drunk bugs: Chronic vapor alcohol exposure induces marked changes in the gut microbiome in mice

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Introduction: The microbiota-gut-brain axis is a bidirectional pathway that has recently been shown to influences brain and behavior. Moreover, gut microbiota have been implicated in several psychiatric conditions such as depression and anxiety. To date, minimal research has investigated gut microbiota in addiction including alcohol abuse. Furthermore, the effect of vaporized ethanol administration on the gut microbiota is unknown.

Methods: Adult male C57BL/6J mice were exposed to 4 weeks of either chronic intermittent vaporized ethanol (Vapor ETOH, N=10) or air (Vapor Control, N=10). A previously described vapor inhalation procedure was employed for the test group (Holmes et al., 2012; Lopez & Becker, 2005). In short, test mice received IP injections of 1.5g/kg of 20% ETOH (v/v) with 71.6 mg/kg alcohol dehydrogenase (ADH) inhibitor pyrazole (Sigma, St. Louis, MO, USA) prior to vapor ethanol exposure (16 hours/day, 5 days/week). Control group received IP injections of saline with 68.1 mg/kg ADH inhibitor pyrazole and only fresh air in vapor chambers. Fecal samples were collected at the end of the experiment and 16S prokaryotic DNA was sequenced. Bioinformatic analysis of sequenced 16S Operational Taxonomic Units (OTUs) assessed bacterial composition.

Results: Phylogenetic measures of alpha (p<0.05) and beta (ANOSIM, p=0.001) diversity revealed significant differences between groups, with alpha diversity decreased in the Vapor ETOH group. Genus level bacterial composition showed significant differences (ANOSIM, p=0.002) between groups with the most significant changes in Alstites (Kruskal-Wallis, p<0.00024) and Clostridium IV and XIVb (Kruskal-Wallis, p<0.00045).
Conclusion: These results demonstrate that chronic vapor alcohol exposure is capable of significantly altering the gut microbiota in mice. These findings align with previous findings of microbiota changes associated to liver disease, inflammation, and psychological distress (Hartmann et al., 2015; Leclercq et al., 2014; Mutlu et al., 2012).

Research supported by the National Institute on Alcohol Abuse and Alcoholism Intramural Research Program. Fecal microbiota analysis research was conducted with the financial support of the APC Microbiome Institute and Science Foundation Ireland (SFI) under Grant No. 12/RC/2273. We would like to thank Ms. Katherine Kaugars and Dr. Gerard Moloney for their superb technical assistance.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB274

Effect of Pomegranate Flower on Plasma and Brain Tissues Oxidant System and Reactive Gliosis in Hyperhomocysteinemic Rat Model

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Hyperhomocysteinemia is an important risk factor in some cardiovascular diseases and brain damage circumstances by inducing free radical production and reactive gliosis. Punica granatum L. (PGL) is known as a powerful antioxidant substance. It is aimed to investigate the effect of homocystein on oxidant-antioxidant systems and reactive gliosis in hyperhomocysteinemic rat in the present study. Additionally, the possible protective effect of PGL against the hyperhomocysteinemia induced oxidative stress was determined.

Totally 21 adult Wistar rats were used in this study. Animals in control group (n=7) were only fed with standard food for six weeks. Hyperhomocysteinemia was constituted in second group (n=7) by applying L-methionine (1.5 g/kg/day) in drinking water in addition to standard feed for the same time period with the control. The rats in third group were received PGL extract (500mg/kg/day) in standard rat feed in addition to L-methionine for six weeks (n=7). All rats were decapitated at the end of experiments and blood samples and brain tissues were collected for analyzing of oxidant parameters.

Some parameters revealing lipid peroxidation increased in hyperhomocysteinemia group whereas PGL treatment significantly decreased these parameters. Additionally, hyperhomocysteinemia caused augmentation on glial fibrillary acidic protein and S100 protein levels and neuron specific enolase activity. PGL treatment prevented these augmentations.

In conclusion, it is revealed that PGL diminished hyperhomocysteinemia induced lipid peroxidation, increased antioxidant activity and decreased reactive gliosis in rat brain tissues. Based on these results, we could express that PGL has neuroprotective effect in rat brain.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
A novel aligned dorsal root ganglion explant culture system using 3D printed substrates

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Directing axonal growth to targets is challenging and expensive, including techniques that utilise microfluidic devices and nanofiber scaffolds. 3D printing is an inexpensive, efficient, versatile and rapidly evolving method of building 3D constructs, and may provide opportunities to build systems to study directed neural migration and nerve regeneration. Dorsal root ganglion (DRG) explants are used to elucidate the migration of sensory neurons and their attendant glia, Schwann cells. If cultured on flat surfaces, nerve fibres grow radially from the ganglion. A method of culturing DRG explants where axons grow linearly would greatly assist in the measurement and analysis of growth. Substrates were 3D printed with polylactic Acid (PLA), a biocompatible polymer, using an Ultimaker original printer. Each 60 µm layer of substrate was printed upon the previous one, resulting in grooves or microchannels travelling in parallel across the surface of the print. The substrates were coated with poly D lysine or laminin to improve cell adhesion to the surface. P4 Wistar rat pups were euthanised in accordance with institute guidelines and relevant legislation (directive 2010/63/EU) and their DRGs were cultured on 3D printed substrates in the absence or presence of the antimitotic agent flourodeoxyuridine (FDU) which prevents Schwann cell proliferation. The orientation of the axons was analysed by measuring the angle they projected at relative to the parallel microchannels, and close alignment was seen in both the control and FDU groups (-2.9±1.4°, n=68 and -2.7±1.4°, n=67 respectively; Mean±SEM). This suggests that the axons directly contact and respond to the 3D surface of the microchannel regardless of the presence or otherwise of Schwann cells. By modifying the topography of the substrates, this alignment could also be demonstrated with curved microchannels, further establishing a relationship between the 3D topography of the substrate and the directionality of axon growth. This topographical control of growth can also be combined with molecular cues. DRGs were cultured for 7 days on printed substrates coated with either lysine or laminin. All cultures demonstrated axonal growth and Schwann cell proliferation and migration that closely followed the microchannels, but the addition of laminin extended growth when compared to lysine (5.18±0.60 mm; n=6 and 3.52±0.21 mm; n=6 respectively; Mean±SD). Topographical cues can independently direct the growth of axons and Schwann cells, where growing neurites somehow sense the spatial gradient provided by microchannels. Varying the surface architecture of 3D prints may offer an efficient and rapid method of studying a regenerating nerves response to a spatial gradient, enabling the design of tissue engineered scaffolds that induce a particular response to a specific topography.

Supported by Irish Research Council (GOIPG/2013/921)

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Analysing the effect of body growth on epithelial nerve net structure in Pleurobrachia pileus

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Genetic studies suggest that the marine invertebrate, the ctenophore, evolved a neuromuscular system independently to all other animals (1). They therefore represent an exciting opportunity as a new invertebrate model organism to study the evolution of the nervous system and basic principles of neurophysiology.

In order to understand how a nervous system functions we must first understand how this system is organised structurally. The challenges associated with mapping network architecture are more tractable in small, simple nerve nets. In addition, Pleurobrachia pileus adult forms have significant variability in body size. This study therefore aims to investigate how nervous system architecture adapts to a changing body size.

P. pileus were collected from Irish coastal sites, fixed in 4% paraformaldehyde and classified into large (>0.2g) and small (<0.2g) cohorts. An antibody against tyrosylated α-tubulin was used to visualise the epithelial nerve net. The polygonal shapes were manually traced and the average surface area at the aboral organ and body wall were calculated.

The size of the nerve net polygons at the aboral organ was significantly increased in large animals (mean±SEM: 1477±76 µm², n=5) compared to small animals (966±137 µm², n=5; p=0.0129, t-test), which suggests the basic architecture of the network is persistent as the body size changes in this region. However, the polygons of the network in the body wall show no difference in size between large (1104±99 µm², n=4) and small animals (820±140 µm², n=3; p=0.1652), which suggests that this region of the network responds to increasing size by adding additional structural elements.

Our findings suggest a previously unidentified functional distinction of sub regions of the nerve net in P. pileus. Nerve nets are often considered global through conducting pathways in which the same behaviour can be evoked by stimulating any part of the network. Previous electrophysiological studies have shown that this is not the case in ctenophores as ciliary and muscular responses are confined to specific sites and only interact with other pathways indirectly (2). This study provides structural evidence to support these claims.

The larger polygonal surface area of the aboral organ (‘rudimentary brain’) as the animal increases in size suggests that this structural difference is important in terms of relaying information to and from the body to a defined location. This contrasts a smaller and consistent polygonal size on the body wall as the animal grows which indicates an increase in the number of polygons. This might suggest the development of new sensory receptors through new polygonal formation. Moroz LL et al. The ctenophore genome and the evolutionary origins of neural systems. Nature. 2014; 510:109–114


This work is supported by University College Dublin

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Prenatal high fat diet exposure in mice impairs maternal behaviour and primes offspring for increased neuroinflammation and altered hypothalamic stress markers

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Introduction & Aims: The obesity epidemic is leading to an increase in obesity during pregnancy, which has implications for the health and wellbeing of the offspring. Maternal obesity may influence maternal behaviour which may subsequently affect offspring behaviour and stress responses, however the mechanisms remain unclear. One possibility is that stress responses are mediated by an inflammatory response to a high fat obesogenic diet. To test this hypothesis, this study investigated stress markers involved in hypothalamus-pituitary-adrenal (HPA) axis function, and neuroinflammation in offspring from high-fat (HF)-fed obese mothers, with or without subsequent postnatal HF-feeding, and measures of early maternal and pup behaviour.

Methods: Female C57BL/6 mice were fed either a high fat (HF; 45% kcal fat) or control diet (C; 7% kcal fat) 6 weeks before mating, throughout pregnancy and lactation. Weaned offspring were fed C or HF diet resulting in 4 offspring groups: C/C (n=5), C/HF (n=4), HF/C (n=6), HF/HF (n=4) (pre/postnatal diet). In 15 week-old male offspring brain, gene markers of inflammation (IL-1β, TNF-α, CRH, GR) and HPA function (corticosterone, glucocorticoid receptor [GR]) were measured by RT-PCR in the hypothalamic paraventricular nucleus (PVN). In 7 day-old male and female pups (C, n=20; HF, n=6) anxiety was assessed by ultrasonic vocalisation (USV) frequency during 4 min isolation from the dam. Maternal behaviour (C, n=44; HF, n=8) was assessed by recording times of first dam-pup interaction and pup retrieval to the nest in the 2 min following pup return. Data analysis: 2-way ANOVA and t test.

Results: IL-1β but not TNF-α was significantly increased (p<0.01) and GR was significantly decreased (p<0.05) in HF-fed male offspring PVN, but only when their mothers were HF-fed (HF/HF). Maternal HF diet also increased CRH and decreased FCyR1 in offspring PVN, regardless of postnatal diet (p<0.05). Pup USV was not different between C and HF pups. The time taken to interact with pups and then return them to the nest was significantly (p<0.0001) longer for HF-fed dams.

Conclusions: These results suggest that changes in markers of both neuroinflammation and stress are altered in young adult offspring from obese mothers, but only when fed a HF postnatal diet. Although pup anxiety appeared unaffected by maternal HF-feeding, the reduction in mother-to-pup interaction at postnatal day 7 may have longer-term effects on stress responses and behaviour in HF offspring. Further studies of behaviour of these offspring later in their lifespan will determine whether potential behavioural changes, including anxiety and memory and learning, can be specifically linked to altered hypothalamic inflammatory and stress markers.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Zn²⁺ induces microglial cytotoxicity via NADPH-dependent oxidase-mediated ROS production and TRPM2 channel activation

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The accumulation of ROS resulted from the activation of the enzymatic activity of NADPH oxidases (NOX) elevates the cytosolic Ca²⁺ concentration [Ca²⁺], causes cell death through the activation of poly-ADP-ribose (ADPR) polymerase (PARP). ROS-dependent PARP-1 activation has been act as the main mechanism in promoting ADPR generation resulting in the activation of the transient receptor potential melastatin-related 2 (TRPM2) channel, a Ca²⁺-permeable channel. In this present studies, we have provided compelling evidence using both pharmacological and genetic approaches to demonstrate that the microglial TRPM2 channel plays a critical role in mediating ROS-induced microglial cell death. Measurements of the [Ca²⁺]c showed that H₂O₂ induced robust increases in the [Ca²⁺], in a concentration-dependent manner and such Ca²⁺ responses were strongly inhibited by pretreat ment with Pj-34, a PARP inhibitor, and also by pretreatment with 2-APB, a TRPM2 channel inhibitor. Indeed, H₂O₂-induced Ca²⁺ responses were largely decreased in microglial cells isolated from trpm2-/- mice. H₂O₂ applied at 30-300 µM induced concentration-dependent microglial cell death and H₂O₂-induced cell death was significantly attenuated by pretreatment with 10 mM IM-54 from 90%±2.1 % to 55%±8%, suggesting microglial cell death preferentially via the necrotic mechanism. H₂O₂-induced cell death was significantly decreased from 91%±2.5 to 57%±4, 59%±10 and 18%±6% by pretreatment Pj-34, DPQ and 2-APB respectively. In fact, a significant reduction in microglia cell death was seen in TRPM2-deficient microglial compared to WT which was about 16%±3.6% and 99%±0.6% respectively. Zinc at 300 µM caused necrotic cell death in microglial cells (3%±1% vs. 89%±4%) and this response was significantly inhibited by NOX inhibitors, DPI and GKT 137831, from 88%±5 to 44%±12% and 32%±3% respectively. Furthermore, immunofluorescence studies showed a strong inhibition by Pj-34 or DPQ in the increases of the PAR level induced by Zn²⁺ and H₂O₂, but treatment with DPI or GKT attenuated the increases in the PAR level induced by Zn²⁺ but not by H₂O₂. Consistently, treatment with Pj-34 and DPQ strongly inhibited Zn²⁺-induced cytotoxicity (91%±1.1% vs. 30%±3.8% for 10 mM Pj-34 and 42%±3.6% for 10 mM DPQ). Zn²⁺-induced cell death was significantly attenuated by pretreatment with 2-APB (90%±1.1% vs. 32%±3.9%) and TRPM2 channel deficiency (93%±1.9% vs. 17%±3.8% at 300 mM Zinc for WT and KO-TRPM2 respectively). Taken together, these results provide clear evidence to support that activation of the TRPM2 channel is a key molecular mechanism responsible for ROS-dependent Zn²⁺-induced microglial cell toxicity.

Ministry of Education, Malaysia and University Putra Malaysia

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Peripheral nerve striations represent the sinuous path of axons and are indicative of axonal length

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The bands of Fontana are striations visible to the naked eye on peripheral nerves, their appearance varying depending on the nerve under observation and its illumination. Their origin and development remains a subject of some debate. A deeper understanding of the development and variability of these bands may help elucidate the mechanisms behind lengthening of axons in peripheral nerves. We provide evidence that the bands are caused exclusively by an axonal wave, and propose a model where band patterning may be used to predict the length of axons within nerves. Adult Wistar rats and C57BL/6 mice were euthanised in accordance with institute guidelines and relevant legislation (directive 2010/63/EU). Sciatic and phrenic nerves were dissected from the animals, fixed and stained with the fluorescent lipophilic dye DiO to highlight the myelinated axons of the nerve using confocal microscopy. The confocal images of nerves were then compared to images of the nerves under oblique illumination that revealed the bands of Fontana. Linear regression analysis showed the number of axonal sine waves in mouse sciatic nerves correlated precisely with the number of bands visible (r²=1; slope=1; n=4), and the bands aligned with the axons, irrespective of the direction of illumination. Band intervals closely correlated with wavelengths in rat and mouse sciatic and phrenic nerves. (1) slope=0.97; r²=0.99; p=0.0001; n=16). The nonlinear course of axons results in axon length which is longer than nerve length, the magnitude of which can be expressed as an axon/ nerve length ratio (ANLR). Axon wavelength also correlated with ANLR (1/Slope=-0.002; r² = 0.48; p=0.0007; n = 20). The ANLR for sciatic nerves (mouse: 1.07±0.01; rat: 1.09±0.02, n=5; Mean±SD) was lower than for phrenic nerves (mouse: 1.18±0.02; rat: 1.16±0.03, n=5; Mean±SD). While the physiological process that regulates ANLR is unknown, our observation that the differences between nerves are greater than the differences between species suggests ANLR is a functional property, rather than simply related to scale. ANLR correlates with axon wavelength, and wavelength correlates with band interval, suggesting that simple inspection of nerve striations may allow calculation of the true axonal lengths in peripheral nerves, relevant for study of peripheral nerve conduction velocity in particular.

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Neuroprotective effects of egg white hydrolysate on recognition memory impairments associated to low mercury concentration chronic exposure

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Mercury (Hg) is a known environmental neurotoxicant associated to oxidative damage on the Central Nervous System (CNS) and memory injury (Mello-Carpes et al. 2013). In this context, egg white protein hydrolysates (EWH) have demonstrated to possess antioxidant action and beneficial effects for human health (Garcés-Rimón et al. 2016) Objectives: The aim of the study was to investigate if the dietetic supplementation with EWH acts as a neuroprotective agent on the recognition memory disorders associated with long-term Hg exposure in rats. All experiments were approved by the Ethical Commission for the Use of Animals of Universidade Federal do Pampa, Brazil (0052014). 8-week-old male Wistar rats were treated for 60 days with: a) Untreated - saline solution (i.m.); b) Mercury - HgCl2 (1st dose 4.6 µg/kg, subsequent doses 0.07 µg/kg/day, i.m – Wiggers et al. 2008); c) Hydrolysate - EWH (1g/kg/day, gavage - Life Sci. 78:2960, 2006); d) Hydrolysate-Mercury. Object recognition memory test (OR) was performed to verify Short (STM) and Long-Term Memory (LTM) and Open field (OF), plus maze (PM) and tail flick (TF) tests were performed as control for behavioral experiments. Reactive Oxygen Species (ROS) in hippocampus were determined by dichlorofluorescein diacetate (DCFH-DA) method, malondialdehyde (MDA) levels by TBARS, antioxidant power by FRAP assay and total Hg concentration by atomic fluorescence spectrometry. Histological studies in hippocampus were carried out in formaldehyde fixed sections. We confirmed that the STM and LTM were impaired in adult rats exposed to Hg at low concentrations and proved that this damage is related to increased metal deposition and subsequent ROS production and apoptosis in hippocampus. In addition, we demonstrated for the first time that EWH treatment is able to prevent memory impairment induced by Hg exposure reducing Hg content, ROS production and cell death in hippocampus (STM - Untreated: 66.96±3.14; Mercury: 55.29±8.35*; Hydrolysate: 65.74±5.27; Hydrolysate-Mercury: 73.55±3.78*; LTM - Untreated: 69.31±4.91; Mercury: 53.80±7.15*; Hydrolysate: 71.81±6.42; Hydrolysate-Mercury: 61.47±1.07*, n=8; One-Way ANOVA, *p<0.05 vs Untreated and #p<0.05 vs Mercury). EWH exert potent neuroprotective effects on memory impairments induced by chronic exposure to low doses of Hg. These findings may represent a good public health strategy since they indicate that EWH is a promising candidate as a new natural therapy for heavy metals intoxication.


This research was supported by a Brazilian Government (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq – 203440/2014-5) and a Spanish Government (MINECO – AGL2012-32387; CSIC – Intramural 201570028).

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Participation of the purinergic receptor P2X7 in modulation of rod- and cone-mediated electroretinographic responses

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The purinergic receptor P2X7 (P2X7R) is widely distributed in both neurons and glial cells of the retina. Its contribution to the electroretinographic (ERG) a- and b-waves has been proved by the results of application of the relatively selective agonist BzATP. The aim of our study was to clarify the P2X7R-mediated effects of the endogenous ATP on the ERG responses. Our interest was focused mostly on the comparison of the P2X7R-mediated effects on the ON (b-wave) and OFF (d-wave) responses as well as on the rod-driven and cone-driven responses. For this purpose, we studied the effects of the selective P2X7R antagonist A438079 on the frog (Rana ridibunda) ERG responses to stimuli of long (5 s) duration allowing for separation of ON and OFF responses. White diffuse light stimuli, varying within an intensity (I) range of 11 log units, were presented in the dark or under a rod-saturating background. Pure rod-driven responses were obtained by using very dim white stimuli below the cone threshold. Dark red stimuli (> 670 nm) were applied in order to isolate pure cone-driven responses in the dark. The antagonist A438079 was applied in 200 µM concentration by perfusion of frog eye cup preparations. The eyecups were prepared after anesthetizing the frogs with 500 mg/l tricaine methanesulfonate in the bathing water, followed by decapitation and pithing (AVMA Guidelines for the Euthanasia of Animals: 2013). Standard ERG recording (0.1-1000 Hz bandwidth) was made. When a single photoreceptor type was stimulated in the dark, the amplitude of both ON (b-wave) and OFF (d-wave) responses was diminished by A438079. (RM-ANOVA; p<0.01, N=14 for rod responses; p<0.01 (b-wave), p<0.001 (d-wave), N=7 for cone responses). The effect was maximal at the lowest I and gradually diminished with increasing I. Different result was obtained when stimuli, activating both rods and cones, were applied. In the dark, mesopic stimulation produced enhancement, rather than diminution, of the b-wave amplitude (RM-ANOVA; p<0.001, N=14). In the same I range, the effect on the d-wave did not change its sign (the d-wave enhancement was diminished). Similar result was obtained under photopic background, when low-intensity cone stimulation was presented on the background of maximal, although not dynamic, rod response. The b-wave amplitude was increased (RM-ANOVA; p<0.01, N=12), while that of the d-wave was decreased (p<0.001, N=12), although the d-wave diminution was less than that obtained in the dark (p<0.01). The effect
on the a-wave was always a small diminution of its amplitude, mostly in the lower I part of the dynamic range. Our results show that the endogenous ATP, through its P2X7R, potentiates the pure rod- and pure cone-driven responses to weak light stimuli. They also demonstrate that P2X7R is involved in rod-cone signal interaction, most likely at a postreceptorial level.

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**PCB283**

**Characterisation of endogenous lipid ligands that activate the G2A receptor and modulate intracellular calcium signalling**

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The G2A receptor (GPR132) is an orphan G-Protein Coupled Receptor (GPCR) from the “pH sensitive family” of GPCRs. It is expressed in both cells of the innate and adaptive immune system, and has a role in both inflammation [1,2]. G2A is observed to be activated by oxidised fatty acids in cellular expression systems, most notably 9-Hydroxyoctadecadienoic acid (9-HODE) [3]. It is unknown if the G2A-mediated actions of 9-HODE are through direct binding to G2A, or if 9-HODE exerts an indirect action on G2A by binding to other proteins expressed within these cellular systems. In addition, effects of G2A activation on intracellular calcium signalling have only been characterised using a CHO cell expression system. Thus it is important to establish whether this is a consistent downstream signalling pathway for G2A in other cell types. Initially G2A activation was studied using a yeast assay. Unlike mammalian cell lines, yeast can be modified to express only G2A and no other receptor at the cell surface. Activation of G2A is coupled to yeast growth to give a readout for direct G2A activation. The DiscoverX PathHunter assay system gives a direct readout for receptor activation using enzyme complementation upon association of beta-arrestin 2 with G2A. Effects on intracellular calcium signalling were evaluated in rat basophilic leukemia (RBL) cells stably expressing G2A and in control RBL cells using single cell, fura-2 microfluorimetry. We show that 9-HODE over the concentration range 1-10 uM activates the human and rat orthologues of the G2A receptor using the yeast (n=4) and beta arrestin (n=4) assays. In addition, 9-HODE (10 uM) activates calcium transients in G2A-RBL cells (n=30 cells from 3 experiments), but not control RBL cells (n=30 cells from 3 experiments). These data suggest that 9-HODE binds directly to the G2A receptor, where it can influence intracellular calcium signalling, which might contribute to its functional activity in the immune system.


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**PCB284**

**60-day AlCl₃ exposure at human dietary levels reaches a threshold necessary to promote memory impairment in rats**

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Aluminum (Al) is a significant environmental contaminant nowadays. While a good deal of research has been conducted on the acute neurotoxic effects of Al, little is known about the effects of longer-term exposure at human dietary Al levels (Bondy 2015). The purpose of this study was to investigate the effects of 60-day Al exposure at low doses for comparison with a model of exposure known to produce neurotoxicity in rats. Three-month-old male Wistar rats were divided into two major groups: 1) Low aluminum levels, and 2) High aluminum level. At group 1 rats were treated orally for 60 days as follows: a) Control – ultrapure drinking water; b) Aluminum 1.5 mg/kg b.w. (Walton 2007) and, c) Aluminum 8.3 mg/kg b.w. At group 2 rats were treated for 42 days as follows: a) Control – received ultrapure water through oral gavages; b) Aluminum 100 mg/kg b.w., protocol known as able to promote cognitive impairment in rats (Prakash and Kumar 2009).

We analyzed cognitive parameters, biomarkers of oxidative stress and acetylcholinesterase (AchE) activity in hippocampus and prefrontal cortex. Results were expressed as mean and SEM, compared by t-test and ANOVA followed by Bonferroni test (*P< 0.05). Ethics Committee Approval 028/2014 - Unipampa. To investigate the effect of AlCl₃ on object recognition long-term memory (LTM) consolidation, rats were trained in the object recognition learning task where all rats explore the two new objects for a similar percentage of the total exploration time. After the end of the treatment periods and 24 h after training, one of the objects was randomly exchanged for a novel one object, and the rats were reintroduced into the apparatus to freely explore the objects (familiar and new ones). Control rats explored the novel object significantly more than 50% of the total exploration time (P= 0.0028 for control of group 1, P= 0.0001 for control of group 2). However, in all the Al-treated groups, animals spent about 50% of the total exploration time exploring each object, without differences between the time spent for exploring the familiar and the novel object (P= 0.55 for Al 1.5 mg/kg and P= 0.76 for Al 8.3 mg/kg of group 1; P= 0.69 for Al 100 mg/kg of group 2). Al increased hippocampal reactive oxygen species and lipid peroxidation, reduced antioxidant capacity and decreased AchE activity. Our data demonstrates that 60-day chronic exposure to low doses of AlCl₃, which reflect common human dietary Al intake, reaches a threshold sufficient to promote memory impairment and neurotoxicity. The elevation of oxidative stress and cholinergic dysfunction highlight pathways of toxic actions for this metal.

Marked gastrointestinal dysfunction and microbiome composition alterations in a mouse model of autism

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Introduction Autism spectrum disorders (ASD) are characterized by deficient social interaction. Moreover, epidemiological data indicate that children and adults with ASD display marked gastrointestinal (GI) comorbidities and some evidence of altered microbial composition in the GI tract. Intriguingly, preclinical research has demonstrated that interventions to the gut microbiome can reverse both autism-like behaviour symptoms and GI abnormalities in animals. In conjunction, these findings suggest that disconnection within the microbiome-gut-brain axis can contribute to the manifestation of ASD. The BTBR T+tfpr31J mouse, a well-known animal model of ASD, exhibits a robust deficit in sociability. However, there is limited information on the gut-brain signalling in this model of ASD.

Methods BTBR and C57BL/6 adult male mice (n=10) were subjected to a battery of behavioural tests. To estimate changes in the gut motility, we analysed intestinal transit time with Carmin Red dye in vivo, as well as faecal water contents and colon length. To examine intestinal permeability, 4kDa FITC-dextran flux was analysed in distal ileum segments in Ussing chambers ex vivo. Ileum and colonic tissues were collected to measure tight junction proteins and serotonin-associated gene expression levels (qPCR), as well as serotonin contents (HPLC). Caecal microbiota composition was analysed by Illumina MiSeq. Data (mean±SEM) were compared by unpaired t-test; a p value <0.05 was deemed significant in all cases.

Results BTBR mice showed more stereotyped behaviours in the marble burying and grooming tests, as well as reduced sociability in the 3-chamber and resident-intruder tests. On the GI side, BTBR mice demonstrated a significant delay in whole intestinal transit time (262±4 vs 238±4min), increased colon length (86±1 vs 70±1mm), and decreased faecal water content (47±1 vs 52±2%). These indicative of a deficit in intestinal propulsive activity were accompanied by altered expression of genes involved in gut serotonin turnover. The analysis of FITC mucusal-to-serosal flux in ileum tissue revealed dramatic increase in the epithelial permeability (14.0±2.2 vs 5.5±1.8ug FITC/cm²) in BTBR animals. BTBRs showed a significant decrease in microbiota diversity and Firmicutes/Bacteroidetes ratio. On-going work is aimed to explore the associations between changes in gut and brain function and alterations in the gut microbial consortium.

Conclusion Here we demonstrate that in BTBR mice autistic-like behaviours are associated with significant GI malfunction, which compromises such keystones of gut health as the integrity of intestinal barrier function and peristalsis. Our findings support the implication of the gut-brain axis paradigm in autistic disorders. The study is supported by Science Foundation Ireland (SFI) through the Irish Government’s National Development Plan (12/RC/2273).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB286

An investigation into the role of the putative cannabinoid receptor GPR55 in rat cortical neurons; relevance to Alzheimer’s disease

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Alzheimer’s disease (AD) is a neurodegenerative disease associated with neuronal loss and cognitive decline. A neuropathological feature of AD is the deposition of ß-amyloid (A β) which is proposed to contribute to neuroinflammation and neuronal cell death. A therapeutic approach that can halt the actions of A β is a potential strategy to impede disease progression. The orphan G-protein coupled receptor GPR55 is responsive to cannabinoids (Brown & Wise, 2002) and is widely expressed in the neurons and glia of the brain (Henstridge et al. 2011; Pietr et al. 2009). The suggested endogenous ligand for GPR55, L-α-lysocephatidylinositol (LPI), modulates inflammatory responses (Balenga et al. 2011). This evidence suggests that GPR55 may have a regulatory role in neuroinflammation. The present study aims to examine the role of GPR55 and its signalling pathways in the regulation of neuroinflammation and neuronal cell death using an in vitro model of AD.

Cultured primary rat cortical neurons were treated with LPI (1 μM-10 μM). LPI-induced signalling effects were assessed using phospho-cAMP element binding protein (pCREB) immunocytochemistry and confocal microscopy and Fura-2 imaging of intracellular calcium responses. Cortical neurons were also treated with LPI (1 μM) in the presence or absence of AG09 (10 μM) for 72 hours. The conditioned neuronal medium was applied to the BV2 microglial cell line and migration of BV2 cells was assessed using a Boyden chamber assay. Neuronal apoptosis was assessed by caspase-3 immunocytochemistry and terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL).

LPI (10 μM) induced a significant 4-fold increase in CREB phosphorylation after 15 minutes of treatment (p<0.01 vs control, ANOVA and Student Newman-Keuls, n=30 cells measured from 3 independent cultures). LPI (10 μM) induced calcium responses (all results herein are presented as mean±SEM; 0.12±0.05 ratio units, n=30 cells measured from 3 independent cultures).
Are P2X3 receptors in the carotid body (CB) a viable target for controlling blood pressure in hypertension? A translational study

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Increase in peripheral chemoreceptor reflex sensitivity and sympathetic nerve activity (SNA) are associated with the development and maintenance of hypertension. Previously, we have shown that denervation of the carotid body (CB) in spontaneously hypertensive (SH) rats reduces arterial pressure (McBryde et al. 2013). Since ATP is a known mediator of signalling in the human CB (Kahlin et al., 2014) and P2X3 receptors upregulate expression during cardiovascular ageing (Ford et al. 2015), we hypothesized that P2X3 receptor upregulation in the CB contributes to increases in arterial pressure. We have validated the expression of P2X3 receptors in the CB of humans and SH rats and initiated functional studies to assess whether a highly selective non-competitive P2X3 receptor antagonist (AF-219) would reduce chemoreflex hypersensitivity, arterial pressure and sympathetic activity in the SH rat. CBs from twelve human cadavers and 12 rats were used for analysis. In human and rat CB, western blotting and immunohistochemistry were performed for the level of expression and localization of P2X3 receptor protein, respectively. Hypertensive and normotensive rats were implanted with radio-telemetry devices to record arterial pressure (AP) and renal SNA (RSNA) in conscious animals. Animals were infused with AF-219 (8 mg/kg/h) or vehicle i.v. for 1h. The CB was stimulated with sodium cyanide (120 µg/kg i.v.). CBs from both human and rats expressed P2X3 receptors. P2X3 receptor protein from the CB was upregulated four fold in SH vs Wistar rat (P<0.05). Glomus cells of human and rat were identified as expressing tyrosine hydroxylase. P2X3 receptor immunofluorescence was detected encapsulating glomus cells. In SH rats, infusion of AF-219 reduced: CB hyperreflexia (P<0.05); AP (systolic -28±3mmHg; P<0.001) and RSNA (-34%; P<0.01). No such changes were detected after vehicle or AF-219 infusion in Wistar rats. In conclusion, P2X3 receptors are present in human CB. In SH rats, P2X3 receptors are upregulated in the CB and contribute to chemoreflex hypersensitivity. Antagonism of P2X3 receptors systemically lowered both AP and RSNA substantially in SH but not Wistar rats. Antagonism of P2X3 receptors in humans as a novel approach for controlling hypertension awaits a clinical trial.

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Distinct functional roles for different types of calcium channels in facilitation at mossy fiber to CA3 pyramidal cell synapses

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Neurotransmitter release from presynaptic terminal is ensured by calcium entering through several types of VGCCs. Although the spatial distribution of distinct VGCCs impacts calcium dynamics and neurotransmitter release, the specific contribution of individual types of VGCCs to neurotransmitter release remains poorly defined. To dissect the roles of P/Q- and N-types VGCCs, we used random-access two-photon calcium imaging, electrophysiology and electron microscopy. Our results show that P/Q- and N-types VGCCs support different calcium dynamics with specialized functions in triggering neurotransmitter release. First, bouton calcium imaging revealed that P/Q-type VGCCs contributed a larger but more spatially homogeneous fraction of calcium than N-type VGCCs. Consistent with a global calcium increase, blockade of P/Q-type VGCCs prevented the recruitment of additional release sites during trains of activity. This effect could be mimicked by ECTA-AM, indicating that P/Q-type VGCCs can be loosely-coupled to the sensor. In contrast, antagonizing N-type VGCCs decreased the overall amplitude of EPSCs but had no effect on short-term facilitation. Altogether, our results demonstrate the highly specialized roles of P/Q- and N-type VGCCs in neurotransmitter release. While N-type VGCCs are tightly-coupled to calcium sensor and provide local calcium elevations, P/Q-type VGCCs are well-suited to support global calcium elevations and promote the recruitment of additional release sites during trains of activity.

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PCB289

Which Biological Relevant Odor Stimuli Elicit Positive Anemotaxis in the House Cricket, Acheta domesticus?

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Insects use their antennae to locate food sources, mates, and oviposition sites. The main olfactory sensory organs or sensilla are housed on the antennae. Odorants pass through small pores in the cuticle where they interact with the underlying dendrites, delaying information to the brain of the insect. House crickets, Acheta domesticus, bear sensilla contained on their long easily accessible antennae that have been identified as possessing an olfactory function via electron microscopic studies, allowing this species to be used as an excellent model to study olfaction. In addition, information is sparse regarding the olfactory system of crickets. In this study, we used a behavioral bioassay using a y-tube olfactometer to test biological-relevant odor stimuli. We were interested in determining which headspace volatiles (those odor compounds present in the air surrounding various plants found in the natural habitat of the cricket) elicited upwind walking behavior (anemotaxis) toward the odorant source. The results of this research will advance our understanding of insect olfaction in this species.


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PCB291
Defect in adenosinergic vasodilatory mechanisms in the brainstem of the spontaneously hypertensive rat
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Abnormalities in adenosinergic regulation of peripheral vascular function have been implicated in the development of hypertension in spontaneously hypertensive rats (SHR) (Illes et al., 1989). Here, we examined the role of adenosine receptors in regulating cerebrovascular reactivity in pre-hypertensive SHR, an animal model with high cerebrovascular resistance (Cates et al., 2011) and brainstem hypoperfusion (Marina et al., 2015).
Changes in the diameter of pial arterioles (20-100 µm) in response to adenosine agonists were assessed after pre-constriction with the thromboxane agonist U46619 in 400-µm-thick horizontal slices from the cortex and ventral brainstem of 4-6 week-old Wistar and SHR. Measurements were made using a Leica confocal (SP) microscope. Values are means ± S.E.M., compared by ANOVA.
U46619 (200 nM) induced a similar vasoconstriction in arterioles from Wistar and SHR. In brainstem slices, adenosine (10 µM) reversed the U46619-induced vasoconstriction in Wistar but not SHR (+41±5% vs. +10±5%, n=6/8, respectively, p<0.05). In contrast, in cortical slices, the vasodilator effect of adenosine was similar between Wistar and SHR (+45±3% and +47±2%, n=11/13, respectively). The selective A2A receptor agonist (CGS-21680, 1 µM) dilated pre-constricted brainstem arterioles from Wistar but not SHR (+31±9% vs. -9±7%, n=6/5, respectively, p<0.01), while its vasodilator effect in the cortex was similar between strains (+38±8% and +33±12%, n=8/8, respectively). Likewise, the vasodilator effect of the A1/A2 agonist S’-N-ethylcarboxamidoadenosine (NECA, 1 µM) tended to be higher in brainstem slices from Wistar than SHR (+27±5% vs. +8±4%, n=7/7, respectively), while its efficiency in cortical slices was very similar between strains (Wistar: +54±11%; SHR: +55±10%, n=8/7, respectively). Pre-treatment with the selective A2A receptor antagonist (ZM-241385, 0.1 µM) fully blocked the vasodilator effects of adenosine, CGS-21680 and NECA. In contrast to A2 receptor-related compounds, the selective A1 receptor agonist N6-Cyclopentyladenosine (1 µM) produced moderate vasoconstriction of a similar magnitude in the brainstem of Wistar and SHR (-17±7% and -24±9%, n=5/6, respectively), but no change in the cortex (+3±17% vs +1±8%, n=6/6, respectively).
These results suggest that the vasodilator efficiency of adenosine A2A receptors is attenuated in the brainstem but not cortical arterioles of pre-hypertensive SHR. This brainstem-specific reduction in A2A receptor sensitivity could contribute to inadequacies of functional hyperaemia and brainstem hypoperfusion in the SHR, which may trigger sympathetic activation and hypertension.

PCB292
Pallidorecticular pathway influence the functional activity of reticular thalamic nuclei
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The reticular thalamic nucleus (RTn) coordinates the overall traffic information to the cerebral cortex through modulates axons from cortico-thalamic (CT) and thalamic-cortical (TC) fibers. There are two spiking modalities of RTn neurons: electrical burst activity (synchronized mode) and electrical tonic activity (desynchronized mode) that occurs during awake state and REM sleep. Furthermore, burst activity synchronizes rhythmic oscillations into CT-TC fibers and its lost has been associated with pathological states such absence epilepsy and schizophrenia. The electrical activity of RTn depends on its afferent fibers and the membrane properties of reticular neurons. According with this, a GABAergic pathway was described from the globus pallidus externus (GP) an important nucleus that integrates and controls the information of basal ganglia. This afferent suggests important participation of GP in functional activity of RTn by influence of spiking activity.
We studied the spontaneous electrical activity of RTn neurons by extracellular unit recording in vivo on male Wistar rats (300-350 g). To determine if the pallidorecticular pathway could modify the spontaneous RTn neurons discharge activity, we either activated it or blocked it by ipsilateral intrapallidal infusion of different concentrations of glutamate or GABA respectively (values are mean ±, compared by Student t-test and one-way ANOVA). Burst activity is reported in burst index (a minimum of two spikes and maximum interspike interval of 10 ms) total events >10 ms) Rats were anesthetized with chloral hydrate (400 mg/kg i.p) and were placed in stereotaxic frame. Supplementary doses of anesthetic (50-70 mg/kg) were used as needed according to the presence of corneal reflex. We found that activation of pallidal neurons modify the spontaneous firing rate of RTn neurons. In a dose-dependent manner, glutamate decreases the spontaneous spiking rate of RTn neurons relative to basal values (30 PMol decreased the spiking rate by 35.13 ± 5.86%, n=11; 300 PMol by 45.82 ± 6.07%, n=10 and 3 nMol by 56.2 ± 6.81, n=13), without significance change in either the bursting index. Additionally, intrapallidal GABA application increases RTn neurons spiking rate in eight neurons tested per concentration (30 PMol increased the spiking rate by 25.96 ± 6.08%, 300 PMol by 64.3 ± 19.57%, 3 nMol by 77.16 ± 19.89%). Burst index remained without changes.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Poster Communications

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Our results suggest that the GP exerts tight control over RTn activity. Asanuma C, Porter L L. Light and electron microscopic evidence for a GABAergic projection from the caudal basal forebrain to the thalamic reticular nucleus in rats. J Comp Neurol 302 (1999):159-72.


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PCB293

Functional Magnetic Resonance Imaging meets Doppler: Contradictory or complimentary measures of cerebrovascular reactivity?

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The responsiveness of the cerebrovasculature to alterations in arterial content of carbon dioxide (PCO2) is a common functional test to assess brain health (i.e., CBF-CO2 responsiveness, also referred to as cerebrovascular reactivity (CVR)). Regular exercise has a positive effect on brain function, and a greater CVR is associated with higher aerobic fitness. Further, an attenuated CVR has been observed in disease populations with impaired brain function (e.g., dementia). The most common and simplest test to assess CVR is the inhalation of a predefined concentration of CO2 via a Douglas bag open circuit (e.g., 5% CO2 in air). This test can be performed and measured using functional magnetic resonance imaging (fMRI), transcranial Doppler (TCD) and/or Duplex Doppler. However, the calculated CVR measure is derived differently between approaches. This study aims to compare CVR measures obtained using these three approaches in active/sedentary and young/old groups where differences in CVR are expected. Ten young healthy volunteers (6 active and 4 sedentary) have been recruited so far, with the aim of recruiting 40 (active/sedentary; young/old). Following full screening and a fitness test, participants completed a gas familiarisation visit. The gas challenge consisted of 4-min room air baseline, followed by two 4-min periods of CO2 inhalation (5% and 7% in air) via the open-circuit Douglas bag method with a 4-min recovery period in-between (room air). Participants attended two experimental sessions on separate days (randomised and counter-balanced). During a session, CVR was either measured using fMRI (DABS sequence to obtain simultaneous blood-oxygen level dependent and perfusion responses) or TCD (measures of middle cerebral artery flow velocity) and Duplex Doppler (measures of vessel diameter and velocity in internal carotid artery). Breath-by-breath measures of end tidal CO2 were obtained in both visits. fMRI and Doppler CVRs were calculated using a linear regression including baseline and both stimulus concentrations and separately for each stimulus concentration (5% and 7% CO2).

Preliminary analysis indicates that there are differences between these CVR measures when comparing between active and sedentary groups; however, initial findings also indicate that there are differences between the neuroimaging methodologies used (e.g., CVR calculated from MRI/TCD/Duplex) as well as variations within the methodologies (e.g., 5% versus 7% CO2 and methods of data extraction). Ongoing data collection and analysis will enable us to better understand these methodological differences. More research is needed to investigate how these measures differ and relate to health and disease, how they vary during healthy ageing, and how they change in response to a clinical intervention targeting improved brain health.

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PCB294

The role of cloroxquine treatment on vasodilation capability of cutaneous microvasculature

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Background. Antiphospholipid syndrome (APS) is defined by the combination of thrombotic events and/or obstetrical morbidity associated with the presence of antiphospholipid antibodies. The pathogenesis of obstetrical APS remains poorly understood, but the role of the complement system and of endothelial cells, platelets, and monocytes seems important in the occurrence of placental thrombosis. Hydroxychloroquine is widely used in patients with various autoimmune diseases, particularly systemic lupus erythematosus. Antiinflammatory properties of cutaneous microvasculature to local heating of APS patients

Ten young healthy volunteers (6 active and 4 sedentary) have been recruited so far, with the aim of recruiting 40 (active/sedentary; young/old). Following full screening and a fitness test, participants completed a gas familiarisation visit. The gas challenge consisted of 4-min room air baseline, followed by two 4-min periods of CO2 inhalation (5% and 7% in air) via the open-circuit Douglas bag method with a 4-min recovery period in-between (room air). Participants attended two experimental sessions on separate days (randomised and counter-balanced). During a session, CVR was either measured using fMRI (DABS sequence to obtain simultaneous blood-oxygen level dependent and perfusion responses) or TCD (measures of middle cerebral artery flow velocity) and Duplex Doppler (measures of vessel diameter and velocity in internal carotid artery). Breath-by-breath measures of end tidal CO2 were obtained in both visits. fMRI and Doppler CVRs were calculated using a linear regression including baseline and both stimulus concentrations and separately for each stimulus concentration (5% and 7% CO2).

Preliminary analysis indicates that there are differences between these CVR measures when comparing between active and sedentary groups; however, initial findings also indicate that there are differences between the neuroimaging methodologies used (e.g., CVR calculated from MRI/TCD/Duplex) as well as variations within the methodologies (e.g., 5% versus 7% CO2 and methods of data extraction). Ongoing data collection and analysis will enable us to better understand these methodological differences. More research is needed to investigate how these measures differ and relate to health and disease, how they vary during healthy ageing, and how they change in response to a clinical intervention targeting improved brain health.

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HIF-1α and its role in normal and eclampsia pregnancy

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Introduction

Placental development is resulted from angiogenesis. It takes place in a hypoxic environment & involved hypoxia inducible factor (HIF). On the other hand, overexpression of HIF1α also correlates with preeclampsia (PE) pathogenesis.

Materials and Methods

We reviewed ScienceDirect, Medline, & Pubmed literatures under the MeSH: “HIF1α” & “Pregnancy”, with limitations on English article. 12 studies were reviewed. The inclusion criteria was trials studies on human. Data & studies were analyzed based on PICOs checklist to create a comprehensive literature review.

Results

Early placental development takes place in hypoxic environment. Cellular response to chronic & acute hypoxia are mediated through HIF protein. HIF1α is the HIF protein that is regulated by oxygen. Under hypoxic condition HIF1α is stable & bind to ARNT (a beta subunit of HIF protein).

Hypoxia is necessary for embryogenesis, especially during the implantation process of trophoblast. During this time, O2 levels is 2-3% & HIF1α (a fusion of HIF1α & ARNT) will activate genes transcription to survive under hypoxic condition, such as angiogenesis, cell survival, cell proliferation, & glycolysis. On the 10-12 weeks, fetus placenta & trophoblast will shift to a normoxic condition with O2 levels is 8%-10%. In this time, there will be expression, formation, & activation of the VHL complex, protein that control & regulate HIF1α. After weeks 12th, HIF1α is no longer needed. Thus, HIF1α will be rapidly degraded by means of ubiquitination & proteosomal degradation.

Unfortunately, in PE, there is an abberant in the process of placentation. The cytotrophoblasts fail to adopt the invasive endothelial phenotype that is necessary for normal physiologic remodeling. Therefore, the spiral arteries becomes abnormal & thus, reduce blood flow to placenta. As a result, there will be hypoxic placenta (HIF1α overexpression) which releases numerous vasoactive factors that cause a decrease in NO & an increase in ROS. Anti-angiogenic factors such as sFlt1 & sENG also present in preeclamptic placenta along with decrease level of VEGF/PIGF. A combination of these two events results in total peripheral resistance & manifests as hypertension.

Regulation of HIF1α in normal & PE pregnancy

HIF1α is necessary in the first trimester, but overexpression of HIF1α after the first trimester is strongly correlated with PE. Regulation of HIF1α may be needed in the future to prevent preeclampsia in woman with high risk.


Conclusion

HIF1α has important roles both in the normal & PE pregnancy. HIF1α is necessary in the first trimester, but overexpression of HIF1α after the first trimester is strongly correlated with PE. Regulation of HIF1α may be needed in the future to prevent preeclampsia in woman with high risk.

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Effects of acute hyperbaric oxygenation on vascular relaxation mechanisms to hypoxia in healthy Sprague-Dawley rats

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Introduction: Our previous study showed that acute exposure to hyperbaric oxygen (HBO2) increase oxidative stress parameter leading to impaired vasorelaxation to acetylcholine (ACh), while it was preserved in 4 days intermittent HBO2 protocols (1). Vasodilation in response to hypoxia is mainly mediated by cyclooxygenase (COX) activation and production of prosta-cyclin (PGI2) (2,3). However, cytochrome P450-epoxigenase metabolites may contribute to vasodilation in healthy vessels (4). It is known that changes in pO2 may affect the synthesis of metabolites of arachidonic acid (5).

The aim of this study was to examine if acute single HBO2 exposure affects the reactivity of isolated rat aortic rings to hypoxia and to investigate if there are changes in vasorelaxation mechanisms due to HBO2.

Poster Communications
Implication of microRNAs 199a3p and 199a5p in vascular function: modulation of the nitric oxide (NO) pathway

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MicroRNAs (miRs) 199a3p and 199a5p have been mainly implicated in proliferation, cell survival and remodeling in context of cancerogenesis but more recent data proposed a role in cardiovascular functions. We seek to assess how miR199a3p and miR199a5p modulate endothelial function by identifying their molecular targets in endothelial cells.

Mice were treated with miRs199a3p/5p inhibitors (antago-miRs, 75mg/kg/day) by injection in the caudal vein and were sacrificed after 30 days. Contractile profile and endothelial function were evaluated ex vivo in the aorta. Cultured endothelial cells (Sigma) were transfected with miRs199a3p/5p inhibitors (locked-nucleic acid (LNA)) or a scramble sequence. NO production, expression and/or activity of endothelial nitric oxide synthase (eNOS) and its major regulators were measured by electroparamagnetic resonance (EPR) and Western blot respectively. Implication of protein kinase B (Akt or PKB) pathway was analysed using LY294002 treatment (20μM) or Akt1 small interfering RNA (siRNA, 50nM). Angiogenesis was investigated in 2D-culture in matrigel support.

Vessels from mice treated with antagonirs against miR199a3p or 5p showed a larger NO-dependent relaxation compared to controls (ANOVA: p<0.001 ctf 37.4±4.2 (n=4) vs antagoniR a3p: 72.6±2.8 (n=5) and antagoniR a5p: 69.0±8.8 (n=5) maximal relaxation). Circulating haemoglobin-NO measured by EPR in venous blood collected from these mice was significantly increased compared to controls (ANOVA, Tukey-Kramer post-test: p<0.05, ctf 100±17% (n=4) vs antagoniR a3p: 339±59% (n=5) and antagoniR a5p: 240±41.2% (n=5)) suggesting a role of both miRs in the control of the NO/S NOS pathway. Repression of miR 199a3p/5p improved eNOS activity through an increased eNOS phosphorylation at serine 1177 (N=6) and a decreased phosphorylation status of Akt (N=5). The eNOS allosteric regulator, caveolin-1, was not modulated by LNA treatments (N=3). Both treatments promoted calcineurin expression (N=4) and Akt activation (N=5). Furthermore, addition of LY294002, a Akt inhibitor (N=3), or specific knockdown of Akt1 by siRNA (N=3), inhibited the increase of eNOS phosphorylation at serine 1177 induced by LNA treatment. Interestingly, repression of miR199a5p promoted superoxide dismutase (SOD) expression (N=3), suggesting that miR199a5p also improved NO bioavailability. In addition, inhibition of miR199a5p upregulated vascular endothelial growth factor (VEGF) production and promoted tubes formation in bovine endothelial cells (N=3).

Our results demonstrate that miRs199a3p/5p modulate the NO/S pathway in the endothelium by promoting NO production and repressing NO degradation. These results also suggest a strong implication of the Akt pathway in this modulation. Interestingly, miR199a5p shows additional control of angiogenesis.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Endothelium-dependent and endothelium-independent vasodilation of skin microcirculation during high salt loading in young healthy women

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Introduction: Recent studies have shown that endothelial dysfunction is an early manifestation of adverse effect of high salt (HS) loading (1). In our previous study we have demonstrated that one week of HS diet significantly impaired skin microvascular reactivity in young healthy women independently of changes in blood pressure (BP) (2). The present study was designed to determine if one week of HS diet affects endothelium-dependent and/or endothelium-independent vasodilation of skin microcirculation in young healthy women.

Methods: Twenty four young healthy women were assigned to a 7-days low salt (LS) diet (<3.5 g NaCl/day) followed by a 7-days HS diet (~14 g NaCl/day). Skin microvascular blood flow was measured by Laser Doppler Flowmetry in response to iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP) before and after LS and HS diet protocols. BP, heart rate (HR), 24h-urine sodium, potassium, urea and creatinine levels were measured before and after diet protocols. Salt intake was estimated based on calculation of 24-hour urinary sodium excretion. The study protocol and procedures conformed to the standards set by the latest revision of the Declaration of Helsinki and the Ethical Committee of Faculty of Medicine University of Osijek approved them.

Results: Changes in 24h urinary sodium excretion and calculated salt intake confirmed that subjects conformed to the HS diet (13±5.4, P<0.001). Systolic blood pressure (SBP) was unchanged after HS diet, but significantly reduced after LS diet (SBP, mmHg: baseline 114±8, LS diet 106±7, HS diet 108±9, P<0.001). HR was not significantly changed after both diet protocols (HR, per minute: baseline 75±12, LS diet 71±5, HS diet 73±6, P=0.377). ACh induced dilation (endothelium-dependent) was unchanged after LS diet, but significantly impaired after HS diet protocol (flow change compared to basic flow: baseline 16.80±7.71, LS diet 17.96±6.23, HS diet 13.08±7.5, P=0.012 baseline and LS diet vs. HS diet). SNP induced dilation (endothelium-independent) was not significantly changed after both LS and HS diet protocols (flow change compared to basic flow: baseline 18.95±7.77, LS diet 19.38±7.49, HS diet 18.98±6.96, P=0.939).

Conclusions: This study confirmed our previous findings that one week of HS loading significantly affects skin microvascular reactivity without changes in BP levels in young healthy women. Furthermore, since one week HS diet significantly impaired ACh, but not SNP-induced dilation, these data suggest that short-term HS intake affects microvascular function in young healthy women through its direct adverse impact on endothelium function.


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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Influence of high salt diet on the interrelation of HIF-1α and antioxidative genes in microcirculation of Sprague-Dawley rats


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Our recent data showed that mRNA expression of hypoxia inducible factor 1 alpha (HIF-1α) in brain blood vessels (BBV) depends on the level of superoxide and suggested that COX-2 can be interrelated with HIF-1α (1). This study aimed to assess the effects of HS and a superoxide scavenger TEMPO (T) on expression of antioxidative genes (Cu/ZnSOD, MnSOD, EC-SOD, catalase (CAT) and glutathione-peroxidase 1 and 4 (GPx1 and GPx4)) in regard to HIF-1α expression and their interrelation to each others in microcirculation.

Four groups of 11-weeks old healthy male Sprague-Dawley rats were included in the study: low salt group (LS); standard rat chow (0.4% NaCl) and HS group (4% NaCl in chow) for 1 week with or without TEMPOL in vivo (drinking water, 1nM/L) (LS+T and HS+T). Following diet protocol, rats were anesthetized with ketamine (75 mg/kg) and midazolam (2.5 mg/kg) decapitated and their BBV were harvested. mRNA levels were determined by real-time qPCR. Data were analyzed by One-way ANOVA (SigmaPlot 11.0) and MANOVA test (IBM SPSS Statistics 20). p<0.05 was considered significant. All experimental procedures conformed to the European Guidelines (Directive 86/609) and were approved by the local Ethical Committee (Class:602-04/14-08/06, #2158-61-07-14-04). Expression of MnSOD and CAT was significantly increased in LS+T group compared to other groups (p<0.05). Cu/ZnSOD, EC-SOD and GPx4 expression was decreased in HS and LS groups compared to LS+T group and Cu/ZnSOD, EC-SOD, GPx4 expression were decreased in HS group compared to HS+T and to LS+T (p<0.001). EC-SOD expression was decreased in LS group compared to HS+T group (p<0.005). GPx1 expression increased in LS and HS compared to HS+T (p<0.001, p=0.002 respectively). Expression of HIF-1α was significantly increased in both T groups compared to both, LS and HS groups (LS vs. LS+T p=0.005; LS vs. HS+T p=0.042; HS vs. LS+T p=0.001; HS vs. HS+T p=0.003).

Correlations of HIF-1α with antioxidative genes were found only in HS and HS+T groups. In HS group, HIF-1α positively correlated with MnsSOD (r=0.935, p=0.002) and GPx1 (r=0.834, p=0.02) and in HS+T group with CAT (r=0.925, p=0.024).

Positive correlation of HIF-1α gene expression with the antioxidative enzymes in HS groups suggests that a longer period of NaCl intake could significantly modify its expression. The expression of HIF-1α gene depends largely on expression of MnSOD (2). Since the expression of MnSOD did not significantly change compared in HS diet to LS diet, it is possible
that this is additional reason for unchanged HIF-1α expression. Increased expression of HIF-1α in TEMPO, together with increased MnSOD expression suggest that HIF-1α expression depends on other antioxidant genes’ expression and the level of oxidative stress.


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PCB300

Characterisation of cyclic nucleotide mediated platelet inhibition

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Platelets are important mediators of haemostasis and thrombosis. Current research on antiplatelet therapy gives little attention to the role of the atypical endothelium in atherothrombosis and there is a demand for treatments that reduce thrombosis but concurrently preserve haemostasis. RhoA is a member of the Rho Family of small G proteins that act as the bridge between extracellular signals and the swift cytoskeletal restructuring of platelets leading to spreading, aggregation and thrombus formation. Vascular endothelial cells release prostacyclin (PGI2) and nitric oxide (NO) to maintain platelets in a resting state. PGI2 and NO increase intracellular levels of cAMP and cGMP causing cyclic nucleotide dependent kinase activation (PKA/G) which phosphorylate a substantial group of substrate proteins leading to functional modifications. In platelets this RhoA activity has previously been shown to be inhibited by cyclic nucleotide signalling which potentially has significant ramifications in the capacity of cyclic nucleotides to inhibit platelet activation. Through bioinformatic screening of RhoA regulatory proteins we have identified Rho GTPase activating protein 6 (RhoGAP6 - terminates RhoA signalling) and Rho guanine nucleotide exchange factor 2 (RhoGEF2 - activates RhoA) as potential new PKA/G targets. We have shown that RhoGAP6 and RhoGEF2 are expressed in platelets and have confirmed their activity towards RhoA in transfected cells using pull down assays. RhoGEF2 is phosphorylated on serine 886 by PKA/G in platelets. This phosphorylation may be linked to binding of RhoGEF2 with the 14-3-3 adaptor protein. RhoGAP6 phosphorylation sites are currently being investigated with functional studies on these sites to be conducted. The discovery of new platelet proteins RhoGAP6 and RhoGEF2 and PKA/G phosphorylation targets in platelets provides a deeper insight into the molecular mechanisms of platelet regulation which might aid in the development of new diagnostic and/or therapeutic options in thrombotic vascular disease.

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PCB301

Biogenesis, localization and mobilization of two abundant platelet thiol isomerases

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Background: Thiol isomerases facilitate the rearrangement of protein disulphide bonds required for the correct folding of secreted proteins. They typically localize in the endoplasmic reticulum but some cells have thiol isomerases in granular structures or on the cell surface. Thiol isomerases are key regulators of platelet activation and blood coagulation and this requires that they are exposed on the surface of activated platelets. However, understanding the origin and localization of platelet thiol isomerases and the mechanisms underlying their release to the platelet surface is necessary to further elucidate their role in thrombosis and haemostasis.

Aim: we explored the biogenesis and trafficking of the thiol isomerases PDI and ERP57 in megakaryocytes, identified the compartment where they reside in platelets and characterized the cellular events responsible for their movement to the platelet surface.

Methods and Results: Using immunofluorescence microscopy we observed the distribution of thiol isomerases throughout mouse and human megakaryocyte development. We determined that the biogenesis of thiol isomerases is distinct from that of cargo proteins destined for the platelet granules, that are trafficked through the trans-Golgi defined by TGN46 (red) or recycling endosomes containing the transferrin receptor (TFR). Immunofluorescence microscopy and subcellular fractionation of platelets revealed that thiol isomerases are mainly present in the inner cell-surface membrane region defined by sarco/endoplasmic reticulum calcium ATPase 3 (SERCA3) and their distribution does not change in the absence of platelet α-granules (Nbeal2-/- mouse platelets or platelets of patients affected by Gray Platelet Syndrome). Thiol isomerases were secreted to the surface of activated platelets, as measured by flow cytometry and confocal microscopy, and their movement was prevented by Latrunculin A that blocks the polymerization of actin (46±8 % and 77±12% inhibition, for PDI and ERP57, respectively), but not by the absence of membrane fusion mediated by the soluble N-ethylmaleimide-sensitive fusion protein attachment receptor/Munc13-4 (absent in platelets from Unc13d−/− mice).

Conclusions: PDI and ERP57 are synthesized and packaged into a compartment that is distinct from known granules and localize to the inner surface of the platelet outer membrane identified by SERCA 3. The movement of thiol isomerases on the surface of activated platelets is driven by the polymerization of actin, but does not depend on the vesicular-membrane fusion mediated by Munc13-4.

Defining the origin, trafficking and mechanism of secretion of platelet thiol isomerases may aid the development of selective inhibitors that could represent new antithrombotic strategies.
AMPK-activated protein kinase α1 controls cytosolic calcium concentration and induces calcium sensitization in resistance arteries

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AMP-activated protein kinase (AMPK) is an important sensor and regulator of cellular energy state activated by physiological or pathological stimuli. Our previous work has shown that in large conductance vessels, AMPK also regulates vascular contractility through phosphorylation and inhibition of smooth muscle myosin light chain kinase. Its implication in the control of vascular tone in resistance arteries has never been studied and constitutes the aim of the present work.

Vascular tone was assessed ex vivo by pressure myography in small mesenteric resistance arteries (SMRA) and cytosolic calcium concentration measured using FURA2. The role of AMPK was assessed by the use of mice genetically deficient for the α1 isoform of the protein (AMPK-KO) and their littermate controls (AMPK-WT). Results are expressed as means ± S.E.M. SMRA of AMPK-KO mice showed increased cytosolic calcium concentrations at basal state (t-test; 104.2 ± 12.65 vs 60.3 ± 16.5nM in AMPK-WT, p=0.016, n=6/8) and after contraction induced by depolarization (t-test; 183.3 ± 14.2 vs 104.3 ± 16.5nM in AMPK-WT, p=0.005, n=6/8) or with phenylephrine 1μM (t-test; 241.5 ± 27.2 vs 104.2 ± 16.5nM in AMPK-WT, p=0.001, n=5/7). Contractile tone was modified neither by depolarization (t-test; 29.6 ± 7.9 vs 33.4 ± 3.7% AMPK-WT, p=0.631, n=5/9), nor by the use of 1μM of phenylephrine (t-test; 17.0 ± 2.3 vs 17.4 ± 1.4% AMPK-WT, p=0.833, n=5/8). In consequence contraction/calcium ratios were significantly reduced in the absence of AMPK, both in response to 1μM of phenylephrine (t-test; 0.3 ± 0.2 vs 1.8 ± 1.1μm/nM in AMPK-WT, p=0.03, n=5/6) and to depolarization (t-test; 0.9 ± 0.3 vs 2.3 ± 0.4μm/nM AMPK-WT, p=0.02, n=6/8).

Thus, our data first demonstrate that AMPK is involved in the regulation of basal cytosolic calcium concentration in SMRA. Whether this relates to an increased calcium intracellular uptake by SERCA pump (through phosphorylation of phospholamban) is currently investigated. The discovery that AMPK induces a calcium sensitisation in SMRA shows for the first time that this protein could differentially modulate vascular tone in conductance vessels.

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Pre-eclamptic toxemia (PET) is characterised by maternal hypertension and proteinuria due to widespread endothelial dysfunction (1). PET plasma induces endothelial junctional breakdown and permeability modulation (2). Altered circulating vascular endothelial growth factors (VEGF) placental growth factor (PIGF), VEGF<sub>165α</sub> and VEGF<sub>165β</sub> may contribute to endothelial dysfunction (3). Flt-1 receptor coated ELISA plates were co-incubated with biotinylated-VEGF<sub>165β</sub> and PIGF or non-biotinylated-VEGF<sub>165β</sub>. Percentage optical density (OD) was recorded. 0.4µm pore polyester membrane transwells seeded with human umbilical vein endothelial cell (HUVEC) monolayer were exposed to FITC-BSA (1mg/mL) tracer and incubated with VEGF<sub>165α</sub>, followed by VEGF<sub>165β</sub> and PIGF for 2 hrs. Membranes were fixed and probed for VE-cadherin junctions. Tracer samples were collected over time and OD recorded. VE-cadherin junctions were visualised via fluorescence microscopy and quantified by percentage junction gap, thinning junctions, or thickening junctions. Values are expressed as mean ± SEM, compared by ANOVA. Co-incubation with non-biotinylated-VEGF<sub>165β</sub> and PIGF decreased biotinylated-VEGF<sub>165β</sub> to Flt-1 binding (p<0.0001, n=4). PIGF co-incubation further decreased biotinylated-VEGF<sub>165β</sub> binding, resulting in an IC<sub>50</sub> of 18.37nM (p<0.0001, n=4). VEGF<sub>165α</sub> increased FITC-BSA OD after 2 hrs (1.85 ± 0.23OD) (p=0.0001, n=4), but not VEGF<sub>165β</sub> (0.77 ± 0.09OD) (p=0.05, n=4) or PIGF (0.93 ± 0.25OD) (p=0.05, n=2) vs. vehicle (0.77 ± 0.06OD). VEGF<sub>165α</sub> inhibited VEGF<sub>165β</sub>-induced permeability (0.99 ± 0.15OD) (p<0.01, n=3), but not PIGF (1.72 ± 0.18OD) (p=0.05, n=3). PIGF co-incubated with VEGF<sub>165β</sub> abolished VEGF<sub>165β</sub>-induced permeability rescue (2.07 ± 0.15OD) (p=0.05, n=2). VE-cadherin expression after 2 hours revealed increased gaps and decreased thin junction percentage after VEGF<sub>165α</sub> (Gaps: 49.14 ± 6.2%Thick: 20.95 ± 1.57%)(p<0.01, n=4), but not VEGF<sub>165β</sub> (Gaps: 26.52 ± 2.96%Thick: 40.30 ± 5.79%)(p=0.05, n=4) or PIGF (Gaps: 28.05 ± 3.42%Thick: 49.11 ± 0.24%)(p=0.05, n=2) compared to vehicle (Gaps: 23.50 ± 2.93%Thick: 49.73 ± 4.57%). VEGF<sub>165α</sub> co-incubation with VEGF<sub>165β</sub> decreased gap and increased thin junction percentage (Gaps: 17.43 ± 0.52%Thick: 41.38 ± 2.77%)(p=0.001, n=4), but not with PIGF co-incubation (Gaps: 62.68 ± 5.30%Thick: 16.60 ± 0.44%)(p=0.05, n=2) compared to VEGF<sub>165α</sub> alone. Co-incubation with VEGF<sub>165α</sub>, VEGF<sub>165β</sub> and PIGF (Gaps: 55.87 ± 0.35Thick: 20.41 ± 0.44) showed no significant difference in gap or thin junction percentage compared to VEGF<sub>165α</sub> alone (p=0.05, n=2). These observations indicate VEGF interplay resulting in endothelial permeability and junctional modulation, suggesting a possible role for circulating VEGF alterations in pre-eclampsia.


This project was funded by the BHF. Many thanks to all members of the Tumour and Vascular Biology Labs, QMC, Nottingham

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PCB305

Shear stress prevents oxidative stress induced loss of the human microvascular endothelial cell glycocalyx

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The glycocalyx is crucial for normal endothelial function and microvascular responses to intraluminal blood flow. The glycocalyx tethers and concentrates the extracellular superoxide dismutase (ecSOD) which scavenge reactive oxygen species (ROS). We have previously found that ROS activated matrix metalloproteinases (MMPs) enzymes resulting in glycocalyx shedding in endothelium. We sought to test the hypothesis that shear stress protects against ROS induced reduction in glycocalyx by reducing MMP expression and restoring glycocalyx structure in human microvascular endothelium. Human adipose microvascular endothelial cells (HAMECs) were cultured in a custom built flow chamber designed for a wide surface area and subjected to no (NSS), low (LSS: 5 dynes/cm²), high (HSS: 20 dynes/cm²) shear stress for 8 hours. Experiments were performed in the presence and absence of exogenous [hydrogen peroxide (H₂O₂, 2X10⁻⁴ mol/L) and endogenous ROS [buthionine sulfoximine (BSO; 10⁻³ mol/L)] following shear stress treatment (n=5). H₂O₂ and BSO reduced the glycocalyx of HAMECs which was measured by fluorescence detection of the cell surface density of heparan sulfate glycosaminoglycans with fluorescently labeled wheat germ agglutinin WGA (65.7% reduction in intensity; n=5). Both H₂O₂ and BSO increased the mRNA and protein expression of MMPs (2-3 fold; MMP1, MMP2 and MMP9) and ADAMS (3-5 fold; ADAM10 and ADAM17). In addition, ROS generation reduced the protein levels of tissue inhibitor of matrix metalloproteinases (TIMP) -1 and -3. There was a reduction in protein levels of syndecan-1 and ecSOD in the total cell lysate and increased in levels of syndecan-1 ectodomain and ecSOD in cell culture media determined detected by immunoprecipitation and Western blotting following ROS. The MMP inhibitor, marimastat (50µmol/L) effectively restored syndecan-1 and ecSOD on the endothelial cell surface. HAMECs exposed to LSS, and HSS demonstrated an improved glycocalyx density and increased cell surface syndecan-1 and ecSOD to the control levels following ROS generation. HSS reduced the ROS induction of ADAM10 (by 30% in LSS and 50% in HSS) and normalized its mRNA and protein expression to basal levels. Similarly, ROS induction of MMP9 mRNA levels was reduced (LSS: 55% reduction; HSS: 80% reduction). Protein expression of MMP9 was reduced in HAMECs following LSS and HSS, HSS and LSS significantly increased TIMP1 and TIMP3 mRNA (TIMP1 ratio of LSS vs. NSS=2.1±0.1; ratio of HSS to NSS=2.5±0.3, p<0.0001). These data indicate that shear stress maintains the expression of microvascular cell endothelial surface core proteins syndecan-1, ecSOD, and the glycocalyx. Taken together, these data support the conclusion that shear stress protects the expression of enzymes that regulate glycocalyx structure.
Menzies, RL., Unwin, RJ., Dash, RK., Beard, DA., Cowley Jr, AW., Carlson, BE., Mullins, jj., Bailey, MA. (2013) 'Effect of P2X4 and P2X7 receptor antagonism on the pressure diuresis relationship in rats' Frontiers in Physiology 4, 305


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PCB306

The effect of Type 1 diabetes mellitus on the renal expression of purinergic receptors P2X1, P2X4 and P2X7

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Current treatments of diabetic nephropathy (DN) are limited to Renin-Angiotensin system blockade, however DN remains the commonest cause of end-stage renal disease. Thus, novel targets for antagonism are urgently needed. The P2X receptors (P2XRs) potentially provide such targets. P2XRs (P2X1-7R) are ligand-gated ion channels activated by extracellular ATP. They are present within the kidney and increased expression of subtypes P2X4R and P2X7R may predispose it to vascular injury(1). Upregulated glomerular P2X7R expression also occurs in rats with established DN(2). Therefore, we have explored whether the expression of these P2X7R subtypes changes in early Type 1 Diabetes Mellitus (T1DM). T1DM was induced in male Sprague Dawley rats (n=6) via intraperitoneal (IP) injection of streptozotocin (35mg/kg). Blood glucose induction in male Sprague Dawley rats (n=6) via intraperitoneal injection of streptozotocin (35mg/kg). Blood glucose concentration was measured at 48h and all rats were killed after 3 weeks by IP anaesthetic overdose (n=3) or were untouched (n=3). All rats were culled after 3 weeks by IP anaesthetic overdose (n=3) or were untouched (n=3).

cose concentration was measured at 48h and all rats were

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PCB307

The NO donor (RuBPY) does not induce in vitro cross-tolerance with acetylcholine

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Nitric oxide (NO) is known to be critical endogenous regulator of vascular cells function. NO donors are widely used as pharmacological tool to understand the physiological effect of NO and to treat cardiovascular diseases. The major clinical benefit of the organic nitrates like Nitroglycerin (GTN) is attributed to their potent venodilator effect. However, the chronic use of GTN is limited by nitrate tolerance that is characterized by rapid loss of its effects or cross-tolerance to other vasodilator. This study aimed to verify if the new NO donor synthesized in our laboratory (RuBPY) induces in vitro tolerance and cross-tolerance to acetylcholine (ACh) and sodium nitroprusside (SNP) in rat cava vein. All experimental protocols were performed in accordance to the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and were approved by the Ethics Committee on Animal Use (CEUA) of the University of São Paulo (Protocol 11.828.532). Rats (200 to 230g, n=25) were killed under anesthesia with isoflurane (500 µL/animal). The cava vein was isolated and used to vascular reactivity and western blot (WB) studies. The vasodilatation induced by RuBPY and GTN were compared by the maximum effect (ME) and potency (pD2). In vitro tolerance was induced by incubation of the veins for 10, 30 or 60 min with RuBPY (2 µM or 10 µM) or GTN (4 µM or 100 µM). In vitro cross-tolerance to ACh and SNP was induced by the veins pre-exposure for 60 min to RuBPY (2 µM) or GTN (4 µM). The eNOS phosphorylated in the activation site (Ser1177), the inactivation site (Thr495) and the ratio of active eNOS dimers to inactive eNOS monomers was evaluated by WB. Our results demonstrated that RuBPY induced greater relaxation (ME: 92.8 ± 4.4%, n=7; P<0.05) than GTN (ME: 75.3 ± 3.7%, n=6). Previous exposure for 10 min to RuBPY (2 µM or 10 µM) or GTN (4 µM or 100 µM) did not induce tolerance. Pre-exposure for 30 min to 2 µM or 10 µM RuBPY did not alter the relaxation to RuBPY. However, pre-exposure for 30 min to 4 µM or 100 µM GTN reduced the relaxation to GTN (ME: 45.4 ± 2.2%, n=6; P<0.05 and ME: 39.2 ± 1.4%, n=6; P<0.05), respectively. Pre-exposure for 60 min to RuBPY reduced the relaxation in the concentrations of 2 µM (ME: 48.0 ± 2.3%, n=7; P<0.05) and 10 µM (ME: 30.1±1.2%, n=7; P<0.05). Pre-exposure to RuBPY or GTN did not reduce the relaxation to SNP. Cross-tolerance to ACh was induced only with GTN. The ME induced by ACh was reduced from 100.3 ± 5.3% to 75.1 ± 4.2%, n=7; P<0.05). Although RuBPY
and GTN phosphorylated eNOS in the inhibitory site (Thr^495), they did not modify the eNOS dimers/monomers ratio. Taken together, our results show that RuBPY takes more time (60 min) than GTN (30 min) to induce tolerance and it does not induce cross-tolerance with acetylcholine.

Supported by FAPESP and CNPq

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**PCB308**

**TRPV2 channels are critical in retinal arteriolar myogenic signalling**

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By acting as a pathway for depolarisation and Ca^{2+}-entry mechanoTRP channels are thought to play a key role in the pressure-induced vasoconstriction or “myogenic response” in vascular smooth muscle. Here we present evidence that TRPV2 forms a mechanoTRP channel in rat retinal arteriolar smooth muscle cells (SMCs) which is crucial in the generation of myogenic signalling in this tissue. Using RT-PCR and immunohistochemistry we have previous reported that TRPC1, M7, V1, V2 and P1 are expressed in the cytosol and membrane of SMCs surrounding rat retinal arterioles1; while TRPV4 expression was more prominent in the SMC nuclei. Additionally stretch-sensitive Ca^{2+} influxes were reversed by the TRPV2 inhibitor tranilast and the non-selective TRP1/V2 antagonist amiloride, while inhibitors of TRPC1, M7, V1 and V4 had no effect1. In the present study we provide more definitive evidence that TRPV2 mediates stretch-activated cation currents and underpins myogenic activity in these microvessels.

Sprague Dawley rats (300-500g) were sacrificed according to Schedule 1 methods; retinas extracted and arterioles isolated by titration. Cell-attached patch-clamp and pressure myography recordings were carried out; data was tested for normality using the D’Agostino-Pearson normality test and analysed using appropriate statistical tests as indicated.

Direct membrane stretch during cell-attached recordings (45mmHg negative pressure) triggered cation currents (0.38±0.10pC/s to 2.10±4.30pC/s; n=14, P<0.001, paired t-test) which were absent when either an externally targeting TRPV2 antibody (Ext TRPV2 Ab; 1:100; 0.43±0.32pC/s to 0.45±0.30pC/s; n=6, P>0.05) or tranilast (100 µM) were included in the patch pipette (0.42±0.24pC/s to 0.53±0.23pC/s; n=10, P>0.05) while channel activation was still apparent in the presence of an antibody targeted towards an internal epitope of TRPV2 (Int TRPV2 Ab; 0.19±0.18pC/s to 3.90±0.42pC/s; n=7, P<0.001).

Pre-incubation of vessels fragments with Ext TRPV2 Ab (1:100, 2hrs room temp.) reduced the development of myogenic tone upon pressurisation (32.6±2.1µm diameter at 0mins after inflation with 40mmHg compared to 32.0±2.1µm after 15mins inflation) and eliminated the subsequent dilatory action of tranilast (32.0±2.1µm; P>0.05, one-way ANOVA; n=11). Arterioles pre-incubated with Int TRPV2 Ab (1:100, 2hrs) developed tone (33.7±2.0µm) at 0mins after inflation compared to 31.3±2.1µm after 15mins inflation; (P<0.01) and dilated with application of tranilast (32.6±2.1µm; P<0.05, one-way ANOVA with post-hoc Dunnett’s multiple comparison tests; n=11).

Our results confirm that TRPV2 channels are critical for the generation of myogenic tone in retinal arterioles and provide an important basis for future studies investigating why myogenic signalling and blood flow autoregulation are disrupted in ocular disease states such as diabetic retinopathy and glaucoma.


Funded by a grant from BBSRC.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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**PCB309**

**Role of reactive oxygen species in perivascular adipose tissue-induced vasoconstriction: Impact of sex**

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Complex mechanisms appear to regulate the perivascular adipose tissue (PVAT) function and more than one pathway, including reactive oxygen species (ROS), may be responsible for the effect of PVAT on vascular physiology (1, 2). We have found sex differences in the regulation of vascular tone by PVAT in the porcine coronary artery (PCA) (Data not published). As there are sex differences in oxidative stress in the vasculature (3), the aim of the present study was to determine whether there are sex differences in production of ROS from NADPH oxidase (Nox) in PVAT and the effect on vasomotor function of isolated porcine coronary artery. Coronary arterial responses to PVAT were recorded in an isometric tension recording system in the presence and absence of a range of Nox inhibitors. Nox activity (superoxide anion production (O-2)) in PVAT and PCA homogenates was assessed using lucigenin-enhanced chemiluminescence. Western immunoblotting was performed to examine the expression of different Nox isoforms in PVAT and PCAs. t-test or ANOVA was used to analyse the data depending on the number of factors analysed. Data are expressed as mean±S.E.M. In male and female PCAs, PVAT caused significant vasoconstrictor responses (p<0.001, n=6). Pre-incubation with non-selective Nox antagonist diphenyleneiodonium (DPI) (10µM) (p<0.01, n=5), selective NOX1 antagonist (ML171) (100µM) (p<0.001, n=6), selective NOX2 antagonist PhoxI2 (100µM) (p<0.001, n=6) and selective Nox1 and Nox4 antagonist (GTK137831) (10µM) (females: p<0.01; males: M; p<0.001, n=8) significantly reduced PVAT-induced vasoconstriction in PCAs from both females and males. NOX1, NOX2 and NOX4 were expressed in PVAT with no difference detected between females and males (n=6F and 5M). However, in PCAs, NOX1 expression was greater in females (p<0.05, n=6F and 5M) whilst NOX4 was higher in males (p<0.01, n=6F and 5M). NOX2 was expressed equally in PCAs from different sexes (n=6F and 5M). NOX activity was detected in PVAT and PCAs with no sex differences. DPI (10µM), ML171 (100µM) and phox-I2 (100M) reduced significantly O-2 production in PVAT and PCA from both sexes (Table 1). In contrast, GTK137831 did not inhibit O-2 production in PVAT and PCA from both sexes (Table 1). This study illustrates that PVAT is an important source of Nox-derived ROS that can induce contraction of porcine coronary artery with no effect.
of sex difference on the crosstalk between PVAT and vascular tone despite a significant variation in the expression of NOXs isoforms.

Table 1

<table>
<thead>
<tr>
<th>Drugs</th>
<th>PVR (Male)</th>
<th>PVR (Female)</th>
<th>PVR (Tamoxifen)</th>
<th>PVR (Normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPI</td>
<td>106.1 ± 1.2</td>
<td>298.8 ± 2.8</td>
<td>320.5 ± 3.3</td>
<td>320.5 ± 3.3</td>
</tr>
<tr>
<td>ML171</td>
<td>106.1 ± 1.2</td>
<td>298.8 ± 2.8</td>
<td>320.5 ± 3.3</td>
<td>320.5 ± 3.3</td>
</tr>
<tr>
<td>Pos</td>
<td>65.1 ± 3.5</td>
<td>43.2 ± 3.6</td>
<td>43.2 ± 3.6</td>
<td>43.2 ± 3.6</td>
</tr>
</tbody>
</table>

Effect of Nox antagonists on ROS production in PVAT and PCA from both sexes. (vehicle control vs drugs) Data are expressed as mean±S.E.M.


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PCB310
The role of endothelial gremlin in hypoxic pulmonary hypertension
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Introduction
Pulmonary hypertension (PH) is a common complication of many chronic lung diseases which significantly reduces life expectancy. Previous work identified an important role for Gremlin1, a Bone Morphogenetic Protein antagonist, in the development of PH (Costello et al., 2008). Furthermore, ubiquitous haploinsufficiency of Gremlin1 in mice resulted in augmented BMP signalling and significantly reduced pulmonary vascular resistance (PVR) after 3 weeks of hypoxia exposure (Cahill et al., 2012). Gremlin within the lung is produced by endothelial cells (EC), type II pneumocytes and macrophages. Microvascular EC play an important role in the vascular changes associated with hypoxia. The specific role of endothelial derived gremlin1 in the development of pulmonary hypertension is unknown nor is it known if the different sources within the lung are functionally redundant. To define the role of endothelial-derived gremlin in PH, an inducible endothelial gremlin deleted model was used.

Methods and Results
A novel inducible endothelial gremlin knock-out mouse model was generated by crossing mice where gremlin1 was flanked by loxP sequences (Gazzero et al., 2007) with mice expressing the Cre recombinase under the control of the vascular endothelial cadherin promoter. Adult mice (age 6 weeks, n=10 per group) received chow with added Tamoxifen or a normal Chow diet for 4 weeks, followed by a 2-week "wash-out" period on a normal Chow diet. Groups of mice were then exposed either to 3 weeks normoxia or hypoxia (F02=0.10). Gremlin deletion was confirmed by the detection of the appropriate truncated amplicon. Mice were anaesthetised with urethane (1500mg.kg\(^{-1}\)), anti-coagulated using heparin (1000 I.U.kg\(^{-1}\)) and euthanased by ex-sanguination. Similar elevation of haematocrit was observed in both hypoxic groups (tamoxifen or normal diet) confirming a normal haemopoesis response to hypoxia. PVR was measured using the isolated ventilated lung preparation, perfused at a constant flow rate. Hypoxic exposure significantly increased haematocrit levels and the PVR of mice fed either a tamoxifen diet or normal diet (P<0.01). Mice with a reduced endothelial gremlin expression, fed tamoxifen, had an increased PVR compared to normal diet fed mice (P=0.023) following both normoxic and hypoxic exposure.

Conclusion
In conclusion, endothelial gremlin deletion increased pulmonary vascular resistance under both normoxic and hypoxic conditions suggesting that it plays an important role in normal pulmonary vascular homeostasis.


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PCB311
N-terminal membrane flanking residues of BKβ1 contribute to the effects of the BK channel opener GoSlO-SR-5-130

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Large conductance, voltage and calcium activated K+ (BK) channels are formed by four pore forming α subunits. These channels are modulated by regulatory β (β1-4) and γ (γ1-4) subunits, which alter the biophysical and pharmacological properties of the channel. The β1 subunit, expressed in smooth muscle, shifts the voltage sensor activation to more negative membrane potentials. Recently, we identified a BK channel opener, GoSlO-SR-5-130 (GoSlO130) that required β1 subunits to mediate its full effects, but little is known about which β1 residues contribute to this enhanced effect. Currents from I/O patches were recorded using the patch clamp technique from HEK cells expressing BKα and mutant BKβ1 δ subunits. Experiments were performed at 37°C and the cytosolic surface of patches bathed in symmetrical (140 mM) K+ and 100 mM free Ca2+. Patches were held at -60 mV and stepped from -100 mV to +200 mV in 20 mV increments.
Mutant β1 subunits were transiently transfected into HEK cells along with BKα subunits using the Ca2+ phosphate technique. GoSlo130 (10 µM) shifted the voltage required for half maximal activation by -28±3 mV (ΔV1/2,n=6) and this was increased to -94±6 mV (n=5, p<0.05) when applied to patches coexpressing WT BKβ1 subunits. The BKβ1 subunit consists of two transmembrane domains, a large extracellular loop and small intracellular N and C termini. To investigate if the intracellular termini were essential for the effects of GoSlo130, we deleted the N terminus alone (BKβ1ΔNT2-15) and in combination with the C terminus (BKβ1ΔNT2-15&CT179-191). When GoSlo130 was applied to the BKβ1ΔNT construct, the ΔV1/2 was significantly reduced to -25±7 mV (n=5, p<0.05). Deletion of both termini had little further effect (ΔV1/2 = -20±4 mV, n=6), suggesting that the NT of the β1 subunit played a role in mediating the effects of GoSlo130.

To investigate which NT residues were important, we utilized a sequential deletion approach5. The effects of GoSlo130 were not significantly altered when residues 2-12 were deleted (ΔV1/2 = -105±8 mV, n=6). However, deletions of membrane flanking residues significantly reduced the effect of GoSlo130 (ΔV1/2 = 78±6 mV, n=6; -47±5 mV, n=6) in the BKβ1ΔNT2-13, BKβ1ΔNT2-14 constructs, respectively. These data suggested that residues 13-15 of the NT were essential for mediating the effects of GoSlo130. We next mutated residues 13-15 from ETR to GGG on the full length BKβ1 construct and found that GoSlo130 still shifted the activation V1/2 by -87±2 mV (n=6) compared to -94±6 mV in the WT BKβ1 construct. These data suggest that the backbone of residues E13, T14 and R15 in BKβ1 may contribute to the effects of GoSlo130 in BKαβ1 channels.

2. Large et al., (2015) Effects of the novel BK (Kca 1.1) channel opener GoSlo-SR-5-130 are dependent on the presence of BKβ1 subunits. BJ 172(10):2544-56

This work was supported by Enterprise Ireland’s CFTD and a Research Frontiers Programme grant from SFI. SD was supported by an IOTI award and DIK Tre Research Office.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB312

K7 channel activators inhibit pregnant mouse myometrium uterine contractility and delay RU486 induced preterm birth

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Introduction: This study focussed on the potential of K7 channel activators as a new class of tocolytics. Building upon our previous work demonstrating K7 channels (alpha subunits KCNQ1-5 and auxiliary proteins KCNE1-5 genes) are expressed in myometrium from women at term and preterm gestations, and functional in pregnant human myometrium, our aim was to assess the function of K7 channels and determine whether K7 activators could delay birth in in murine model of preterm birth.

Methods: Myometrium was isolated on day 16 of gestation from C57BL/6j pregnant mice treated with RU486 to induce preterm labour (PTL, 150 µg S.C., day 15) or DMSO (preterm not in labour, PTNL) (n=6 per group). Tissues were used for isometric organ bath studies (% of baseline mean integral tension) with K7 channel activators retigabine and ML213 (5-20 µM) or stored (-80°C) for analysis (qPCR). CCTV recording used to determine the onset of labour and time gestation length (delivery of first pup) following a maximum of six doses (given once an hour) of either retigabine or ML213 (20 mg/kg P.O.) or vehicle. In vivo uterine telemetry determined the impact of acute doses (2 doses P.O.) of retigabine on intrauterine pressure (IUP). Data are mean ± S.E.M. or median [interquartile range] and assessed using Kruskal-Wallis test with Dunn’s multiple comparison post hoc test or Mann-Whitney U test (significance, P<0.05).

Results: mRNA for KCNQ1-5 and KCNE 1-5 mRNA were detected in PTL and PTL mouse myometrium (n=4-6, expression profile KCNQ1>Q5>Q4>Q2>Q3). mRNA expression for KCNQ4 and KCNQ5 slightly decreased after labour onset (P <0.05). KCNE expression profiles in PTL myometrium (E4>E3>E2>E5>E1) slightly differed to those in PTLN (E4>E2>E3>E5>E1) due to a reduction in KCNE2 expression in labour. Retigabine and ML213 suppressed myometrium contractions in vitro in PTL at all concentrations studied (e.g. MIT: retigabine at 5 µM, 32.7 ± 4.5% versus DMSO 79.2 ± 4.5%, p<0.05; ML213 at 5 µM 11.9 ± 3.6% versus DMSO 75.8 ± 4.5% p<0.05, n=6). In vivo, acute doses of retigabine transiently reduced IUP when analysed relative to time of labour, but did not increase gestation length. However, both retigabine and ML213 when given in 6 repeated doses caused significant 6 and 3 hour delays in preterm labour onset respectively compared to the K7 activator vehicle (p<0.01).

Conclusions: K7 channels are present and functional in myometrium from the RU486 mouse model of preterm birth, and treatment in vivo with K7 channel activators can delay preterm birth. Taken together with our previous in vitro human myometrium data this suggests that K7 channels are a viable tocolytic target. Development of activators that target the predominant myometrium K7 channel isoforms should be pursued.

Funded by: MRC grant G1100243, MRC/KCL studentship and Tommy’s charity (No.10605080).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB313

Activation of calcium/calmodulin-dependent kinase 2 (CaMKII) mediates Epac-induced increases in spontaneous transient outward current in rat mesenteric artery smooth muscle

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Exchange protein directly activated by cAMP (Epac), a major cAMP effector, induces smooth muscle relaxation by increasing the frequency of localised Ca2+ release from
**Novel mathematical model for glucose transport in skeletal muscle interstitium**

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Type II diabetes is characterized by a decreased sensitivity to insulin resulting in an impairment in glucose uptake and high blood glucose concentrations [1]. Measures of insulin resistance involve blood glucose measurements following an insulin clamp [2]. These global measurements fail to account for the spatial complexity of glucose uptake into the muscle.

Therefore, in this study, we developed a spatial mathematical model for glucose transport in skeletal muscle to assess the effects of insulin resistance on glucose uptake. The mathematical model describes the steady-state diffusive transport of both insulin and glucose in the interstitial space between capillaries and muscle fibers in skeletal muscle. The insulin and glucose concentration at the capillary wall was fixed to the blood insulin and glucose concentrations respectively.

We simulated glucose and insulin transport for both fasting and post-prandial conditions by altering the blood insulin and blood glucose concentrations. Insulin resistance was simulated by decreasing the rate constant describing the kinetics of glucose uptake by a factor related to the severity of the resistance. Glucose concentration for fasting and post-prandial conditions for the case of no insulin resistance is shown in Figure 1.

This figure shows the heterogeneous nature of glucose in the interstitial space. Glucose uptake rate was 165% higher in post-prandial conditions when compared to fasting conditions. The effect of insulin resistance on glucose uptake for both fasting and insulin resistance is shown in Figure 2.

Overall, we wish to elucidate the importance of geometry on glucose transport. Here, we demonstrate how glucose uptake rates change with varying levels of insulin resistance in both fasting and post-prandial states. Additionally, capillary density may be altered in type II diabetics [3], and thus may be an important factor in glucose uptake. With this spatial model, we will be able to explore the effects of capillary density in various metabolic conditions.

**Figure 1.** Glucose concentration (mmol/L) for fasting (A) and post-prandial (B) conditions as a function of position in the skeletal muscle interstitium.

Note: colour bars have different scales in order to elucidate the gradients in the interstitial space. The capillaries are represented as small circles and the muscle fibers are represented by large circles.
Figure 2. Glucose uptake rate as a function of percent insulin resistance for both fasting and post-prandial conditions.

Tam CS et al (2012). Diabetes Care 35:1605-10

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Role of Soluble Frizzled Related Protein 5 in post ischemic arteriogenesis

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Diabetes and obesity are major sources of disability in UK. A common consequence of diabetes is Peripheral Vascular Disease (PVD), represented mainly by limb ischemia and is a principal cause of leg amputation (1).

The ischemic insult causes poor blood supply and inflammation. In healthy individuals the inflammation contributes to vascular remodeling, which restores vasculature and blood perfusion, whereas in diabetes this step is impaired leading to poor revascularisation and necrosis (2). The mechanisms behind impaired collateral revascularisation in diabetes are still unclear, but it should require both angiogenesis and arteriogenesis. Vascular Endothelial Growth Factor (VEGF) is understood to drive angiogenesis, but VEGF mRNA alternative splicing results in both pro- and anti-angiogenic VEGF isoforms (3) Wnt5α has been shown to increase in anti-angiogenic VEGF in circulating monocytes, preventing revascularisation (4). In healthy subjects circulating Wnt5α is inactivated by binding Soluble Frizzled Related Protein (SFRPS); in PVD patients levels of SFRPS are downregulated (5), allowing Wnt5α to stimulate antiangiogenic VEGF expression.

To determine whether Sfrp5 contributes to revascularization in ischemia we performed an unilateral hind limb ischemia in SFRP5 knockout mice (SFRP5⁻/⁻) or on wild type littermates as control. All animal procedures met the requirements dictated by the Animals (Scientific Procedures) Act 1986 / ASPA Amendment Regulations 2012. Mice were anaesthetized with 2% isoflurane, blood flow was measured using Laser Speckle imaging, the left femoral artery ligated, the animal sutured and recovered. Laser Speckle imaging was carried out on both hindpaws 3 days and then weekly after the ischemia. Mice were killed after 28 and stained for blood vessels with Isolectin B4 and arterioles with smooth muscle actin antibody.

Results
SFRP5⁻/⁻ mice had reduced recovery of blood flow after ischemia (figure 1). After 28 days blood flow to the ipsilateral limb was 0.84±0.7 of contralateral in SFRP5⁻/⁻ mice, which was significantly lower than in wild type littermate controls 0.65±0.06 (Fig 1).

Conclusions
The finding that Sfrp5⁻/⁻ mice, which have raised VEGF-A165b expression after hindlimb ischemia, have impaired arteriogenesis (4), indicates that VEGF-A165b may be able to inhibit arteriogenesis as well as anti-angiogenesis, either directly or indirectly.

Fig. 1. SFRP5 knockout mice have impaired recovery after ischemia. A. Speckle imaging of the paws of wild type and a SFRP5⁻/⁻ mouse where femoral artery ligation has been undertaken in the right leg. B. Quantitation of impaired recovery of blood flow. Values are normalised to the contralateral side. P<0.01 two way ANOVA. *=p<0.05, **=p<0.01. n=14

Fig. 2. Arteriole density was increased in wild-type but not SFRP5⁻/⁻ mice after 28 days ischemia. A. Representative images of arteriolar (top images) and capillaries (bottom images) immunofluorescence. Muscles were fixed in PFA and OCT embedded, cryosections were stained for α-smooth muscle actin Cy-3 conjugated (red), white arrows indicate some examples of arteriole. Capillaries have been stained for Isolectin GS-IB4, followed by secondary antibody Alexa Fluor 488 (green). B. Arterioles and capillaries were counted on ischemic and contralateral gastrocnemius and average per mm² has been plotted. *=p<0.05, n= 8 mice per group.
while force remains unchanged. Interestingly, some quiescent neurons and electric field-stimulated (EFS) contractile activity are studied in vessels normalized to a transmural pressure of 21 mmHg. After equilibration vessels are challenged by acute and prolonged incubations with somatostatin (SST-14, octreotide. The therapeutic rationale is that SST or octreotide have treated patients with SST or its stable synthetic analogue SRFP5\(^5\) have treated patients with SST or its stable synthetic analogue SRFP5\(^5\). We gratefully acknowledge Einar Pahle and the operation team from Viborg Hospital and Hans Pilegaard and operation team from Aarhus University Hospital Skejby.

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PCB316

**Action of somatostatin and analogues on human lymphatic vessels**

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Lymph is actively transported from lymphatic capillaries to the venous circulation by intrinsic contractile activity in the collecting lymphatic vessels. The largest collecting lymphatic is the thoracic duct (TD), which terminates into the veins of the neck. Accumulation of lymph in the thorax (chylothorax) caused by damage to the TD occurs in congenital heart diseases, e.g. univentricular circulation, as well as in connection with thoracic operations or trauma. With protracted chylothorax pharmacological options are desired to resolve this problem more quickly. Since the first reports of somatostatin (SST) infusion to treat chylothorax, many clinicians worldwide have treated patients with SST or its stable synthetic analogue octreotide. The therapeutic rationale is that SST or octreotide reduce lymph production: we hypothesize that an additional direct action of SST on lymphatic contractility also occurs. To investigate SST reactivity we use isolated lymphatic vessels; thoracic duct from (oesophagus cancer) and intestinal lymphatics (from gastric bypass) mounted in wire myographs for isometric force measurement. Spontaneous, non-spontaneous and electric field-stimulated (EFS) contractile activity are studied in vessels normalized to a transmural pressure of 21 mmHg. After equilibration vessels are challenged by acute and prolonged incubations with somatostatin (SST-14 and -28) and octreotide. Results to date suggest that the acute exposure we test in our system does not decrease contraction frequency but rather tends to stimulate rate of activity, while force remains unchanged. Interestingly, some quiescent vessels were also activated with 10nM Oct (P<0.09) and 10nM SST-14 (P=0.17). The EFS experiments revealed no effect on nerve-stimulated contraction for any of the drugs. We conclude that human lymphatic vessels respond to acute SST exposure by increased activity, which would be contrary to reducing lymph flow in vivo in treatment of chylothorax. However, we cannot yet conclude what effect chronic exposure in vivo could have on lymphatic pumping ability.

We gratefully acknowledge Einar Pahle and the operation team from Viborg Hospital and Hans Pilegaard and operation team from Aarhus University Hospital Skejby.

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PCB318

**Human platelets use a cytosolic nanodomain to control thrombin-evoked Ca\(^{2+}\) signalling**

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Previous work in our lab has demonstrated that human platelet Ca\(^{2+}\) signaling utilises a pericellular recycling system.\(^1,2\) This model predicts that Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores occurs initially into a cytosolic nanodomain enclosed within the membrane complex (MC; a close apposition of an invagination section of the platelet plasma membrane and endoplasmic reticulum - analogous to the cardiac diad). From this cytosolic nanodomain, the Ca\(^{2+}\) is spread via removal by the Na\(^{+}\)/Ca\(^{2+}\) exchanger (NCX) into the lumen of invaginated membrane system, where it can then accumulate and recycle back into the cytosol through Ca\(^{2+}\)-permeable ion channels.\(^2\) This study aimed to test the hypothesis that Ca\(^{2+}\) release initially accumulates within an NCX-associated cytosolic nanodomain. If Ca\(^{2+}\) release occurs onto a close associated NCX within a nanojunction then the NCX-mediated Ca\(^{2+}\) removal should be largely unaffected by the presence of the fast Ca\(^{2+}\) chelator Dimethyl BAPTA (DM-BAPTA), which buffers the bulk cytosol but will not significantly affect Ca\(^{2+}\) signals within about 10 nm of its point of entry into the cytosol.\(^3\) Platelets were isolated from blood obtained by venepuncture of healthy volunteers under informed consent and with local ethical committee approval in accordance with the declaration of Helsinki. Thrombin-evoked changes in cytosolic- and extracellular Ca\(^{2+}\) concentration were monitored in Fura-2-loaded platelets and washed platelet suspensions containing 2.5 \(\mu\)M Fluo-4 salt respectively, using our previously published methodologies.\(^2\) Single cell imaging was performed in washed platelet suspension containing 5 \(\mu\)M Fluo-4 salt using a Fluoview FIV1200 laser-scanning confocal microscope. Data are presented as mean % of control + SEM of the number of samples (n) indicated. Statistical significance was tested by Student’s t-test and one way ANOVA followed by post-hoc Tukey test. DM-BAPTA-loading prevented any notable rise in the cytosolic Ca\(^{2+}\) concentration after stimulation with 0.5 U/ml Thrombin (1.0% ± 0.6%, P<0.05, n=4), yet in the absence of a rise in Ca\(^{2+}\) in the bulk cytosol it was possible to identify a Ca\(^{2+}\) removal in the extracellular fluid (69.7% ± 10.5% of untreated controls, P<0.05, n=7). This extracellular Ca\(^{2+}\) accumulation in DM-BAPTA-loaded cells was inhibited by pre-treatment with the NCX inhibitor, KB-R7943 (35.4% ± 7.7%, P<0.05, n=7). Single cell imaging demonstrate the presence...
of a single hotspot in each cell, which were rarely observed in KB-7943-pretreated cells. In addition, Ca\(^{2+}\) removal from DM-BAPTA-loaded cells was significantly inhibited in cells in which the PMC was disrupted by treatment with nicergoline (50.4% ± 19.6%, P<0.05, n=9). These results suggest that NCX removes Ca\(^{2+}\) from an isolated cytosolic nanodomain which is not readily reported by Fura-2. Its sensitivity to nicergoline suggest this is likely to be enclosed within the MC.\(^1\)


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**PCB319**

**Involvement of ORAI/STIM store-operated Ca\(^{2+}\) Channels in hyperosmolarity-induced endothelial dysfunction**

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Endothelial cell dysfunction is a major cause of vascular disease that can be propagated by multiple pathological conditions, although the mechanisms of endothelial cell dysfunction are not fully understood. We have recently found that the activity of the store-operated Ca\(^{2+}\) channel molecules, STIM/ORAI, was enhanced by high glucose in vascular endothelial cells, which causes abnormal Ca\(^{2+}\) homeostasis and endothelial dysfunction under hyperglycaemia [1]. Since hyperglycaemia is usually accompanied by hyperosmolality in diabetes, particularly for the critical condition called Hyperglycaemic Hyperosmolar State (HHS), we aimed to study the effect of hyperosmolality on the expression and activity of STIM/ORAI channels in vascular endothelial cells.

Using Fura-PE3 AM Ca\(^{2+}\) dye, we found that store-operated Ca\(^{2+}\) entry (SOCE) evoked by thapsigargin was inhibited in endothelial cells (EA.hy926) by the pre-treatment with hyperosmotic conditions via addition of 30, 60 and 90 mM mannitol in normal bath solution. The inhibition of SOCE by hyperosmolality was also seen in the HEK293 cells overexpressing EFYP-STIM1, mCherry-ORAI1, EFYP-STIM1/mCherry-ORAI2, and EFYP-STIM1/mCherry-ORAI3 channels. The currents for ORAI1, ORAI2 and ORAI3 were examined by whole cell patch-clamp, and the thapsigargin-evoked currents of ORAI1 and ORAI2, and 2-APB-induced ORAI3 were inhibited by hyperosmolar condition (19.5 mM mannitol) in the HEK293 overexpressing STIM1/ORAI1-3 channels. The mRNA and protein expression of ORAI1-3 STIM1-2 was detected by real-time PCR and Western blotting, respectively, and their expression levels were down-regulated by hyperosmotic conditions.

Endothelial cell (EA.hy926) migration was significantly inhibited by hyperosmolality after incubation with 19.5 mM mannitol or 19.5 mM sucrose for 24 hrs using a scratch wound technique. The cell volume was significantly reduced by hyperosmotic conditions (30, 60 and 90 mM mannitol), but such cell volume change could be unrelated to the expression of ORAI/STIM channels, because there was no significant difference between the non-transfected cells and the ORAI/STIM transfected cells.

Our findings suggest that SOCE is regulated by hyperosmolality in vascular endothelial cells and ORAI/STIM channels contribute the cellular processes. The relevance of SOCE to osmotic stress could provide a new insight to the pathophysiology of endothelial dysfunction in diabetes.


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**PCB320**

**PIP\(_2\) and G\(_{\beta\gamma}\) subunits are synergistic regulators of Kv 7.4 channels**

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G-protein \(\beta\gamma\) subunits have recently been shown to be positive regulators of KCNQ4-encoded voltage-gated potassium channels, Kv 7.4 (PNAS, 112: 6497-6502, 2015). The aim of the present work was investigate whether there was an interplay between G\(_{\beta\gamma}\) subunits and phosphatidylinositol 4, 5-bisphosphate, (PIP\(_2\)) an established positive modulator of Kv 7.4 (J. Gen.Physiol. 140: 41-53, 2012). Electrophysiological recordings were performed using standard perforated, whole-cell, cell-attached and inside-out configurations of patch-clamp technique on HEK293 cells heterologously expressing Kv 7.4 (Am.J.Physiol. 280: C859-C866, 2001). PIP\(_2\) levels under different conditions were estimated using an In-cell Western Blot technique (Biochem.Biophys.Res.Commun. 452: 852-857, 2014).

Data are presented as mean ± S.E.M. Bath application of PIP\(_2\) (1-300 \(\mu\)M) to inside-out patches increased apparent NPo of Kv 7.4 in dose-dependent manner with a EC\(_{50}\) of 117.4 ± 54 \(\mu\)M (n = 4-11). Similarly, application of G\(_{\beta\gamma}\) subunits (0.4-50 ng/ml) dose-dependently increased NPo and a notable increase was observed at concentrations higher than 2 ng/ml. After PIP\(_2\) depletion by wortmannin (an inhibitor of PIP\(_2\) re-synthesis), in combination with a short stimulation of G-protein-coupled proteinase-activated receptors or purinoceptors, currents through Kv 7.4 channels were decreased by 95% in perforated-, cell-attached and inside-out patches. Bath application of PIP\(_2\) re-established channel activity of inside-out patches, whereas G\(_{\beta\gamma}\) subunits had no effect. Inhibitors of G\(_{\beta\gamma}\) subunits (gallein, M201or GRK2i) also depressed Kv 7.4 currents, but PIP\(_2\) was not able to enhance channel activity in this case. Application of a low concentration of G\(_{\beta\gamma}\) subunits (1 ng/ml) to inside-out patches was augmented the action of PIP\(_2\). Thus, 3 \(\mu\)M of PIP\(_2\) increased NPo to 0.077 ± 0.017 (n = 8) alone and to 0.255 ± 0.073 (n = 6) in the presence of G\(_{\beta\gamma}\) subunits. This study shows that Kv 7.4 channels are regulated by an interplay of PIP\(_2\) and G\(_{\beta\gamma}\) subunits.


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PCB321

Early-life stress in male mice induces increased vascular oxidative stress in adulthood

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Epidemiological studies indicate that early-life stress (ELS) is an independent risk for cardiovascular disease (CVD). Endothelial dysfunction and oxidative stress are mediators of CVD. We hypothesized that ELS induces oxidative stress in adulthood using a mouse model of maternal separation and early weaning (MSEW). We first assessed vascular reactivity and superoxide generation of aortic rings in the presence and absence of superoxide scavengers from control and MSEW male mice. Mice were anesthetized with methohexitol sodium (Brevital; 50 mg/kg IP), and aortae and plasma collected. Compared to control mice, aortic rings from MSEW mice displayed endothelial dysfunction that was reversed by superoxide scavenger, polyethylene glycol-superoxide dismutase (p<0.05, n=5-7). Superoxide production, assessed with dihydroethidium (DHE) HPLC assay, in aortae from MSEW mice was significantly greater than observed in control aortae (p<0.05, n=4), although unaffected by NO synthase inhibition. Increased expression of the NADPH oxidase subunits, NOX2 and NOX4, was evident in the aortae of MSEW mice (p<0.05, n=5). Moreover, NADPH oxidase inhibitor, apocynin, prevented vascular endothelial dysfunction (p<0.05, n=5-7), and reversed increased aortic superoxide generation (p=0.05, n=4) in MSEW mice, indicating that MSEW induces superoxide production and endothelial dysfunction, at least in part, via increased NADPH oxidase expression and activity. In a second series of studies, we assessed whether circulating factors induce endothelial oxidative stress. Mouse aortic endothelial cells (MAECs) were incubated with plasma from control mice or MSEW mice assessing superoxide production. Plasma from MSEW mice showed a significant increase in superoxide compared to control (p<0.05, n=10). Plasma free heme levels were significantly increased in MSEW mice (p<0.05, n=11), thus we assessed superoxide production in the presence or absence of hemopexin, a heme scavenger. Hemopexin reduced superoxide in MAECs treated with MSEW plasma (p<0.05, n=10), whereas no difference was found with control plasma (n=10). We further hypothesized that the heme-induced superoxide production in MAECs is through a toll-like receptor 4 (TLR4)-mediated pathway. Treatment with TAK242, TLR4 antagonist, significantly reduced superoxide production in MAECs treated with MSEW plasma (p<0.05, n=10), whereas no effect was found with control plasma (n=10). Furthermore, aortic tissue from MSEW mice displayed significantly greater expression of TLR4 compared to control mice (p<0.05, n=8). These observations suggest that MSEW induces TLR4-dependent aortic oxidative stress and endothelial dysfunction. In conclusion, ELS may lead to increased risk for cardiovascular disease through increased heme, oxidative stress, and TLR4 activation.

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PCB322

Atorvastatin-induced vasodilation is amplified by cilostazol in female rat aorta during aging

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Problem statement: Statins have been broadly used in clinical practice owing to its both potent lipid-modifying and other cardio-protective effects including increasing nitric oxide production, enhancing endothelial progenitor cells migration and now are well-known as pleiotropic effects. The present study was designed to determine the effect of age on a statin-induced relaxation on rat thoracic aorta, whether pre-treatment with cilostazol affects the vascular reactivity to atorvastatin. Also the role of nitric oxide was examined in this interaction.

Methods: Female Wistar rats, aged 3–4 months (young) and 14–15 months (adult), were sacrificed by cervical dislocations, the thoracic aorta was dissected and cut into 3-4-mm long rings. The rings were mounted at 1 g tension in a 20 ml organ bath containing Krebs–Henseleit solution. Rings were pre-contracted with phenylephrine (10^-6 M), and the presence of endothelium was confirmed with acetylcholine (10^-6 M) and as well as those treated with NO nitro-L-arginine methyl ester (L-NAME, 10^-4 M) in young and adult rat aortas.

Results: Atorvastatin (10^-10^-10^-3 M) (control), in the presence of cilostazol (10^-6 M) and as well as those treated with NO nitro-L-arginine methyl ester (L-NAME, 10^-4 M) in young and adult rat aortas. Results: Atorvastatin (10^-10^-10^-3 M) induced relaxation in a concentration-dependent way in young and adult rat aortic rings precontracted with phenylephrine. In young rat aortas, the relaxation response to atorvastatin was significantly greater than the adult rats. The sensitivity of atorvastatin was decreased significantly in mature rat aortas (pIC50 = 7.9 ± 0.1 in young and 5.6 ± 0.1 in adult rats, p<0.05).

Atorvastatin evoked relaxations of young and adult rat thoracic aorta rings in a concentration-dependent manner after incubation with the selective phosphodiesterase 3 inhibitor cilostazol (10^-6 M). Atorvastatin induced relaxation in the presence of cilostazol in low concentrations. Cilostazol enhanced the potency of atorvastatin compared to control in both ages significantly (pIC50 increased from 7.9 ± 0.1 to 10.4 ± 0.8 in young and from 5.6 ± 0.1 to 10.4 ± 0.8 in adult group, n = 6 in all groups, p < 0.05). Cilostazol enhanced the sensitivity more potently than the young group (1.9 times and 1.3 times, respectively, p<0.05) in adult rat aortas. Both in young and adult aortas, pre-incubation with the non-selective nitric oxide synthase inhibitor L-NAME, atorvastatin-induced relaxations were suppressed. Incubation of aortic rings with L-NAME in the presence of cilostazol, did not completely eliminate the relaxation to atorvastatin but significantly reduced the potency of atorvastatin-induced relaxation in both age groups.

Conclusion: The results show that combined application of cilostazol with atorvastatin was significantly more potent than
atovastatin alone. Combined drug therapy may be efficacious in delaying the occurrence of cardiovascular events.

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PCB323

Effect of cilostazol on tadalafil-induced relaxation in urinary bladder of female rats: comparison by age

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Various studies have detailed age-related changes in the structure and function of the bladder. Aging may change the bladder functions during the filling, storage and emptying. These changes cause urinary incontinence, especially in women. The aim of the present study was to determine the age-related response to tadalafil and to combination of tadalafil with cilostazol on the female rat urinary bladder. Urinary bladder preparations isolated from young (2-3 months) and old (14-15 months) female Wistar rats were obtained from Necmettin Erbakan University Experimental Medicine Research and Application Centre (Konya, Turkey). The protocols of the animal experiments were approved by the Internal Ethical Committee of Necmettin Erbakan University Experimental Medicine Research and Application Centre. The rats were housed in wire-topped opaque polycarbonate cages and maintained under constant environmental conditions with a 12 h light/dark schedule. The environmental temperature was 20±2 ºC and humidity was 50 %. The preparations were contracted with carbachol (10^-6 M), after the contraction had reached steady state, tadalafil (10^-9, 10^-6 M) was added to the organ bath. The relaxation responses induced with tadalafil were compared between control and cilostazol administered preparations of young and old groups. The cumulative addition of tadalafil (10^-9-10^-3 M) caused concentration-dependent relaxation after the maximal contractile response to carbachol. In young and old rat urinary bladders the pD2 (–log IC50) values of tadalafil were 6.14 ± 0.09 and 8.59 ± 0.01, respectively. Aging increased the sensitivity to tadalafil, significantly (p < 0.05). The sensitivity of old rat bladders to tadalafil was 1.4 times higher (p < 0.05) than young rats. Incubation of urinary bladder preparations with the selective PDE5i cilostazol (10^-6 M), increased the pD2 values of tadalafil in both age groups, significantly. In young and old rat urinary bladders, the pD2 values of tadalafil in the presence of cilostazol were 8.87 ± 0.01 and 9.19 ± 0.01, respectively. In conclusion, young and old rat urinary bladders which were pre-contracted with carbachol (10^-6 M) produced concentration-dependent relaxation after tadalafil administration. In both age groups cilostazol (10^-6 M) administration increased the sensitivity to tadalafil, significantly. The combined cilostazol and tadalafil application was more potent than tadalafil alone.

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PCB324

The vascular endothelium acts as a networked collective and detects muscarinic and purinergic agonists using clusters of cells that are tuned to concentration

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While agonist-mediated signaling by the endothelium is acknowledged to control virtually all vascular functions, how the endothelium distinguishes low-level concentration fluctuations from noise, and decodes and integrates physiologically relevant information is unclear. Recently we demonstrated that the endothelium decodes agonist signaling by using cells with differing affinities that are organized into spatially-restricted clusters of cells (Wilson, 2016). By utilising cellular hubs to integrate population-wide inputs the endothelium enhances the bandwidth of collective responses (Wilson, 2016). Here we have investigated whether or not different agonists use the same cell hubs to integrate signals or if signals are routed via agonist specific detector cells. In surgically-opened carotid arteries obtained from male (Sprague-Dawley, 150-250g) rats killed by overdose with Pentoject (Schedule 1; Animals (Scientific Procedures) Act 1986), calcium signalling was studied in a large field of endothelium (~150 cells). The muscarinic agonist carbachol and purinergic agonist ATP evoked repeatable, complex calcium signalling across the endothelial field. The calcium signals for each agonist began in discrete clusters of cells and progressed from there as propagating waves of calcium. The number of different clusters of cells activated and the extent of propagation of the calcium wave increased with agonist concentration. With increasing concentration of each agonist, the amplitude of the calcium rise in each activated cell also increased. Carbachol produced a response in 50% of cells (EC50 = 200.5 nM; 95% confidence interval, 113.3–354.9 nM; n = 3) at a lower concentration when compared with ATP (EC50 = 11.68 nM; 95% confidence interval, 9.28–14.69 nM; n = 3). Furthermore, the lowest concentrations of each agonist that produced activity 100% of cells did not elicited maximum calcium rises. High concentrations of ATP (~ 10 µM) produced an inhibitory effect of endothelial calcium signals. Together, these results suggest the endothelium is organized to function as a collective in detecting and relaying signals. Cells are recruited based on their affinity for the concentration agonist and the endothelium integrates population-wide inputs for efficient high-fidelity signaling. The spatial relationship of various agonist detector cells will be discussed.


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The altered gene expressions induced by the lysine deacetylase (KDAC) inhibitor trichostatin A in HAP1 cells is unaffected by knockout of KDAC8 or KDAC1

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It is now apparent, partly through the use of class I lysine deacetylase (KDAC) inhibitors such as trichostatin A (TSA), that regulation of the acetylation status of protein lysine (K) residues is an important determinant of smooth muscle (SM) function (1). Although nuclear-resident KDACs, such as KDAC1, are thought to regulate the transcriptional machinery controlling much gene expression, another class I KDAC, KDAC8, is localised predominantly outside the nucleus in SM and regulates acetylation of non-nuclear proteins. However, it is uncertain if KDAC8 influences global gene expression changes. This study utilised a model system of human cells with selective knockout (KO) of KDAC8 or KDAC1 to compare the relative influences that absence of each protein had on basal and TSA-mediated gene expression.

Control (WT), and 3 separate clones each of KDAC8 KO and KDAC1 KO HAP1 cells, were produced using CRISPR-Cas9 technology (Horizon Discovery, Vienna). The presence and localisation of KDAC8 and KDAC1 were assessed using Western blot and immunofluorescence microscopy. Cells were treated for 16 hours with 0.5µM TSA or vehicle controls. RNA was extracted and gene expression assessed on Illumina HT-12 chips (n=3 per group). Analysis was performed with Perseus software, comparisons made by 2 sample t-test and bioinformatic pathway analysis performed in STRING (v10).

KDAC8 was expressed predominantly in non-nuclear regions, whereas KDAC1 was mostly in the nucleus, suggesting similar localisations to SM cells. KDAC8 and KDAC1 proteins were absent in their respective KOs. TSA treatment increased acetylation of α-tubulin and histone H3, indicating TSA action on non-nuclear- and nuclear-resident KDACs. TSA affected the expression of 3693 genes in WT, 2955 genes in KDAC8 KO and 2770 genes in KDAC1 KO cells (FDR 0.01,>2 fold change). Of note, TSA altered expression of 1624 genes (>2-fold) common to WT, KDAC1 KO and KDAC8 KO. In addition, direct comparison of KDAC1 KO and KDAC8 KO clones showed no differences in basal or, surprisingly, TSA-induced gene expression profiles.

Pathway analysis of the 400 most downregulated genes by TSA in each group indicated prominent alteration of transcriptional machinery components, including genes encoding KDAC7, KDAC9 and many zinc finger proteins. Genes upregulated by TSA (top 400 in each group) included many encoding proteins involved in membrane transport. The absence of non-nuclear KDAC8 protein, or nuclear KDAC1 protein, has little effect on basal or TSA regulated gene transcription, suggesting a robust and modular control of gene expression by the KDAC superfamily. The aforementioned TSA-mediated alteration of expression of genes encoding transcriptional proteins may be influential in this regulation.


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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Studying peripheral microcirculation by a murine model

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The murine model of hindlimb ischemia (HLI) is among the most popular useful models to study vascular disease. However, from a physiological perspective, the available information regarding the murine vascular function regulation is practically inexistent. Our objective was to characterize the bilateral vascular response to a normobaric oxygen breathing (NOB) protocol in two groups (i) a control group, consisting of 16 healthy male C57BL/6 mice (8-27 weeks old), and an (ii) HLI model group, consisting of 9 male C57BL/6 mice (16 weeks old). All procedures involving animal experimentation were ethically supervised. Ischemia was surgically induced on the left limb under isoflurane anesthesia, while the right limb remained as the control. The NOB protocol included a 10 min stabilization period, a 10 min breathing a saturated oxygen atmosphere, and further 10 min for recovery. Mice were maintained under ketamine-xylazine anesthesia. The NOB procedure was applied once to the control group, and on day 0 (surgery) and 4, 6, 9, 12, 15 and 21 post-surgery days in the HLI group. Blood flow was recorded in both paws by LDF. On the HLI group perfusion was recorded with both LDF and LDI. Spectral characterization of the LDF signal was performed with Fourier and wavelet transforms. Nonparametric statistics were applied (p<0.05). Three main vascular responses were detected on the control group with NOB – bilateral perfusion decrease, bilateral perfusion increase and mixed response. For the HLI group no differences in perfusion between paws were found in day 0. In the HLI group, after surgery, the control paw consistently responded to NOB with a perfusion decrease, while for the ischemic paw, an increase in perfusion was consistently observed in all days. In these animals LDF and LDI perfusion signals were positively correlated in all recovery days in both paws. This approach also allowed the spectral characterization of the murine LDF signal and respective perfusion-regulating components frequency ranges (heart, respiration, myogenic, sympathetic and endothelial) which reinforces the potential usefulness of this animal model to look deeper into vascular physiology.


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.
Postnatal high fat diet alters gene expression in mouse mesenteric peri-vascular adipose tissue, but a pre-natal high fat diet does not

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The association between obesity and cardiovascular disease has been long established. More recently, the importance of the perivascular adipose tissue has been identified as a key mediator of vascular dysfunction (Van de Voorde et al., 2014). In addition to postnatal obesity, maternal obesity has also been shown to lead to long term changes in vascular function (Torrens et al. 2012; Stead et al. 2016) but whether maternal obesity alters the perivascular adipose remains unknown. The aim of this study was to investigate the effects of maternal and post-natal high fat diets on gene expression in perivascular adipose tissue.

Female C57BL/6J mice were fed either standard chow (C; 7% kcal fat, 18% kcal protein, 75% carbohydrates) or an obesogenic high-fat diet (HFD; 45% kcal fat, 17% kcal protein, 35% kcal carbohydrates) for 4-6 weeks prior mating and throughout gestation and lactation. At weaning, pups were transferred to either C or HFD to give four dietary phenotypes, CC, HFC, CH & HHHF (n=4-5 males). At 15 weeks of age, offspring were killed by cervical dislocation and perivascular fat from around the mesenteric arteries was dissected and snap frozen. Expression of genes involved in inflammation (IL6, CCL2, ChemR23) and adipokine signalling (adiponectin, cystathionine γ-lyase) were measured by qPCR. Data was analysed by 2-way-ANOVA for associations between prenatal and post-natal diet; significance was accepted at p<0.05. Post-natal high fat diet (CHF, HHHF) was associated with an increased expression of IL-6 (p<0.0005), CCL2 (p<0.05) and cystathionine γ-lyase (p<0.005) in perivascular adipose tissue relative to prenatal diets (CC, HFC). Expression of both the adipokine, adiponectin and the chemerin receptor (ChemR23) were similar across the four dietary groups (p>0.05). These data suggest that while a current obesogenic diet can impact upon expression in the perivascular adipose tissue, maternal obesity does not appear to be to lead to any long-term changes. Furthermore, the upregulation of IL-6 and CCL2 in the post-natal high fat groups fit with the previous findings that obesity leads to a pro-inflammatory phenotype.

Stead et al. (2016). J. Hypertens 34, 452-463

Torrens et al. (2012). PLOS one 7(12): e00671.


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Planar cell polarity genes frizzled-4 and frizzled-6 exert specific shaping role in arterial vessel morphogenesis

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Precise and comprehensive measurements of vascular network anatomy are crucial steps for the analysis of normal and pathologic vascular networks, and is of paramount importance for the understanding of several aspects of microcirculation [1]. Wnt/planar cell polarity signaling (PCP) pathway was found to be involved in angiogenesis [2]. The aim of this study was hence to investigate the contribution of 2 Wnt/PCP pathway receptors, frizzled 4 and frizzled 6, in 3D vascular network morphogenesis.

Experiments were performed on 10 to 12-week old frizzled 4 (Fzd4-/-; n=4) and frizzled 6 (Fzd6-/-; n=3) deleted mice and littermate controls (WT; n=7), in accordance with national and European institutional ethical rules. 2 hours after injection of anticoagulant and vasodilator treatment, mice were euthanized by pentobarbital intraperitoneal injection, exsanguinated, and perfused with a contrast medium (Neoprene latex and barium sulphate) via the brachiocephalic artery trunk. After dissection and fixation, kidneys were imaged with a high-resolution micro-CT imaging system with a voxel volume of 16 μm3, followed by subsequent 3D reconstruction of the arterial vascular networks. Computational treatment includes decompression of 3D networks into subtree data structures based on diameter-defined Strahler order (DDSO, fig. 1), calculation of overall geometric parameters, and fractal and branching pattern analyses. Statistical comparisons were performed by one-way ANOVA with post-hoc Tukey test, and considered significant when P<0.05. Statistical data are given as mean±SD.

DDSO number was 5 in WT and Fzd4-/-, and only 4 in Fzd6-/-, Both total vessel length L (in mm) total vessel volume V (in mm3), and fractal dimension Df were significant lower in fzd-deleted kidneys (WT:L=0.847±0.158; V=5.95±0.77; Df=2.07±0.11) (Fzd4-/-:L=0.26±0.086; V=2.07±0.17; Df=1.71±0.04) (Fzd6-/-:L=0.175±0.061; V=1.59±0.15; Df=1.54±0.09). Scaling characteristics such as vessel diameter were lower in Fzd4-/- and Fzd6-/-, whereas bifurcation angle distribution, around 90° form each DDSO, was not different from WT. Estimation of vessel resistance for each DDSO (R (DDSO)) based on Hagen-Poiseuille equation, showed a significant increase in R (DDSO) in Fzd4-/- and Fzd6-/-.

Taken together, our results evidence important differences between WT, Fzd4-/- and Fzd6-/- mice in the size and the complexity of the arterial vasculature, whereas on the other hand, the branching patterns were not found to be significantly affected. They show that the core Wnt/PCP PCP genes frizzled 4 and frizzled 6 play a pivotal role for vessel-branching morphogenesis. The proposed methodology was found
suitable for quantitative comparisons of vascular networks between different subgroups.

Fig 1: Characteristic kidney vascular networks for WT (A), frd4-/- (B), and fz66-/- (C) mice. DSSO of individual vessel segments are color coded.


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PCB330

Impaired microvascular function in metabolic syndrome is driven by macrophage-dependent hydrogen sulfide depletion

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Altered paracrine signaling from the perivascular adipose tissue (PVAT) to the underlying microvasculature is a major determinant of endothelial dysfunction in metabolic syndrome. The composition of PVAT is complex, comprised of both adipocytes and cells of the immune system, however, the relative contribution of individual cell types to vascular dysfunction remains obscure. This study tested the hypothesis that proinflammatory macrophages impinge on microvascular function by reducing the bioavailability of hydrogen sulfide (H₂S) in a mouse diet-induced obesity model of metabolic syndrome. Mesenteric resistance arterioles isolated from 20 week-old lean and obese mice were loaded with the fluorescent H₂S indicator SFT-AM (5 µM), mounted in a pressure myography chamber, pressurized and imaged confocally to assess [H₂S] in the smooth muscle and endothelial layers, and reported as mean ± S.E.M. of background corrected fluorescence units (F). In the smooth muscle layer, [H₂S] was 6.4 ± 0.5 F and 3.0 ± 0.2 F in lean and obese respectively. Similarly, [H₂S] was lower in the endothelium of vessels from the obese (3.4 ± 0.4 F) compared with lean (6.4 ± 0.4 F), P<0.001, one-way ANOVA, n=8. The same vessels were then precontracted with phenylephrine (1 µM), the vasodilatory response to acetylcholine (0.01-10 µM) measured, and differences between dose-response analyzed as an index of endothelial function. Vasodilation was impaired in vessels from obese animals (P<0.001; two-way ANOVA, n=4). Importantly, incubation with the H₂S donor GYY4137 (50 µM) restored H₂S levels and endothelial function in obese mouse vessels to levels comparable to those seen in lean controls. To assess the role of macrophages in driving these phenotypes, vessels from lean and obese mice were co-cultured overnight in the presence of macrophages purified by immunomagnetic separation from either lean or obese mice. In vessels from lean mice, [H₂S] in both smooth muscle and endothelium was decreased by exposure to macrophages from obese but not lean mice (P<0.001, one-way ANOVA, n=8), which was observed in parallel with impaired vasodilation (P<0.001; two-way ANOVA, n=4). Collectively, these data support a model in which proinflammatory macrophages resident in the PVAT interact with the microvasculature to promote endothelial dysfunction by reducing the bioavailability of H₂S.

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What about the vascular response to hyperoxia?

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Normobaric oxygen breathing (NOB) is a proven provocation test useful to assess the in vivo peripheral vascular function, especially by noninvasive technologies (e.g. Laser Doppler flowmetry - LDF and photoplethysmography - PPG). However, the vascular effects of hyperoxia are controversial, and regional differences in vascular reactivity are also known as a major source of inter and intraindividual variability. In this study we aim to characterize the human vascular response in the lower limbs assessed by LDF and PPG, to a 10 min NOB challenge. A group of 10 healthy subjects (mean age 20.9 ± 3.0 years old, both sexes) was selected after informed written consent and submitted to a standardized NOB procedure. Variables included, blood flow, measured on the inferior aspect of both feet toes by LDF and reflection PPG; transcutaneous (tc) O₂ pressure measured on the dorsum of one foot only by tc gasimetry; heart rate, calculated by PPG; and respiratory rate and depth by pneumography (PNG). Variables were recorded continuously for 30 min and measured in three phases: (i) for 10 min resting period while breathing room atmosphere, (ii) for 10 min NOB and (iii) for 10 min after, corresponding to recovery. Spectral analysis of the PPG, LDF and PNG signals was performed with both Fourier and wavelet transforms, and their range and maximal amplitude frequency compared. Non-parametric statistics were applied (p<0.05). NOB significantly increased ventilation depth and reduced the respiratory rate evoking hyperoxia, as evidenced by the significant increase in tcpO₂. Hyperoxia, in turn, produced two distinct vascular responses - 6 subjects responded with a significant perfusion reduction in both limbs while 4 responded with a significant perfusion increase in both limbs. In both vascular responses the LDF and PPG signals were found to be positively and significantly correlated. Spectral analysis revealed the same frequency amplitude for cardiac activity registered by LDF and PPG. The same coherence was found regarding respiratory activity signals from LDF, PPG and PNG. Significant differences for these bands’ frequency of maximal amplitude could not be found. So these opposite vascular effects registered under these conditions must be related with the modification of myogenic and/or endothelial activity induced by hyperoxia and should be further investigated. The present results confirm the controversy around this issue but also, suggest PPG, a long known accessible technology, as a useful tool to look deeper into vascular function quantification.
Peripheral microvascular reactivity might be easily challenged in vivo by postural modifications. Laser Doppler flowmetry (LDF) and photoplethysmography (PPG) are two well-known noninvasive optical technologies capable of providing reliable, continuous flow recordings by using different wavelengths to quantify perfusion at different levels of the dermal vascular networks. Our aim was to compare the bilateral vascular response to a leg lowering maneuver using both LDF and PPG. Ten subjects (both sexes, 26.0 ± 5.0 years old), were selected after informed written consent. The challenge maneuver consisted in three phases - 10 min baseline record keeping after informed written consent. The challenge maneuver consisted in three phases - 10 min baseline record keeping after informed written consent. The challenge maneuver consisted in three phases - 10 min baseline record keeping after informed written consent. 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Chronic exposure to AlCl₃ at human dietary levels increases blood pressure and promotes vascular dysfunction in rats
C.S. Martinez¹, J. Piagette¹, A. G. Escobar¹, D. V. Vassallo¹, M. Jesus Alonso², M. Salaises², F. Pecanha¹, M. Miguel² and G. Wiggers¹

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Aluminum (Al) is a significant environmental contaminant. This non-essential metal has been related with several diseases, mainly age-related neurological changes which oxidative and inflammatory disorders are the postulated toxicity mechanisms (Bonyd 2015). At cardiovascular system, there is not enough evidence of Al induced toxicity. We aim to investigate the effects of a 60-day Al exposure at doses similar to human dietary levels on cardiovascular system. 20 three-month-old male Wistar rats (± 300 g) were divided into two groups and received for 60 days in drinking water: a) Control - tap water; b) AlCl₃ - aluminum chloride at a dose of 8.3 mg/kg bw. Systolic blood pressure (SBP) was measured by plethysmography. Vascular function was studied in aortic and mesenteric resistance arteries (MRA) in isolated organ bath (Alvarez et al. 2007; Mulvany & Halpern 1977). Concentration-response curves to acetylcholine (ACh) and sodium nitroprusside were performed. Vasoconstrictor response to phenylephrine (PHE) in presence and absence of endothelium and in presence of NOS inhibitor (LNAME), potassium channels blocker (TEA), NAD(P)H oxidase inhibitor (apocynin), superoxide dismutase (SOD), non-selective COX inhibitor (indomethacin), selective COX-2 inhibitor (NS 398), and AT₃, selective receptor blocker (losartan), were analyzed. Systemic and vascular reactive oxygen species (ROS), lipid peroxidation and total antioxidant capacity, were measured. Results were expressed as mean and SEM, compared by t-test and ANOVA followed by Bonferroni test (α=0.05). Ethics Committee Approval 028/2014 - Unipampa. AlCl₃-exposure at low doses increased SBP (Ct: 120.3 ± 89.7 ± 3mmHg, P<0.05). Concentration-response curves to acetylcholine (ACH) and sodium nitroprusside were performed. 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Aqueous leaf extract of *Ageratum conyzoides* ameliorated indomethacin – induced gastric lesions in male albino rats

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In the folkloric medical practice, leaves of *Ageratum conyzoides* have been widely used for treatment of various ailments especially as an abrasive on surface wounds (1, 2). This six-week study investigated the activity of aqueous leaf extract of *A. conyzoides* in indomethacin-induced gastric lesions in male albino rats. Male wistar albino rats (180-250g body weight, aged 6 weeks) were randomly selected and divided into two groups - control and test made up of nine (9) rats each. Gastric lesions were induced in both the control and test groups by using indomethacin as indocid capsule (25mg/ml) in saline administered at a dose of 10mg/kg body weight subcutaneously according to the methods of Njar et al(3, 4). After four hours of ulcer induction, the rats in the test group were given 2mls of leaf extract of *A. conyzoides* orally three times each day up to forty days that chronic administration lasted and the animals had free access to normal rat feed with water ad libitum while the control group were maintained on normal rat feed and free access to water. At the end of 40 days, the animals were euthanized by inhalation of overdose of chloroform, the stomach was carefully dissected out and immediately immersed in 10% normal saline then in gradation of ethanol prior to histological processing and staining. Microscopically, the walls of stomach showed a marked reduction in the lesions’ sizes and submucosal oedema compared to the control group. These results are indicative of cytoprotective actions on indomethacin –induced lesions in the stomach by leaf extract of *A. conyzoides* in male rats.


Kasturi TR, Thomas M, Abraham EM (1973) J. Chem. 11: 91 - 95


Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

**PCB335**

**Pressure-induced oxidative activation of Protein Kinase G enables Ca\(^{2+}\) spark/BK channel-mediated vasoregulation of myogenic tone in resistance arteries**

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Activation of Ca\(^{2+}\)-sensitive, large-conductance potassium (BK) channels in vascular smooth muscle cells (VSMCs) by local, ryanodine receptor-mediated Ca\(^{2+}\) signals (Ca\(^{2+}\) sparks) acts as a brake on pressure induced (myogenic) vasoconstriction—a fundamental mechanism that regulates blood flow in resistance arteries. Here, we report a novel mechanism linking physiological intraluminal pressure within small arteries to Ca\(^{2+}\) spark/BK channel-mediated vasoregulation: oxidative activation of VSMC cGMP-dependent protein kinase (PKG) through formation of an oxidant-induced disulphide bond between cysteine residues (Cys42). Third-order mesenteric arteries were studied from knock-in mice expressing a PKG variant in which Cys42 is replaced with serine; the resulting PKG[PKG[PKG]\(^{C42S}\)] variant is resistant to oxidant-induced activation but can still be activated normally by cGMP. PKG[PKG[PKG]\(^{C42S}\)] arteries displayed significantly enhanced intraluminal pressure-induced constriction (80 mmHg) compared with WT arteries (WT: 32.4 ± 1.2\% [n = 26] vs PKG[PKG[PKG]\(^{C42S}\)]: 39.8 ± 2.5\% [n = 21], P < 0.05) and almost entirely absent BK vasodilation (1 \(\mu\)M Pacitaxel constriction; WT: 11.4 ± 1.8\% [n = 6] vs PKG[PKG[PKG]\(^{C42S}\)]: 1.3% ± 0.6\% [n = 5], P < 0.01). Furthermore, the non-specific PKG inhibitor DT-2 constricted WT arteries but had no effect on PKG[PKG[PKG]\(^{C42S}\)] artery diameters (3 \(\mu\)M DT-2 constriction; WT: 10.9 ± 3.1\% [n = 6] vs PKG[PKG[PKG]\(^{C42S}\)]: 1.7 ± 1.3 [n = 4], P < 0.01). Epifluores-
cent imaging of CM-H2DCFDA loaded arteries demonstrated pressure-induced oxidant production and Western blot protocols demonstrated both oxidant- and pressure-induced PKG dimerization in mesenteric arteries. Perforated patch clamp studies of mesenteric VSMCs revealed absence of spontaneous transient outward currents from PKG[C42S]VSMCs at -40 mV (WT: 0.92 ± 0.57 Hz [n = 6] vs PKG[C42S]: 0.06 ± 0.04 Hz [n = 6], P < 0.05) but conversely, whole cell voltage step protocols indicated equivalent BK channel I/V characteristics. High speed confocal microscopy of pressurized arteries loaded with the Ca2+ indicator Fluo-4 revealed significant reduction in Ca2+ sparks in PKG[C42S] arteries compared with WT (see figure). Importantly, exogenous H2O2 increased Ca2+ spark frequency in unpressurized WT arteries but not in PKG[C42S] arteries (see figure). Values reported are means ± SEM, compared by Student’s t-test. Our interpretation is that disablation of the oxidant activating mechanism in the PKG[C42S] mice reduces Ca2+ spark frequency, decreasing pressure-induced BK channel activation and thereby deactivating the BK channel-mediated negative feedback regulation of vasoconstriction. Therefore, our results support the novel concept of a negative feedback control mechanism that regulates arterial diameter through mechanosensitive production of oxidants to activate PKG and enhance Ca2+ sparks.

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The contribution of inward rectifying potassium channels to the potassium-induced relaxation of different arteries of rat hindlimb

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Moderate elevation of extracellular K+ ([K+]out) may cause relaxation of some arteries. Local elevation of [K+]out between endothelial and smooth muscle cells may be induced by endothelial-derived hyperpolarizing factor (EDHF). Also, an increase of [K+]out in tissue can happen during intensive organ metabolism. Activation of inward rectifier potassium channels (KIR) may contribute to the K+-induced relaxation because their hyperpolarizing influence increases during elevation of [K+]out. However, the contribution of KIR in vascular tone regulation may vary in different organs. In this regard, we studied the role of KIR in K+-induced relaxation in two functionally different vascular regions of rat distal hindlimb: arteries of skin and skeletal muscle.

We used saphenous artery, ventral branch of saphenous artery and arteries of lateral and medial heads of gastrocnemius muscle (sural artery) from male Wistar rats (277-440 g). Artery segments were placed in isometric myograph (DMT A/S). During preconstriction we registered relaxation induced by: (1) acetylcholine (range from 10-6 to 10-5 M), (2) elevation of [K+]out (from 4.5 mM to 19.5 mM). In order to identify EDHF component of acetylcholine-induced relaxation we inhibited NO-synthase (L-NNA, 10-4 M) and cyclooxygenase (indomethacin, 10-5 M). The contribution of KIR in relaxatory mechanisms was defined by KIR-blocker (Ba2+, 3·10-5 M). Ouabain (10-3 M) was used as a blocker of Na+/K+-ATPase. Expression of KIR mRNA (subtypes K2.1, K2.2, K2.3, K2.4) was studied by qPCR. GAPDH and 18S were used as reference genes. EDHF contribution to acetylcholine-induced relaxation was the most prominent in sural arteries, while in the skin arteries EDHF component of acetylcholine-induced relaxation was almost absent. In the sural arteries in the presence of Ba2+ EDHF-induced relaxation significantly diminished; combined inhibition of KIR and Na+/K+-ATPase completely suppressed EDHF-induced relaxation.

The K+-induced relaxation of the skin arteries was mild, meanwhile, the sural arteries relaxed almost completely (80-100%) at rise of [K+]out. KIR blockade led to a reduction of sural arteries relaxation, especially at high [K+]out concentrations (from 9 to 19.5 mM). Combined inhibition of KIR and Na+/K+-ATPase completely suppressed K+-induced relaxation. KIR2.2 were the most abundant among the KIR subtypes. Its expression was the highest in the sural arteries. Our results demonstrate that the skeletal muscle arteries incline more to relax at the general and at the local elevation of [K+]out than the skin arteries. In both cases K+-induced relaxation diminished during a KIR blockade. All in all, KIR are more essential for the K+-induced relaxation of the skeletal muscle arteries, than of the skin arteries, as it corresponds with the functions of these arteries in the organism.

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The AMP-activated Protein kinase (AMPK) reduces calcium sensitivity of microvascular smooth muscle by interfering with thin filament dynamics

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The AMPK is an enzyme involved in the control of metabolism. Recent studies have shown that in parallel to its metabolic effects, AMPK can also affect blood flow in microvessels, thereby adding to the oxygen supply of the tissue. We have shown before that AMPK can induce short term vasodilation by reducing the cytosolic calcium by activation of SERCA1. Here we studied whether AMPK can also affect the calcium sensitivity of the smooth muscle contractile apparatus in these vessels. Studies were performed in segments of small murine mesenteric arteries which were freshly isolated. They were cannulated at both ends, loaded with FURA 2 AM to allow measurements of smooth muscle calcium changes and
studied under constant pressure in an organ bath containing MOPS buffer. Diameter changes were recorded using videomicroscopy. Exposure of de-endothelialized vessels to the AMPK stimulator PT1 (30 μM) after blockade of SERCA (Thapsigargin 1μM) induced a slowly developing vasodilatation of vessel pre-constricted with KCl (n= 5) which started after about 5 min and reached a maximum dilation of 53 ±5% after 20 min. Increasing the extracellular calcium concentration from 0 to 3 mM in several steps induced a stepwise constriction of vessels pretreated with high potassium solution to open Ca<sub>v</sub> channels. When the vessels were pre-exposed to either of the AMPK activators, A769662, or PT1, the same increase in extracellular calcium (leading to similar increases of Ca<sup>2+</sup>) resulted in a significantly smaller calcium-dependent vasoconstriction as observed under control conditions (reduction by 17 %, n= 4 and 19%, n= 5 for A769662 and PT1, respectively, p<0.01).

This inhibition of constriction went along with a significant increase of G-actin levels in vascular smooth muscle (n=4). Moreover, microscopic analysis of the actin cytoskeleton in smooth muscle cells freshly isolated from mesenteric arteries (n= 12) yielded a reduction of actin branching points and a reduction of actin fibre thickness after treatment with PT1 as studied by Laser scanning microscopy using deconvolution techniques.

These studies suggest that AMPK may reduce calcium sensitivity of the contractile apparatus by affecting cytoskeletal dynamics of microvascular smooth muscle. This mechanism could contribute to the long term maintenance of vasodilatation without alteration of smooth muscle calcium levels which may be important for other non-contractile functions of vascular smooth muscle.


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**PCB339**

**Purinergic-dependent contraction of small intrapulmonary veins: role in a pulmonary arterial hypertension rat model**

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**Background:** For a long time, the vasoactivity of pulmonary veins has been debated. Increasing evidences about the role of pulmonary veins to the total pulmonary vascular resistance has been particularly well supported by studies associated to development of fetal and neonatal pathology. In contrast, the vasoactivity of pulmonary veins in adult mammals has been more controversial and largely unexplored. Nevertheless, the alterations of the vascular tone of pulmonary veins are believed to play an important role during the development of cardiovascular diseases including Pulmonary Arterial Hypertension (PAH). In the lung, nucleotides are released from the cytoplasm of many cells including endothelial, smooth muscle and epithelial cells under physiological and pathological conditions. Particularly, release of ATP and UTP has been found elevated under certain pulmonary diseases. This extracellular ATP and UTP binds to P2Y<sub>14</sub> receptors, widely expressed in blood vessels, attributing a pivotal role in the control of vascular tone. However, there are no studies on either the effects of ATP and UTP on small intrapulmonary vein (SPV) contraction or the mechanisms that couple purinergic signalling to PAH. Here we have used ‘living’ lung slices and phase-contrast video microscopy to investigate, for the first time, purinergic-dependent dynamic changes in SPV contraction in PAH rats.

**Methods and Results:** These studies were approved by the ethics committee of Fac. of Medicine, University of Chile (CBA#0614 FMUCH). Lung slices (150μm thick), from healthy and Monocrotaline (MCT)-induced PAH Sprague Dawley rats (200gr), in a vibratome were performed. ATP and UTP-induced SPV contraction was recorded using phase contrast video microscopy. Statistical differences (p<0.05) were performed using non-parametric tests. After 21 days of a single subcutaneous injection of MCT, (60mg/Kg) the rats develop PAH, including right ventricle hypertrophy. Also, in PAH rats there was an exacerbated venous constriction in response to UTP (EC<sub>50</sub>= 6,9±2,45µM) versus healthy rats (EC<sub>50</sub>= 19±5,01µM). Similarly, ATP-dependent vasoconstriction was strongest in PAH (EC<sub>50</sub>=14,3±1.9µM) in comparison with healthy rats (EC<sub>50</sub>=28.6±3.11µM). ATP and UTP-induced SPV constriction was strongly inhibited by Suramin, a non specific antagonist of purinergic receptors. Significant P2Y<sub>2</sub> inhibition (41.5±9.0%) with ARC118925XX was predominant only in healthy rats, but not PAH rats, suggesting a main role of P2Y<sub>2</sub> in PAH. The presence of P2Y<sub>2</sub> and P2Y<sub>4</sub> receptors in co-localization with smooth muscle cells was demonstrated by indirect immunofluorescence.

**Conclusion:** These results suggest a novel mechanism involving P2Y<sub>2</sub>-receptor in exacerbated vasoconstriction observed in PAH. The study of purinergic therapies to improve quality of life of PAH patients is promising.

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**PCB340**

**Effect of cyclooxygenase inhibition on reactive hyperaemia and muscle vasodilator responses to mental stress in young Black Africans (BAs) and White Europeans (WEs)**


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People of Black African ethnicity (BA) have greater prevalence of hypertension than those of White European (WE) ethnicity and greater risk of developing hypertension-associated cardiovascular disease (CVD). Correspondingly, BAs show blunted flow-mediated dilatation of brachial artery and blunted forearm vasodilator responses to mental stress compared with WE:s: decreased Nitric oxide (NO) availability has been implicated<sup>1,2</sup>. Prostaglandins (PGs) also contribute to endothelium-dependent dilatation, but their involvement in the forearm vasodilator response to mental stress, has not been explored in BAs, or WE:s. Thus, we performed experiments on young recreationally-active BAs (18-26 years; n=9) and WE:s (n=10), who were resident in the UK. Arterial blood pressure (ABP) was initially recorded by sphygmometry and continuously monitored by finger photoplethysmography. Forearm blood flow (FBF) was recorded at intervals by venous occlusion.
pulmonary vascular conductance (FVC) was calculated as FBF/ABP. Responses evoked following release of arterial occlusion for 2 min (reactive hyperaemia) and by 5 sound stimuli: S1–5; 100 dB, 2 kHz, for 30 s each at randomized intervals of 5–10 min (mental stress), were recorded on 2 different days >2 weeks apart, without or after the cyclooxygenase, inhibitor aspirin (600 mg p.o.). Without COX inhibition, mean ABP at baseline for the protocol was higher in BAs than WEs: 97 ± 4.1 vs 78 ± 3.3 mmHg respectively ($^*$: BA vs WE; $P<0.05$); after COX inhibition, mean ABP was 93 ± 6.0 in BAs vs 82 ± 3 mmHg in WEs. Baseline FVC was similar in WEs and BAs: 0.06 ± 0.004 vs 0.05 ± 0.01 conductance units (CU) respectively. However, reactive hyperaemia was greater in WEs than BAs and COX inhibition attenuated the peak change in FVC in WEs from 0.51 ± 0.066 to 0.38 ± 0.063 $^*$ CU, but not in BAs: peak change in FVC 0.38 ± 0.063$^*$ vs 0.35 ± 0.04 CU ($^*$: before vs after aspirin; $P<0.05$). Without COX inhibition, WEs showed increases in FVC in response to S1–S5 and they were not affected by COX inhibition: +0.011 ± 0.001 vs +0.019 ± 0.013 at 15 s in S1. By contrast, without COX inhibition, BAs showed an increase in FVC, but after COX inhibition S1–S5 evoked decreases in FVC indicating vasoconstriction: +0.017 ± 0.001 vs -0.011 ± 0.007 CU at 15 s in S1. These results indicate that both reactive hyperaemia and forearm vasodilator response to mental stress are blunted in young BAs relative to WEs. We propose that vasodilator PGs make little contribution to the blunted reactive hyperaemia in BAs, but help preserve the forearm vasodilator response to mental stress in the face of reduced NO availability. These characteristics may be early predictors of hypertension and CVD in BAs. Campia U et al (2002). J Am Coll Cardiol 40, 754–60 Cardillo C et al (1998). Hypertension. 31, 1235–1239 Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB341

Inhibition of NF-kappa B classical activation suppresses the inflammatory response in endothelial cells exposed to acute high shear stress: Implications for vein graft failure

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The long saphenous vein (LSV) is still the most commonly used conduit in coronary artery bypass graft (CABG) due to the ease of harvest and availability. However, its use is complicated by the development of vascular inflammation, neointimal hyperplasia and accelerated atherosclerosis leading to compromised graft efficacy. Venous transposition into the arterial circulation during CABG is a particularly striking example of acute increases of shear stress rates, with venous endothelial cells (ECs) having to adapt to up to 10-fold increases in shear stress. In response, ECs activate MAPK pathways involving p38 and ERK1/2, as well as transcription factors NF-kappa B and Ap-1. However, the upstream activation of NF-kappa B in the vein graft is considerably less well defined.

We hypothesise that, through inhibition of the NF-kappa B canonical pathway, the pro-inflammatory response of endothelial cells to acute shear stress can be dampened and graft patency improved. Human umbilical venous endothelial cells (HUVECs) were cultured either in static conditions or exposed to acute shear stress at 12 dyn/cm$^2$ for different time-points using parallel plate flow chambers. Prior to shear stress, if treatments were used, cells were pre-treated for one with IKK$\beta$ inhibitor, BAY11-7085, or DMSO control. mRNA levels were assessed by RT-qPCR. Total cell or fractionated protein was assessed by Western blotting and an ELISA-based TransAm p65 DNA binding assay (ActiveMotif).

The exposure of HUVECs to acute shear stress for 30 and 90 minutes significantly increased nuclear translocation of NF-kappa B (p65) compared to static controls (n=3, 3.06 ± 0.24 and 1.79 ± 0.008 fold change ± SEM respectively, P<0.05). Nuclear translocation at 30 and 90 minutes was associated with significant I kappa b alpha degradation (n=3, 0.43 ± 0.08 and 0.35 ± 0.1 respectively, P<0.05). Activation of p65 following exposure to shear stress at 30 minutes (n=3, 1.25 ± 0.03, P<0.05) was also confirmed using DNA binding assay. Additionally, p65 was phosphorylated at Serine residue 276 under conditions of acute high shear stress at both 30 and 90 minutes (n=6, 1.34 ± 0.12 and 1.25 ± 0.12, P<0.05). The NF-kappa B inhibitor BAY11-7085 at 10µM significantly reduced the induction of mRNA for the pro-inflammatory genes MCP-1, ICAM-1, IL-8 and IL-6 at 240 minutes (n=3, ~85-95% reduction, P<0.05). Our data suggest that inhibition of the activation of the canonical NF-kappa B pathway appears to dampen the pro-inflammatory response in endothelial cells to acute high shear stress and may provide a novel pre-treatment option for vein graft failure.

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PCB342

Hemin treatment decreases oxidative stress, vascular inflammation and remodelling in chronically hypoxic and pulmonary hypertensive newborn lambs

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The neonatal lamb gestated and born at high-altitude (HA) chronic hypoxia shows increased pulmonary arterial pressure, vascular hyperreactivity and remodelling, a triad that characterizes the neonatal pulmonary hypertension. These changes are mainly the result of a lesser capacity of the enzyme to produce cGMP, by a reduced enzymatic function due to a sGC at low tissue concentration (1). Hemin is an heme oxygenase inducer, resulting in an elevated CO and cGMP production in HA neonatal lambs. Further, hemin administration could also enhance sGC protein expression and function, and decrease cellular processes such as inflammation and oxidative stress. Therefore, we proposed that hemin administration is able to augment cGMP and reduce the pulmonary vascular inflammation and oxidative stress.

Twelve pulmonary hypertensive newborn sheep, born and raised at Putre Research Station, INCAS (3,600 m) were
PCB343

20-HETE impedes trophoblast migration and contributes to hypertension in the RUPP rat model of placental ischemia

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Recent data suggests a possible role of CYP450 vasoactive eicosanoids, particularly 20-hydroxyeicosatetraenoic acid (20-HETE), in preeclampsia (PreE). The goal of this study was to determine a role for 20-HETE in trophoblast proliferation and migration, and maternal hypertension during PreE. Placental vascular CYP450 enzymatic activity and circulating eicosanoids were measured in normal pregnant (NP) and PreE patients and in NP rats and reduced uterine perfusion pressure (RUPP) rat model of placental ischemia. Mean arterial pressure (MAP) was assessed in NP and RUPP rats with and without chronic 20-HETE blockade with HET0016. To examine the functional role for CYP450 metabolites in trophoblasts function, proliferation and migration were assessed in trophoblast cultures in the presence of exogenous 20-HETE and inhibitors of 20-HETE and CYP450. PreE women displayed increased CYP4A expression and 20-HETE compared to NP women. Furthermore, the ratio of circulating 20-HETE:EETs was increased in PreE compared to NP women and this trend was observed in the RUPP rat compared to NP. MAP was elevated in RUPP rats and decreased in RUPP+HET0016 rats. Trophoblasts proliferation increased with exogenous 20-HETE and decreased in the presence of a 20-HETE inhibitor, HET0016. In contrast, trophoblast migration was reduced in the presence of exogenous 20-HETE (1µM). Moreover, specific inhibition of 20-HETE in the RUPP rat significantly lowered blood pressure. Alterations in eicosanoid pathways, specifically 20-HETE, may contribute to pathophysiology of PreE and may be an important target for drug discovery to improve management of the disease.

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PCB344

Hypoxia and ischemia regulate placental syndecan expression and shedding

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Preeclampsia, a hypertensive disorder of gestation, affects ~5% of the population, and is a leading cause of fetal and maternal morbidity and mortality. While the origins of the disease are unclear, it is commonly accepted that placental ischemia/hypoxia is a central causative factor. In response, the placenta secretes soluble factors which cause the maternal manifestation of the disorder. Among the pathways believed to be important in this response is activation of innate immune mechanisms, such as inflammatory cytokines. It has been widely reported in sepsis that vascular inflammation induces remodeling and shedding of the endothelial glycolayx, in particular cleavage and shedding of members of the integral membrane syndecan family. It is now known that these shed fragments have biological activity and act as potent inducers of the innate immune and inflammatory response. What is less well known is the presence of a functional glycolayx in the placenta. Here we hypothesize that hypoxia will induce changes in syndecan production and shedding in vitro, and that chronic placental ischemia will cause syndecan shedding from the placenta in vivo. To examine the effects of hypoxia on placental cells, we cultured BeWo cytotrophoblast cells in oxygen tension mimicking healthy (8%) and ischemic (1%) placentas, and examined syndecan-1 mRNA and soluble SDC1 in the media. In response to hypoxia, SDC1, 2, and 4 mRNA increased significantly (14, 6.5, and 2.5 Fold, respectively, p<0.05 each), as assayed by TaqMan qRT-PCR. SDC3, in contrast, exhibited no significant difference in expression. Soluble SDC1 in the media, meanwhile, was significantly increased by ~50% compared to normoxic control as measured by ELISA (41 ± 4 vs 63 ± 4 pg/ml, p<0.05). To determine the effects of chronic placental hypoxia/ischemia on SDC, we utilized the reduced uterine perfusion pressure (RUPP) rodent model. RUPP rats exhibited significantly increased blood pressure on gestational day 19 when compared to their control counterparts (105 ± 4 vs 122 ± 6 mmHg, p<0.05). Interestingly, soluble circulating SDC1 in the maternal circulation was significantly increased in RUPP rats when compared to their control counterparts (11 ± 4 vs 5 ± 2 pg/ml, p<0.05).
Poster Communications

99 ± 20 pg/ml, p<0.05). These data suggest that hypoxia/ ischemia differentially regulate SDC isoforms in vitro. Furthermore it is possible that isoform specific or pan-syndecan circulating levels of syndecans could serve as a biomarker for preeclampsia. The importance of syndecan fragments in the inflammatory cascade during preeclampsia remains to be determined, as do the mechanisms regulating syndecan production and cleavage in these tissues.

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PCB346

Vascular function in the ANGII DOCA salt mouse model of chronic kidney disease

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Chronic kidney disease (CKD) is a global disease affecting 10% of the world population (Eckardt et al., 2013). Many patients only start treatment once heavy fibrosis/inflammation are established and drugs available to treat CKD at this stage are limited. It is therefore increasingly necessary to target kidney disease at much earlier stages. Many studies point to impaired vascular function and hypoxia as an early pathophysiological event preceding CKD (Fine & Norman, 2008). Understanding this vascular pathology may help identify new therapeutic targets for early intervention in renal disease. Most models of experimental CKD induce rapid progression to fibrosis, inflammation and renal impairment. Here, we describe a low level, multi-hit approach to induce vascular dysfunction in C57BL6 mice prior to development of renal fibrosis. Mice were anesthetised with isofluorane and implanted with a minipump containing angiotensin II (ANGII; 100ng/kg/min) and a deoxycorticosterone pellet (DOCA; 50mg). On recovery, mice were fed a 3% sodium diet (ANGII DOCA salt mice; n=11) for 4 weeks. Control mice were sham-operated, with implantation of minipumps containing 0.9% saline and a blank pellet and were kept on standard chow (SHAM mice; n=12). In one cohort, blood pressure was measured by tail plethysmography and renal function was measured under Inactin anaesthesia, as previously described (Bailey, Mullins, & Kenyon, 2009). In another cohort (of the same group sizes) mice were killed by cervical dislocation, kidneys were harvested and snap frozen or formalin fixed. The aorta was microdissected and mounted on a wire myograph. By week 4, ANGII DOCA salt mice had elevated systolic blood pressure compared to sham-operated control animals (ANGII DOCA salt: 136.5±3.2mmHg SHAM: 113.3±1.9mmHg, p<0.001). Renal function was unchanged: glomerular filtration rate was similar in both groups (ANGII DOCA salt: 0.15±0.05ml min⁻¹ SHAM: 0.23±0.04ml min⁻¹, p=0.05) and there was no evidence of albuminuria. Collagen staining identified perivascular fibrosis in ANGII DOCA salt mice kidneys. Vascular function also differed in ANGII DOCA salt mice compared to SHAM animals. Aortas from ANGII DOCA salt mice were more responsive to phenylephrine (Mean max response, ANGII DOCA salt: 124.5±14.9 %KPSS SHAM: 90.0±11.8 % KPSS p<0.01) and acetylcholine (Mean max response, ANGII DOCA salt: 5.0±12.4 % relaxation, SHAM: 31.0±7.5 % relaxation) than those from SHAM mice. Our multi-hit model captures a phenotype of hypertension with vascular dysfunction in C57BL6 mice. GFR was normal and the absence of proteinuria is consistent with preserved renal function. We are currently using this model to examine the contribution of purinergic signalling to altered vascular function.


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