1. DORSO-VENTRAL EXPRESSION OF POLYSIALYLATED NEURAL CELL ADHESION MOLECULE IN THE RAT DENTATE GYRUS FOLLOWING LEARNING

D. Scully, S.K. Mulvany, K.J. Murphy, C.M. Regan
Applied Neurotherapeutics Research Group, Conway Institute, University College Dublin.

Lesion studies have suggested afferent/efferent pathways distributed through the dorso-ventral axis of the hippocampus to process task-specific information for long-term memory consolidation. As evidence for neuroplastic change that accompanies memory consolidation within these distinct pathways is lacking, we investigated the transient increase in hippocampal granule cell neural cell adhesion molecule polysialylation (NCAM PSA) throughout its dorso-ventral axis following training in spatial learning and avoidance conditioning paradigms. Given the hippocampus is a C-shaped structure, the brain was sectioned in two directions. Initially, coronal sections were collected in a rostro-caudal manner and subsequently horizontal sections in a dorso-ventral manner. Thirteen levels, separated by 0.5mm intervals equally distributed over the entire dorso-ventral axis of the hippocampus, were investigated for polysialylated cell expression. The frequency distribution of polysialylated cells was found to decrease over the dorso-ventral axis of the hippocampus. Following passive avoidance training the greatest proportional increase in polysialylated granule cells was observed at 7.1mm ventral to bregma (Trained: 20.48±0.43 (mean ± sem) vs. Passive: 14.52±0.41). By contrast, water maze spatial learning resulted in an increase of polysialylated cells restricted to a dorsal region 3.3mm caudal to bregma (Trained: 36.28±2.19 vs. Passive: 28.68±1.38, p<0.05, t-test, n=5). Localisation of the neuroplastic response to spatial learning in the dorsal aspect of the hippocampus and to the ventral hippocampal aspect following avoidance conditioning supports the lesioning studies and, further, gives a cellular typography to further investigate the discrete afferent/efferent pathways serving different forms of learning.

Funded by Enterprise Ireland. KJM and CMR are Science Foundation Ireland investigators. The University Ethics Committee approved all experiments.
2. INFLUENCE OF RAT STRAIN AND SUPPLIER ON GENERAL BEHAVIOUR AND SPATIAL LEARNING

J.S. Loscher, C.W. Thomas, K.J. Murphy, C.M. Regan
Applied Neurotherapeutics Research Group, Conway Institute, University College Dublin.

Wistar and Sprague Dawley (SD) rat strains are commonly used in neuroscience to dissect the molecular and genetic underpinnings of learning and memory formation. Surprisingly, few direct comparisons between rat strain, supplier and behavioural outcome have been investigated.

We have sourced Wistar and Sprague Dawley (SD) strains from Harlan (WistarH and SDH) and Charles River Laboratories (WistarCRL and SDCRL), maintained them in our Biomedical Facility, and compared their F1 progeny for spontaneous behaviour in an open field and performance in the water maze spatial learning. Throughout the study all the animals were housed in identical conditions to eliminate environmental variables. When compared in the open-field paradigm, WistarH exhibited greater exploratory behaviour as compared to SDH (WistarH vs. SDH; 231.1 ± 10.31 (mean ± sem) vs 189.9 ± 7.41; p< 0.001, ANOVA, n=30), confirming a strain difference in behaviour. No difference in strain or supplier was found in acquisition of the water maze spatial learning paradigm (e.g. WistarH vs SDH escape latencies; 24.76s ± 6.064 to 7.205 ± 1.943 vs 29.547s ± 3.516 to 12.368s ± 2.882; p> 0.05). The WistarH, however, failed to show a quadrant bias in the 24h probe trial when compared to the WistarCRL (% time spent in target quadrant; 19.9% ± 3.119 vs chance (25%); p> 0.05), suggesting differences in their ability to consolidate the task.

These results suggest that strain and supplier have a strong impact on behaviour and memory consolidation.

*Funded by Enterprise Ireland. KJM and CMR are Science Foundation Ireland investigators. The University Ethics Committee approved all experiments.*
3. TRANSCRIPTIONAL EVENTS IN RAT DENTATE GYRUS FOLLOWING PASSIVE AVOIDANCE LEARNING

N.C. O’Sullivan, L. Conboy, C.M. Regan, K.J. Murphy
Applied Neurotherapeutics Research Group, Conway Institute, University College Dublin.

The consensus view is that a learning event initiates a cascade of molecular signals, which culminates in alterations in gene activity. These, in turn, mediate synaptic remodelling, in particular within the hippocampus, a structure vital to memory formation. To understand the molecular underpinnings of these morphological events we probed DNA microarrays with mRNA isolated from the rat dentate gyrus at increasing times following passive avoidance learning. Using 2-fold change, gene turned ON and gene turned OFF criterion, we found approximately 500 genes to be transcriptionally regulated across a 24h post-training period. Two independent methods of verification were used to show that these transcriptional events were biologically relevant. The transcriptional regulation of 4 genes was validated by real-time PCR; low-density lipoprotein receptor-related protein (LRP3), alpha-actin, synaptosomal-associated protein 25 kDa (SNAP25) and N-ethylmaleimide sensitive factor (NSF). Furthermore, LRP3, SNAP25 and NSF showed corresponding changes in protein expression using immunoblotting procedures. When the master list of 500 genes were clustered, in a functional and temporal manner, they revealed crucial periods of molecular regulation. Genes up-regulated in the 0-3h post-training period included those related to transcription/translation events and structural proteins, consistent with the structural rearrangements previously reported to occur at 3-6h post-training. The most striking feature, however, was the profound down regulation, across all functional groups, at the 12h post-training time during which over 40% of all modulating genes decreased. This latter observation suggests that memory consolidation requires periods of activity-dependent synaptic quiescence.

KJM and CMR are Science Foundation Ireland investigators. The University Ethics Committee approved all experiments.
AN INVESTIGATION OF THE ROLES OF P75NTR AND TRK RECEPTORS IN RECOGNITION MEMORY IN THE RAT.

A. Hennigan, A. Kelly
Trinity College Institute of Neuroscience, Department of Physiology, Trinity College, Dublin 2, Ireland.

The neurotrophins are a group of growth factors associated with the growth, differentiation and survival of neurons. More recently it has been shown that they are intrinsically involved in many forms of synaptic plasticity including long-term potentiation (LTP), learning and memory. Neurotrophins mediate their effects via two classes of receptors; the Trk receptors, associated with cell survival and plasticity, and the p75 neurotrophin receptor (p75NTR), associated with both cell survival and cell death. Here we investigated the effects of alterations in neurotrophin receptor expression on hippocampal-dependent learning in the rat.

An object recognition task was used to test the ability of rats to differentiate between novel and familiar objects after a 24h time delay. An inbred strain of Wistar rat (GH), which we have previously demonstrated to be deficient in nerve growth factor (NGF) and Trk, and normotensive (N) controls were used. N and GH rats (male, 250g, n=24) were injected with either saline (0.9% w/v) or bacterial lipopolysaccharide (LPS; 100ug/Kg, i.p) on the first day of the task 4 hours prior to behavioural training. We administered an LPS injection as we had previously found this to result in increased p75NTR and decreased Trk receptor expression in the rat hippocampus. Animals were sacrificed immediately after testing on day 2 and the dentate gyri and hippocampi were removed for analysis. The saline-treated GH rats demonstrated a significant impairment in learning as indexed by a reduced exploration of the novel object (60.69 ± 9.33%; mean ±SEM, p<0.05, ANOVA, N=6) when compared with N controls (69.83 ± 3.28%). Furthermore, LPS injection resulted in learning impairments in both groups (54.38 ± 13.85% and 54.73 ±12.51% for N and GH respectively). Western immunoblot analysis revealed that the learning impairments were associated with changes in MAPK signalling and neurotrophin receptor expression in the dentate gyrus and hippocampus.

This data is consistent with the hypothesis that changes in the expression of neurotrophin receptors in the hippocampus results in impairments in hippocampal-dependent learning in the rat.

The authors acknowledge grant support from the HEA.
Evidence suggests that inflammation is a significant contributor to pathology in a number of neurodegenerative disease states. In this regard, interleukin-1β (IL-1β) is a pro-inflammatory cytokine that plays a key role in initiating the immune response within the central nervous system (CNS). The actions of IL-1β can be regulated in vivo by interleukin-1 receptor antagonist (IL-1ra); a molecule that prevents IL-1 from binding to, and signalling through, the IL-1 type I receptor. Evidence is emerging to suggest that the monoamine neurotransmitter noradrenaline elicits anti-inflammatory actions in the CNS, and consequently may play an endogenous neuroprotective role where inflammatory events contribute to pathology. In this study we report that noradrenaline induces production of the interleukin-1 receptor antagonist (IL-1ra) in a mixed glial cell preparation (Control: 402 ± 85 pg/ml; Noradrenaline (10 μM): 2055 ± 414 pg/ml) (n=4-8; P<0.01; One-way repeated measures ANOVA followed by a Student Newman-Keuls test), without significantly altering production of IL-1β. The ability of noradrenaline to increase IL-1ra production was blocked by the non-selective β-adrenoceptor antagonist propranolol, and also by the selective β2-adrenoceptor antagonist butoxamine, but not by the selective β1-adrenoceptor antagonist metoprolol. These data indicate that the β2-adrenoceptor mediates that ability of noradrenaline to increase IL-1ra production. Further evidence to support this hypothesis stems from the fact that the selective β2-adrenoceptor agonist salbutamol, mimicked the ability of noradrenaline to increase IL-1ra production (Control: 435 ± 93 pg/ml; Salbutamol (10 μM): 1338 ± 373 pg/ml) (n=8). Consistent with the ability of β-adrenoceptors to activate the cAMP-protein kinase A pathway, the stable cAMP analog di-butryl cAMP mimicked (Control: 2319 ± 462 pg/ml; di butryl-cAMP (100 μM): 3857 ± 919 pg/ml) and the PKA antagonist RP-cAMPs blocked the ability of noradrenaline to increase IL-1ra production. These data further demonstrate that noradrenaline is a neurotransmitter with anti-inflammatory actions, and we suggest that the ability of noradrenaline to increase IL-1ra production from glial cells may contribute to its anti-inflammatory/neuroprotective actions in the CNS.

The authors acknowledge grant support from Science Foundation Ireland.
6.
IDENTIFICATION AND BIOCHEMICAL CHARACTERISATION OF A SERINE PROTEASE-MEDIATED CELL DEATH PATHWAY ACTIVATED IN HUMAN ACUTE MYELOID LEUKAEMIC HL-60 CELLS

A. O’Connell, A. Samali, C. Stenson-Cox
Apoptosis Research Cluster, National Centre for Biomedical Science and Engineering, NUI Galway

An increasing number of studies demonstrate that the general protein kinase inhibitor staurosporine can activate features of cell death under caspase-inhibiting circumstances. The proteolytic mechanisms required for cell death under these circumstances have not been elucidated. Here we set out to determine what proteolytic networks were responsible for sts-induced human acute myeloid leukaemic HL-60 cell death under circumstances of caspase-inhibition.

We found that HL-60 cells exposed to staurosporine (sts) and treated with the general caspase inhibitor Z-VAD.fmk still died in an apoptosis-like fashion, exhibiting cytochrome c release, nuclear shrinkage and fragmentation, DNA laddering and apoptotic-associated morphology. Measurement of DEVD-dependant caspase activity demonstrated a Z-VAD.fmk-sensitive increase in sts-treated HL-60 cells (decreasing from 0.47 ± 0.14 to 0.07 ± 0.05 nM AMC/min/mg protein). This data suggests a simultaneous activation of at least two death systems within the same cell, one regulated via caspase-dependent mechanisms and other being a caspase-independent system. To determine the components of the latter, HL-60 cells were co-treated with sts and various inhibitors of non-caspase proteases. It was found that only inhibitors of chymotrypsin-like serine proteases (TPCK; (N-tosyl-L-phenylalanine chloromethyl ketone), DCI (3,4-dichloroisocoumarin) and FFCK (5(6)-carboxyfluoresceinyl-L-phenylanyl-chloromethyl ketone)) protected the cells from nuclear shrinkage and fragmentation induced by staurosporine. TPCK also abolished apoptosis-associated morphology, oligonucleosomal DNA fragmentation, and partially prevented caspase-3 processing and activity (decreasing from 0.47 ± 0.14 to 0.3 ± 0.02 nM AMC/min/mg protein); however, it did not prevent sts-induced cytochrome c release. Activation of chymotrypsin-like proteolytic activity as detected through cleavage of the substrate AAF.AMC, was observed in apoptotic and apoptotic/Z-VAD.fmk treated cells. Activity measured as nM AMC/min/mg protein rose from basal levels 5.2 ± 1.7 in response to sts to 7.2 ± 0.2, persisted in Z-VAD.fmk treated samples at 6.5 ± 1 but was prevented by TPCK treatment 5.3 ± 1. FFCK, a fluorescent tag of active serine proteases was used in conjunction with anti-fluorescein antibodies in a western blot analysis to demonstrate activation of a 16kDa protein species under conditions of apoptosis. In summary we have identified a novel serine-protease mediated route to cell death that can take place in parallel to caspase activation in HL-60 cells. All results shown are representative of three independent experiments and are represented as means ± SEM (n=3).

The authors acknowledge grant support from IRCSET, SFI, NUI and NUIG.
7.
THE DEVELOPMENTAL ROLE OF THE FIRST ZEBRAFISH GENES ACTIVATED DURING ZEBRAFISH DEVELOPMENT.

S. O’Boyle¹, R. T. Bree¹, S. McLoughlin¹, S. Schindler³, F Müller³, M. Grealy², L. Byrnes¹
¹Biochemistry Department, ²Pharmacology Department, and National Centre for Biomedical Engineering and Science, National University of Ireland, Galway, Ireland. ³Institute of Toxicology and Genetics, Forschungszentrum Karlsruhe, D-76021, Germany.

The midblastula transition (MBT) is a critical event in the early embryo that governs the transition from maternal to zygotic control of development. Prior to the MBT, cell divisions are rapid and synchronous. During this time there is abundant DNA synthesis and little, if any transcription. Occurring at the tenth cell-division cycle in the zebrafish, the MBT marks the activation of zygotic genes, which may function in the entry to subsequent major developmental processes such as gastrulation. Our objective was to identify and characterise these genes in order to better understand vertebrate embryogenesis and the gene interactions therein. To achieve this, we conducted Suppression Subtractive Hybridisation (SSH) on RNA from pre- and post-MBT embryos, and constructed a library of over 1,000 cDNAs enriched for those that are differentially expressed. Differential screening and DNA sequencing yielded 49 different cDNAs. Sequence analysis has putatively identified 38 of these genes and 11 represent novel zebrafish genes. Many of the recognized genes have potential roles in central aspects of subsequent development including transcription, cell motility and cell-cycle control. We have also identified groups of genes as having potential involvement in retinoid acid signalling, protein processing and neural function. Semi-quantitative RT-PCR and whole mount in situ hybridisation confirmed the validity of the SSH screen and illustrated the expression patterns of these genes pre- and post-MBT. The majority of these cDNAs show increased expression after the MBT. Investigation is currently underway into the functional roles of these genes in the later stages of embryogenesis. This analysis establishes a foundation for a better understanding of the key events occurring in the vertebrate embryo at the MBT and thereafter. We have also confirmed the value of SSH in the identification of novel genes expressed at the MBT.

The authors acknowledge grant support from IRCSET (Embank).
8.
TRANSCRIPTOSOME PROFILING OF HUMAN CORONARY ARTERY SMOOTH MUSCLE CELLS IN RESPONSE TO OXIDIZED LOW DENSITY LIPOPROTEIN


1Department of Biochemistry and 2National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland, 3Department of Experimental Pathology, University Hospital MAS, Malmö, Sweden, 4Wallenberg-lab, University Hospital MAS, Malmö, Sweden, 5Department of Medicine and REMEDI, National University of Ireland, Galway, Ireland.

Atherosclerosis, a progressive disease of large arteries, is the principal cause of cardiovascular-related illness. Intimal plaques composed of macrophage; vascular endothelial and smooth muscle cells develop and obstruct blood flow. Coronary artery smooth muscle cell (CASMC) death and plaque instability leads to disease manifestations; myocardial infarction, stroke and gangrene.

Cholesterol-rich low density lipoprotein (LDL) is a critical atherogenic risk factor. LDL cannot accumulate in cells efficiently enough to account for disease progression; instead, oxidatively modified LDL (oxLDL) occurs in vivo and is found abundantly in atherosclerotic plaques and plasma. OxLDL is a known inducer of CASMC proliferation, migration, extracellular matrix production and death. This study investigates the genetic alterations brought about in CASMCs as a result of oxLDL treatment.

Studies employing different levels of oxidation and concentrations of ox-LDL indicated that CASMC cytotoxicity was dependant on dose, length of exposure and extent of oxidation. Cell death was initially studied through morphological characteristics observed using light microscopy. It was decided that primary human CASMC would be treated for 24 h with levels of ox-LDL (15-30 MDA/mg protein) in an attempt at mimicking pathophysiological hyperlipidemia.

This treatment increased expression of stress-responsive genes such as hsp70, MnSOD and Ferritin as well as apoptosis related genes such as Fas ligand, thus, validating our model system.

To generate a broader profile of genes differentially regulated by oxLDL, cDNA microarrays with oligonucleotides representing 10,000 human genes were utilized. Alterations in the expression patterns of approximately 200 genes were determined using Biodiscovery software. These genes were clustered into functionally similar clusters. Those genes associated with oxidative stress, transcription, signal transduction, lipid metabolism and apoptosis were most often differentially expressed in treated cells. Verification of the microarray study is currently underway using RT-PCR, Northern blot analysis and/or Real-Time PCR.

The authors acknowledge grant support from The Irish Research Council for Science, Engineering and Technology (IRCSET) and Science Foundation Ireland.
CHARACTERISATION OF LOW VOLTAGE ACTIVATED AND HIGH VOLTAGE ACTIVATED INWARD CURRENTS IN RABBIT CORPUS CAVERNOSUM SMOOTH MUSCLE CELLS.

Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk

Ca\textsuperscript{2+} influx plays an important role in the contractile activity of erectile tissue. Despite its importance, little is known about the influx pathways present in the corpus cavernosum. In the present study, we used the patch clamp technique to characterise the voltage dependent Ca\textsuperscript{2+} currents in freshly dispersed rabbit corpus cavernosum smooth muscle cells.

Male rabbits were humanely killed with pentobarbitone (I.V.) and their corpora cavernosum removed. Cells were isolated as previously described\(^1\). These were perfused with Hanks solution at 37°C and studied using the ruptured patch technique with Cs\textsuperscript+ rich pipette solutions. When cells were held at -100 mV and depolarised in 10 mV steps for 500 ms, inward currents were evoked at potentials positive to -70 mV. When the current voltage (IV) relationship was plotted, it consisted of two peaks - one at ∼-40 mV and the other at 0 mV. To dissect these two components, currents were evoked from holding potentials of -100 mV and -60 mV. When the IV curve obtained at a holding potential of -60 mV was plotted, the negatively activating component of inward current was abolished and little inward current was observed at potentials negative to -40 mV.

To examine the voltage dependence of inactivation of both currents, cells were stepped to either -40 mV or 0 mV after a series of 2s preconditioning potentials from -110 mV through to 0 mV. Under these conditions the current evoked by a step to -40 mV half-maximally inactivated at -71 ± 4.5 mV (n=5) compared with -42.6 ± 4.4 mV (n=4) for the current evoked by a step to 0 mV.

We next examined if there was a difference in the pharmacology of both components of current. The current evoked by a step to -40 mV from a holding potential of -100 mV was reduced in a concentration dependent manner by Ni\textsuperscript{2+} with an IC\textsubscript{50} of 2.1 ± 0.7 mM. In contrast the current evoked by a step to 0 mV was less sensitive to Ni\textsuperscript{2+} with an IC\textsubscript{50} of 148 ± 110 mM. Application of the L channel antagonist nifedipine (300 nM) only reduced the current evoked by a step to -40 mV by ∼20% (n=3). However, the current evoked by a step to 0 mV from a holding potential of -50 mV was reduced in a concentration dependent manner with an IC\textsubscript{50} of 55 ± 10 nM.

Iso-osmotic substitution of Ca\textsuperscript{2+} with Ba\textsuperscript{2+} also produced quite different effects on the two components of current. The L current amplitude evoked by a step from -50 mV to 0 mV was enhanced from -284±79 pA to -449.14 ±161 pA (n=4) and the time dependence of inactivation was slowed. T current amplitude was -41±11 pA in Ca\textsuperscript{2+} containing solutions compared with -40 ±9 pA in Ba\textsuperscript{2+} solutions (n=4) and no change in the time dependence of inactivation was apparent.

These results suggest that smooth muscle cells isolated from rabbit corpus cavernosum possess both low voltage and high voltage activated inward currents with characteristics typical of T and L type Ca\textsuperscript{2+} currents.

References

Supported by Diabetes UK and C McC was in receipt of a DEL PhD studentship.
10. 
**PURINOCEPTOR STIMULATED Cl- SECRETION IN BOVINE OVIDUCT EPITHELIAL CELLS.**

N. Keating, M.T. Kane & L.R. Quinlan
Department of Physiology, National University of Ireland Galway

Oviduct secretions provide an appropriate ionic environment to support oocyte and sperm viability and promote early embryo cleavage steps. The specific ionic transport mechanisms involved remain largely unknown but are essential to successful reproductive function. Minor changes in secretory function may provide a mechanism for selection of healthy spermatozoa and optimising conditions for embryo development. In this study we used the short-circuit current (I_{SC}) technique to investigate the effect of purinoceptor activation on electrogenic trans-epithelial ion transport in polarized oviductal cell monolayers.

The purinoceptor agonist, ATP, stimulated a dose-dependent increase in I_{SC} when applied apically or basolaterally. However the time course and magnitude of the ATP response was cell surface specific. Basolaterally, ATP (100µM) induced a transient increase in I_{SC}, while apically it induced a more rapid (<1.5s) and larger (>50%) transient, followed by a small, sustained increase.

The basolateral ATP response was unaffected by apical application of amiloride (100µM) but was significantly reduced (>90%) in the absence of extracellular chloride. Blocking basolateral chloride uptake with bumetanide (100µM) or inhibiting apical chloride channels with NPPB (500µM), glybenclamide (100µM) or NFA (100µM) significantly reduced the ATP response, by >50%, >25% and >50% respectively. BAPTA (25µM) or thapsigargin (1µM) completely blocked the ATP response while suramin (500µM) reduced the I_{SC} by 55%. UTP applied basolaterally was equipotent with ATP in stimulating an increased I_{SC}.

We conclude that bovine oviduct epithelial cells express functionally active purinoceptors on both apical and basolateral surfaces. Basolaterally ATP induces a transient activation of chloride secretion through a calcium dependent pathway most likely via the P2Y2 receptor subtype. These results suggest a functional role for ATP signaling in the regulation of oviduct fluid secretion.

**References**

11.
EFFECTS OF ACETYLCHOLINE AND NORADRENALINE ON THE ISOLATED PACHED RAT PULMONARY VEIN

C. M. Sweeney, J. F.X. Jones, S. J. Bund
Department of Human Anatomy and Physiology, University College Dublin, Earlsfort Terrace, Dublin 2.

In the rat, cardiac muscle extends into the pulmonary vein. It has been shown that the paced contractile response is attenuated by acetylcholine (ACh)\(^1,2\) and augmented by noradrenaline (NA)\(^1\). The aims of the present study were to establish the receptor populations responsible for these effects.

Male Wistar rats (281±33g, n=15, mean±SD) were killed by a stunning blow to the head followed by cervical dislocation. A pulmonary vein was dissected free post-mortem and mounted in a myograph for assessment of isometric tension development. The vein was superfused with Tyrode’s solution bubbled with oxygen (100%) at 37°C and set to a pre-tension equivalent to a transmural pressure of 15mmHg. The vein was electrically paced (1msec pulse width, 1 Hz, 10V). The influence of ACh (1\(\mu\)M) was determined before and after application of atropine (1\(\mu\)M). Second, the influence of NA (1nM - 10\(\mu\)M) was determined in the absence or presence of atenolol (10\(\mu\)M) and finally the influence of NA (10\(\mu\)M) on the responses to ACh (1nM -10\(\mu\)M) was determined. Contractile responses were normalised to those prior to drug addition and are expressed as mean±SEM(n).

Atropine inhibited the ACh-mediated attenuation of the paced contractile response (31±5%(4) vs 1±7%(4), p<0.05, paired t-test). The augmentation of the contractile response by NA was partially inhibited by atenolol; for example, the augmentation in 10\(\mu\)M NA was reduced from 169±9%(6) to 135±9%(5), (p<0.05, unpaired t-test). In the final series the attenuation effected by ACh was not significantly different in the presence or absence of NA (n=6, p>0.05, repeated measures analysis of variance).

These results demonstrate that ACh attenuates the paced contractile response of rat pulmonary veins via muscarinic receptor activation. The NA-mediated augmentation of this response is effected in part by \(\beta_1\) adrenoreceptor activation and that ACh-mediated attenuation of the paced contractile response is unaffected by the presence of NA.

References
12.
THE CARDIOPULMONARY REFLEX IN A RAT MODEL OF HIGH OUTPUT HEART FAILURE

M. Buckley, E. J. Johns
Department of Physiology, University College Cork, Cork.

This study investigated whether the cardiopulmonary mediated reflex inhibition of renal sympathetic nerve activity (RSNA) was altered by administration of caffeine and isoprenaline, to induce cardiac damage, and evaluated whether nitric oxide (NO) was involved.

Groups of Wistar rats (n=7-9) received either a regular diet and tap water or 5mg/kg isoprenaline, sc, every 72h and drinking water containing 61.6mg/ml caffeine for two weeks. Blood pressure (BP) was measured from a femoral artery and saline (150mM NaCl) infused at 3ml/h into a femoral vein. The left kidney was exposed and the renal sympathetic nerves sealed onto recording electrodes. After 2h, a 5min baseline was taken, saline infused at 0.25% body wt/min for 30 min and average values recorded every 5min. Following a 30 min recovery period, L-NAME was infused at 10mg/min/kg for 30min and the volume expansion (VE) repeated. Data, means ± SEM, were analysed using one-way ANOVA and significance taken when P<0.05.

In the control group, BP was 86±4 mmHg and after VE increased by 5 mmHg (P<0.05), while RSNA was reduced by 42% (P<0.01). L-NAME infusion had minimal effects on BP or RSNA. The second VE caused similar changes in BP and but the reduction in RSNA, of some 78% (P<0.001), was larger than in the absence of L-NAME (P<0.01). The rats exposed to caffeine and isoprenaline had similar baseline BP and during VE BP decreased slightly but RSNA was changed, a response very different from that of the control rats (P<0.01). L-NAME infusion into the treated rats had no effect on the cardiovascular variables but during the VE RSNA was decreased by 33% which was larger than without L-NAME (P<0.01). These data show that damage to the heart induced by caffeine and isoprenaline blunts the renal sympatho-inhibition resulting from VE suggesting a deficiency of the cardiopulmonary reflex which was dependent in part on NO.
13. MUSCLE TEMPERATURE-RELATED CHANGE IN PEAK POWER AND VELOCITY DURING SPRINT CYCLING IN POWER VERSUS ENDURANCE TRAINED ATHLETES

A. Rossiter, P.M. Jakeman
Human Science Research Unit, University of Limerick.

Studies of rodent muscle show that temperature-related changes in peak power output and peak contraction velocity are dependent on fibre type\(^1\). This study aimed to investigate if the temperature-related changes in peak knee angular velocity (KAV\(_{\text{peak}}\)) and peak power output (PPO) obtained during short-term maximal exercise differ in predominately type II (POWER) versus type I (ENDURANCE) athletes.

With ethical approval, three male and three female power athletes (mean(SD): age 25.8(5.4)\text{y}, height 176.1(6.0)\text{cm}, weight 68.6(9.1)\text{kg}, lean upper limb volume (LULV) 3.9(0.9)\text{L}) and two male and four female endurance athletes (age 33.5(7.8)\text{y}, height 170.5(7.0)\text{cm}, weight 62.2(10.4)\text{kg}, LULV 3.6(0.8)\text{L}) participated in this study. PPO and KAV\(_{\text{peak}}\) were measured during a 7-s maximal sprint on a friction-loaded cycle ergometer. The sprints were performed immediately post-immersion up to the gluteal fold for 30min in water designed to raise (HOT; 45\text{o}\text{C}) or lower (COLD; 10\text{o}\text{C}) skeletal muscle temperature (T\(_m\)) by ±4\text{o}\text{C}. Data were analysed by repeated measures ANOVA.

Table 1. PPO and KAV\(_{\text{peak}}\) values obtained post-immersion in water bath of 10\text{o}\text{C} (COLD), 27\text{o}\text{C} (CONTROL) and 45\text{o}\text{C} (HOT). Data are mean (SEM); *Significantly different from CONTROL.

<table>
<thead>
<tr>
<th>Athlete</th>
<th>PPO (W.L(^{-1}) LULV)</th>
<th>KAV(_{\text{peak}}) (rad.s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Power</td>
<td>Endurance</td>
</tr>
<tr>
<td>COLD</td>
<td>198.9(9.0)*</td>
<td>172.6(8.9)*</td>
</tr>
<tr>
<td>CONTROL</td>
<td>264.8(8.2)</td>
<td>206.7(8.2)</td>
</tr>
<tr>
<td>HOT</td>
<td>267.4(7.0)</td>
<td>215.6(6.2)</td>
</tr>
</tbody>
</table>

In comparison to CONTROL, immersion in COLD resulted in a greater mean decrease in PPO in POWER athletes versus ENDURANCE athletes, 25% and 17% decrease, respectively (p=0.017). Following COLD, the decrease KAV\(_{\text{peak}}\) was also significantly greater in POWER athletes versus ENDURANCE athletes, 18% and 11% decrease, respectively (p=0.009). Raising T\(_m\) resulted in minimal increases PPO and KAV\(_{\text{peak}}\) in both POWER and ENDURANCE athletes. Contrary to studies in isolated rodent muscle, which show that type I muscle is more sensitive to temperature than type II muscle\(^1\), this study suggests that athletes with a greater proportion of type II muscle are more sensitive to changes in T\(_m\) than those with predominately type I muscle.

References
14.
VASOCONSTRICTOR RESPONSES TO NORADRENALINE AND ATP IN STREPTOZOTOCIN DIABETIC RATS.

L. Speirs & C. Johnson
Vascular Biology Group, Queen’s University of Belfast, BT9 7BL, UK

Diabetes mellitus is associated with numerous cardiovascular complications including autonomic neuropathy, orthostatic hypotension and systemic hypertension. Studies on changes in sensitivity to exogenous noradrenaline are conflicting and contributions of other sympathetically released cotransmitters have not been studied.

Sprague-Dawley rats (8 week old, male) were made diabetic by injection (i.p., 60 mg.kg-1) of streptozotocin and maintained for 12 weeks. Animals were killed by cervical dislocation. Injected animals having a blood glucose of less than 10 mM/l were used a controls (non-diabetic controls, NDC: n=9, 500±11 g, blood glucose 5.7±0.5 mM, mean±SE,) while those with higher values were deemed to be diabetic (diabetics, D: n=11, 326±18 g, blood glucose 29±1.8 mM). Normal, untreated animals were also used (normal, N: n=8, 384±32 g). Isometric contractions were measured from tail artery rings with endothelium removed. Noradrenaline (1nM-100µM) and ATP (10nM-1mM) dose response curves were shifted leftwards (EC50 values for noradrenaline, in µM: N: 1.2±0.46; NDC: 0.25±0.02; D: 0.19±0.05; for ATP in µM: N: 103±22; NDC: 34±12; D: 20±4; P<0.0005 for ATP, P<0.05 for noradrenaline, one way ANOVA). There were no differences in KCl-induced constrictions between tissues (N: 0.67±0.60 g, n=16; NDC: 0.81±0.60 g, n=14; D: 0.72±0.50 g, n=23; one way ANOVA), suggesting enhanced vasoconstrictor responses were not due to changes in smooth muscle contractility. Differences in responses were further investigated by examining potentiation of sympathetically evoked responses (electrical stimulation using bursts of 5 impulses @ 20 Hz) in the presence of noradrenaline uptake1 inhibitor, desipramine (0.1µM). Average values for percentage potentiation were not different between tissues (N: 103±14 %, n=9; NDC: 73±10 %, n=13; D: 79±10 %, n=15; one way ANOVA).

Thus, we have shown that in diabetic rats, responses to exogenous noradrenaline and ATP are more sensitive. In the case of noradrenaline, this is not likely to be due to differences in uptake1 mechanisms.

References

15.
CHARACTERISATION OF ELECTRICAL ACTIVITY IN THE RABBIT CORPUS CAVERNOSUM WITH INTRACELLULAR MICROELECTRODES.

R. Dixon¹, K.D. Thornbury², G.P. Sergeant², N.G. McHale² and M.A Hollywood².
¹ Queens University of Belfast, & ² Smooth Muscle Research Centre, DkIT.

The treatment of erectile dysfunction has become a huge area of research in the past decade since the discovery of sildenafil (Viagra). Despite advances in the treatment of erectile dysfunction, little is known about the electrophysiological mechanisms that underlie tone in the corpus cavernosum. The purpose of the present study was to use intracellular microelectrodes to record the electrical activity and examine the effects of a variety of ion channel blockers on this.

Male New Zealand White rabbits were humanely killed with pentobarbitone (I.V.) and their corpora cavernosum removed. Erectile muscle was pinned out on a silicon rubber base and superfused with Krebs solution at 35-37°C. To prevent spontaneous contractions from dislodging impalements, tissues were incubated with 5 mM wortmannin. Smooth muscle cells were impaled with glass microelectrodes filled with 3M KCl and had resistances of 80 - 120 MΩ. In ~80% of impalements (n=44 cells from 16 animals) regular spontaneous transient depolarisations (STDs) were recorded. These occurred at a frequency of 37±5 min⁻¹ (mean ±SEM), had amplitudes of 17±1 mV and their mean half maximal duration was 0.8±0.2 sec⁻¹. The resting membrane potential of impaled cells ranged from –64mV to –37 mV (mean=–44±2 mV). No fast Ca²⁺ spikes were ever observed superimposed upon the STDs. Tetrodotoxin (1 µM; n=3) had no effect on the spontaneous activity suggesting that the activity was myogenic and that fast inward Na⁺ channels did not contribute to the STDs.

To investigate the contribution of Cl channels to this activity, we examined the effects of two Cl- channel blockers - DIDS (100 mM) and niflumic acid (30mM). Neither DIDS or niflumic acid altered resting membrane potential but both significantly attenuated STD amplitude from 14±2mV to 6±1 mV and from 10±1 mV to 2±1 mV respectively (n=5, p<0.05). The contribution of T and L-type Ca²⁺ channels to STDs was examined with the T channel blocker Ni²⁺ (30uM) and the L channel blocker, nifedipine (100 nM). Application of Ni²⁺ failed to alter resting membrane potential, STD frequency or amplitude. However, nifedipine significantly decreased STD amplitude from 19±2 mV to 8±2 mV (n=5, p<0.05, Student’s paired t-test) but failed to significantly alter STD frequency or resting membrane potential.

These results suggest that initial depolarisation of STDs is caused by activation of the Ca²⁺-activated Cl⁻ current (ICl(Ca)). This depolarisation may open L-type Ca²⁺ channels, further stimulating Cl⁻ channels and enhancing STD amplitude.

Acknowledgements
Supported by Diabetes UK.
16.
THE EFFECT OF CARBACHOL ON BKCa CURRENT IN ISOLATED RABBIT URETHRAL SMOOTH MUSCLE CELLS.

E. Dickson¹, G.P. Sergeant², N.G. McHale² and M.A Hollywood² and K.D. Thornbury²
¹ Queens University of Belfast, & ² Smooth Muscle Research Centre, DkIT.

Urethral tone can be augmented by the neuronal release of acetyl choline and noradrenaline. The aim of the present study was to examine the effect of carbachol on freshly dispersed urethral smooth muscle cells by monitoring their effects on Ca²⁺-activated K⁺ currents.

The bladder and urethra were removed from both male and female rabbits immediately after they had been killed by lethal injection of pentobarbitone. The most proximal 3 cm of the urethra was removed and placed in Ca²⁺ free Hanks solution. Cells were isolated enzymatically, perfused with Hanks solution at 37°C and studied using the perforated patch technique with K⁺ rich pipette solutions (ECl=0mV).

When cells were held at 0 mV, spontaneous transient outward currents (STOCs) were observed. The activity was complex and comprised of both fast and slow STOCs. Fast STOCs usually had durations of <100ms, their amplitudes ranged from 45pA to 447pA and occurred at frequencies of up to 468 min⁻¹ (n=20 cells). The slow STOCs had durations >100ms, their amplitudes ranged from 47pA to 393 pA and occurred at a frequency of ~190 min⁻¹ (n=23 cells).

To investigate if these currents were due to activation of BKCa channels, we examined the effect of the BKCa channel blocker penitrem A (100nM, Knaus et al., 1994) on cells held at 0 mV. Application of penitrem A abolished the STOCs (n=6), consistent with the idea that they were due to activation of BKCa channels.

We next examined the effects of the muscarinic agonist carbachol (10 μM) on STOCs. In 37 out of 60 cells, carbachol either induced a series of oscillatory outward currents or evoked a single large amplitude outward current. In the other 23 cells, carbachol application produced a reversible inhibition of STOCs. The excitatory effects of carbachol were also abolished by penitrem A, consistent with the idea that they were mediated via activation of BKCa channels. Application of atropine (1 μM, n=6) failed to alter STOC activity itself, but it abolished the excitatory effects of carbachol suggesting that the effects of carbachol were mediated via muscarinic receptors.

When we examined the effects of noradrenaline on STOCs we found a similar variation in the responses. Application of noradrenaline (10 μM) significantly (p<0.05, ANOVA) increased BKCa activity in 18 of 25 experiments (72%), while it had a significant inhibitory effect on BKCa current in 7 of 25 experiments (28%).

In conclusion, this study has demonstrated that BKCa current in urethral smooth muscle cells can be increased by both sympathetic and parasympathetic agonists. Both carbachol and noradrenaline were seen having both excitatory and inhibitory effects on STOCs, with excitation being more prevalent upon drug addition. Multiple applications of carbachol were found to elicit statistically similar rises in BKCa current, proving that the effect of carbachol is reproducible.

Reference
Knaus et al., (1994). Biochemistry; 33:5819-5828
17. CHONDROGENESIS OF MESENCHYMAL STEM CELLS HARVESTED FROM WISTAR RATS IN STATIC CULTURES

McMahon L¹, Farrell E¹², O’Brien FJ, O’Connell BC¹³, Campbell VA¹², Prendergast PJ¹
¹ Trinity Centre for Bioengineering, Trinity College, Dublin 2. ² Department of Physiology, Trinity College, Dublin 2. ³ School of Dental Science, Trinity College, Dublin 2

Tissue engineering is now looking to mesenchymal stem cells (MSCs) as a cell source for the development of tissues in vitro due to their ability to differentiate into various connective tissue lineages when supplied with appropriate signalling factors. The aim of this project was to examine the ability of a collagen-glycosaminoglycan (GAG) scaffold to support chondrogenesis.

MSCs were harvested from adult Wistar rats sacrificed by CO₂ asphyxiation, and after 3 weeks of expansion the cells were seeded onto 3-D collagen-GAG scaffolds that were fabricated in Massachusetts Institute of Technology. Cell-seeded constructs were cultured statically for 1-3 weeks and treated with the chondrogenic growth factors, TGF-β1 (10 ng/ml), ascorbic acid (0.05 mM) and dexamethasone (100 nM). The production of the cartilage-specific protein, collagen II was assessed by immunocytochemistry, and newly formed GAG was assessed using a ³⁵S radiolabeling technique.

In cell-seeded scaffolds treated with the chondrogenic growth factors for 1 week a 3-fold increase in ³⁵S incorporation (n=4, p<0.05, Student’s t-test) was observed, and this was increased to a 6-fold increase in ³⁵S incorporation in seeded-scaffolds treated with chondrogenic growth factors for 3 weeks (n=5, p<0.05, Student’s t-test). In control seeded-scaffolds (i.e without chondrogenic growth factors) a small amount of collagen II immunoreactivity was observed, and this was increased in those scaffolds exposed to chondrogenic growth factors for 3 weeks.

The data demonstrate that a collagen-GAG scaffold can support the differentiation of adult MSCs along the chondrogenic lineage, and suggest that this scaffold may be beneficial in the tissue engineering of cartilage.

Supported by PRTLI (cycle 3)
18.
A ROLE FOR GROUP 1 MGLUR IN THE INHIBITORY EFFECT OF TUMOUR NECROSIS FACTOR-ALPHA ON RAT LONG-TERM POTENTIATION

D. CUMISKEY & J.J. O’ CONNOR
Department of Human Anatomy & Physiology, Conway Institute of Biomolecular & Biomedical Research, National Neurosciences Network, University College Dublin, Belfield, Dublin 4, Ireland.

Pro-inflammatory cytokines are known to be elevated in several neuropathological states that are associated with learning and memory. We have previously demonstrated in our laboratories that the inhibition of long-term potentiation (LTP) in the dentate gyrus region of the rat hippocampus, by tumour necrosis factor-(TNF) α, represents a biphasic response, an early phase dependent on p38 mitogen activated protein kinase (MAPK) activation and a later phase possible dependent on protein synthesis. Many of the factors involved in the early modulation of LTP by TNFα have yet to be elucidated. We have therefore investigated the effects of mGluR antagonists on the effect of TNFα on LTP in the rat dentate gyrus in vitro. Recordings of field excitatory postsynaptic potentials (EPSPs) were made from the medial perforant path using standard methods. Data are expressed as mean ± sem and analysed using Students t-test.

When TNF-α (5.5ng/ml) was applied to the hippocampal slice 20 min pre-HFS early LTP (TNF-α/LTP 103±9%, n=4 versus control LTP 167±7% 1 h post-tetanus, \( P<0.001 \)) was significantly impaired as previously published1,2. Perfusion of the mGluR5 specific antagonist MPEP (5µM) for 40 min prior to application of TNFa reversed the inhibitory effect of TNFα on LTP (141±4% and 103±9% at 1 h post HFS, n=4). To investigate this further we perfused the MgluR5 specific agonist CHPG (100µM) for 20 mins pre-tetanus. There was no significant difference from control LTP (146±12% and 146±13% at 1 h post HFS, n=4). These results suggest TNFα is not acting solely through an mGluR mediated pathway. To investigate this further we isolated the NMDA mediated EPSP by using the AMPA antagonist NBQX (2µM) and low Mg\(^{2+}\). TNF caused a significant reduction in the NMDA EPSP (50±6% versus control 90±2% at 2 h post drug application, \( P<0.001 \), n=4). This effect was also seen with CHPG (54±6% versus control 90±2% at 2 h post drug application, \( P<0.001 \), n=4). This work implicates NMDAR in the mGluR-mediated TNFα inhibition of LTP. These studies will provide valuable tools to forward our understanding of the mechanisms of action of TNFα on synaptic plasticity.

This work was supported by the Higher Education Authority of Ireland.

References
19.

OXIDATIVE STRESS CONTRIBUTES TO IMPAIRED RESPIRATORY MUSCLE FUNCTION FOLLOWING CHRONIC EPISODIC HYPOXIA IN THE RAT

L. Sills¹, M. Dunleavy², A. Bradford² and K. D. O’Halloran¹
¹Department of Human Anatomy and Physiology, University College Dublin and ²Department of Physiology, Royal College of Surgeons in Ireland.

Sleep disordered breathing is an extremely common condition affecting up to 4% of adults in the developed world. The condition is associated with episodic hypoxia due to recurrent apnoea. Additionally, intermittent hypoxia can occur in normal individuals during severe exercise, air travel, diving, at altitude, post-operatively and during sleep particularly in neonates and may even be induced as a strategy in exercise training and in the treatment of clinical disorders. We have shown that chronic episodic hypoxia impairs respiratory muscle function. The aim of this study was to determine if oxidative stress associated with episodic hypoxic exposure contributes to respiratory muscle dysfunction.

Sixteen adult male Wistar rats (260-310g) were placed in restrainers with their heads in hoods in which the ambient O₂ concentration could be modified by controlling the gas supply to the hoods. Eight rats were exposed to alternating periods of hypoxia and normoxia, twice per minute, 8 hours a day for 1 week (episodic hypoxia). The remaining eight animals were exposed to an Air/Air cycle under similar experimental conditions (normoxic controls). In both groups, half the animals received daily injections of the antioxidant, N-acetyl-cysteine (200mg/kg i.p.) whilst the other half received daily vehicle injections. At the end of the 1-week treatment period, in vitro isometric contractile properties were determined using strips of isolated diaphragm muscle in physiological salt solution at 30°C. Fatigue properties were determined by stimulation of the muscle strips at a frequency of 40Hz (train duration of 300msec), every 2 seconds for 5 minutes.

Episodic hypoxia resulted in a significant increase in diaphragm muscle specific tension (9.4±1.6 (mean ± sem) vs. 14.5±1.1 N/cm², control vs. episodic hypoxia, P<0.05 ANOVA) measured at the beginning of the fatigue trial. Additionally, episodic hypoxia was associated with a significant decrease in diaphragm muscle endurance. Daily treatment with the antioxidant, N-acetyl-cysteine reversed the effects of episodic hypoxia on diaphragm muscle fatigue.

We suggest that oxidative stress likely contributes to impaired respiratory muscle performance following chronic episodic hypoxia. Our results may have particular relevance to respiratory disorders associated with episodic hypoxia such as the sleep apnoea/hypopnoea syndrome.

Funded by University College Dublin and the Royal College of Surgeons in Ireland.
20.
EFFECT OF PRIOR EXERCISE ON THE POST-PRANDIAL LEPTIN RESPONSE TO AN ISOENERGETIC MEAL COMPOSED OF EITHER LOW OR HIGH GLYCAEMIC INDEX FOOD

S. Keenehan, R. Daniels, A. Hunter, P. Jakeman
Human Science Research Unit, University of Limerick, Limerick, Ireland.

Physical activity and the composition of macronutrient intake play a functional role in the regulation of energy balance and satiety. The present study investigated whether a preceding bout of exercise modulates the post-prandial secretion of leptin and satiety following an isoenergetic meal of either low or high GI carbohydrate macronutrient composition.

With ethical approval and informed consent 10 healthy men, age 22 ±3y; BMI 24.2±2.6 kg/m2 were randomly assigned to four trials. Each trial comprised 1h of rest (REST; sitting) or exercise (EXER; 2MJ, walking) prior to the consumption of a 2.8MJ meal composed of either high (HIGH) or low (LOW) glycaemic index food. Blood samples were drawn at the start (T0), following 1h of rest or exercise (T60) and 150 min (T210) and 300 min (T360) after the meal.

No difference was observed in circulating leptin or insulin following 1h (T60) of rest or exercise. In the post-prandial period (T210) the HIGH meal induced a greater increase in insulin than the LOW (Δ31.6 vs 17.4 uIU/ml; P< 0.05), independent of the preceding REST or EXER. At T360 there was no difference in insulin across all four trials. In the LOW/EXER condition leptin was significantly suppressed at both T210 (Δ 3.6 ng/ml; P<0.05) and T360 (Δ 3.7 ng/ml; P<0.05, ANOVA). For all other conditions leptin did not change significantly during the post-prandial period. Ratings of satiety and fullness indicated lower overall satiety and fullness at T210 following the HIGH versus LOW meals but no difference at T360. Prior exercise decreased the rating of satiety and fullness at both T210 and T360 that were independent of LOW or HIGH meal composition.

These observations indicate that rating of satiety and fullness following exercise cannot be accounted for by changes in circulating leptin even though leptin secretion was suppressed following exercise when fed a low glycaemic meal.
21. INTERACTION OF PLATELET INTEGRIN $\alpha_{Ib}\beta_3$ WITH A NOVEL RING-TYPE ZINC-FINGER PROTEIN, IKAP-1

T.M. Brophy, K. Culligan, Chubb. A., N. Moran
Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, 123 St. Stephen’s Green, Dublin.

During the process of platelet activation, the platelet-specific integrin $\alpha_{Ib}\beta_3$ mediates bidirectional signals across the platelet membrane. This is achieved by a regulated conformational change in the integrin, allowing it to bind with high affinity to its ligand, fibrinogen. The highly conserved KVGFFKR motif of the $\alpha_{Ib}$ cytoplasmic tail is critically involved in the regulation of $\alpha_{Ib}\beta_3$ activation. Our group has previously shown that a synthetic biotinylated KVGFFKR peptide binds to several recombinant human proteins immobilized on a high-density protein expression array, including the nucleotide-sensitive chloride channel, ICln, and a hypothetical protein, MGC1. This latter protein was denoted IKAP-1 (Integrin KVGFFKR-Associating Protein-1).

We determined IKAP-1 to be present in platelets by reverse-transcription PCR and Western blotting. IKAP-1 contains one ring-type zinc finger as confirmed by homology searches and computer-aided molecular modeling. This feature is most commonly associated with DNA-binding proteins. His-tagged IKAP-1 protein was expressed in E.coli, and upon nickel agarose purification under non-reducing conditions, thiol-dependent high molecular weight IKAP-1 multimers were observed at 55kDa and 36kDa. Under reducing conditions, a molecular weight of 18kDa was obtained. Polyclonal antibodies were generated by immunisation of rabbits with the purified IKAP-1 protein. IKAP-1 was identified as a cytoplasmic protein in platelets by immunofluorescence microscopy. Using a His-tag pull-down assay, IKAP-1 was found to bind specifically to purified platelet-specific integrin $\alpha_{Ib}\beta_3$, and to endogenous $\alpha_{Ib}\beta_3$ from platelet lysates. This interaction was dose-dependently inhibited in the presence of increasing concentrations (10-100µM) of KVGFFKR peptide. A control peptide had no effect on this interaction.

Therefore, we have identified a novel integrin $\alpha_{Ib}\beta_3$-binding protein in platelets. IKAP-1 associates with $\alpha_{Ib}\beta_3$ via the KVGFFKR motif. IKAP-1 is a novel ring-type zinc-finger binding protein, and as platelets are anucleate, IKAP-1 is not fulfilling a DNA-binding role in the platelet environment. Therefore, an alternative role in platelet signaling processes is strongly implicated.

The authors acknowledge grant support from Higher Education Authority.

Reference
1. Larkin, D., Murphy D., Reilly, D.F., Cahill, M., Sattler, E., Harriot, P., Cahill, D.J., Moran, N. ICln, a Novel Integrin $\alpha_{Ib}\beta_3$-Associated Protein, Functionally Regulates Platelet Activation J. Biol. Chem., 2004 Vol. 279, No. 26: 27286-93
INHIBITION OF ELECTRICAL ACTIVITY IN THE ISOLATED RABBIT URETHRA BY NO DONORS AND ACTIVATORS OF THE GUANYLATE CYCLASE PATHWAY.

A. De Faoite, G.P. Sergeant, N.G. McHale, K.D. Thornbury, & M.A. Hollywood
Smooth Muscle Research Centre, Dundalk Institute of Technology, Dublin Road, Dundalk, Co. Louth.

Although, nitric oxide is the principal inhibitory neurotransmitter in the urethra, the mechanism by which it causes relaxation is unclear. A number of studies have demonstrated that the effects of nitric oxide are not mediated through membrane hyperpolarisation\(^1\). In the present study we investigated the effect of NO analogues on electrical activity recorded from strips of rabbit urethral smooth muscle using the sharp intracellular microelectrode recording technique.

The bladder and urethra were removed from both male and female rabbits immediately after they had been killed by lethal injection of pentobarbitone. The most proximal 3 cm of the urethra was removed and placed in Krebs solution. This was then opened up, the urothelium removed and the preparation pinned out on a silicon rubber base and superfused with Krebs solution at 35-37\(^\circ\)C. To prevent spontaneous contractions from dislodging impalements, tissues were incubated with 5 mM wortmannin. Smooth muscle cells were impaled with glass microelectrodes filled with 3M KCl and had resistances of 80 - 120 M\(\Omega\).

The electrical activity in this tissue was comprised of regularly occurring slow waves which had spikes superimposed upon a plateau, as demonstrated previously\(^2\). Application the stable NO donor (DEA-NO, 10 \(\mu\)M) reduced the slow wave frequency from 3.4 ± 0.15 min\(^{-1}\) under control conditions to 2.3 ± 0.26 min\(^{-1}\) (mean ± SEM; p<0.05; paired t test; n=5). Application of 30 \(\mu\)M DEA-NO further reduced frequency from 3.3 ± 0.26 to 1.2 ± 0.19 min\(^{-1}\) (p<0.05, \(n=5\)). DEA-NO also reduced the number of Ca\(^{2+}\) spikes superimposed upon each plateau from 3.0 ± 0.5 to 2.3 ± 0.6 (10 \(\mu\)M, p<0.05) and from 4.2 ± 1.0 to 2.5 ± 0.7 (30 \(\mu\)M, p<0.05). Resting membrane potential was not significantly altered by either concentration of the drug (Control –50 ± 1 mV compared to -49 ± 2 mV in 10 mM DEA; control –52 ± 4 mV compared to –49± 5 mV in 30 mM DEA-NO).

In order to determine if these effects of DEA-NO were due to activation of the soluble guanylate cyclase (GC) pathway we examined the effect of a number of modulators of this pathway. The membrane permeable soluble guanylate cyclase inhibitor ODQ (10 mM) reversed the effects of DEA-NO (10 \(\mu\)M). In 3 cells, application of DEA-NO in the presence of ODQ only reduced frequency slightly from 4.3 ± 0.7 min\(^{-1}\) to 4.0 ± 0.5 min (p>0.05). Conversely, the membrane permeant activator of GC, YC-1 potentiated the inhibitory effects of DEA-NO. Under control conditions, DEA-NO (10 \(\mu\)M) reduced frequency from 3.5 ± 0.1 min\(^{-1}\) to 2.4± 0.2 min. In the presence of YC-1 (10 \(\mu\)M), DEA-NO further decreased slow frequency to 1.3 ± 0.2 min\(^{-1}\) (n=5; p<0.05).

These data demonstrate that NO donors inhibit spontaneous electrical activity in the rabbit urethra and this effect may underlie the inhibition in tone observed during nitrergic stimulation.

References

Supported by the Medical Research Council
23.

POTENTIAL OF RAMAN SPECTROSCOPY AS AN ASSAY OF CELLULAR METABOLISM POST-IONISING RADIATION EXPOSURE IN HUMAN SKIN CELL ANALOGUES

A.D. Meade\textsuperscript{1,3}, H.J. Byrne\textsuperscript{2}, F.M. Lyng\textsuperscript{2,3}
\textsuperscript{1}School of Physics, Dublin Institute of Technology, Dublin, Ireland. \textsuperscript{2}FOCAS Institute, Dublin Institute of Technology, Dublin, Ireland, \textsuperscript{3}Radiation and Environmental Science Centre (RESC), Dublin Institute of Technology, Dublin, Ireland

Raman spectroscopy, as an evaluation of the products of ionisation radiation exposure in biological systems, has been utilised mainly in the evaluation of the impact of exposure in tissue, cellular constituents and live animals\textsuperscript{1,2,3}. It has also been recently demonstrated that Raman spectroscopy can exhibit key spectroscopic changes in the live cell associated with significant apoptotic and necrotic chemical damage\textsuperscript{4,5}. The present preliminary work utilises Raman spectroscopy at 514.5 nm to evaluate the results of exposure to \(\gamma\)-rays in HaCaT cells (human keratinocyte cell line) from a Co-60 therapy source, in tandem with a fluorometric assay of cellular metabolism (Alamar Blue). Cells were loaded onto glass coverslips for Raman spectroscopy and onto 96 well plates for parallel fluorescence. Three independent biological samples were utilised, with Raman spectra taken from 20 cellular nuclei in a given sample at a given dose point. The final Raman spectra were averaged over all spectral measurements at a given dose point (i.e. 60 spectra per dose point), and the fluorescence measurements were averaged over the three independent samples.

The results demonstrate that spectral changes in the Amide I region (Raman shift \(\sim 1667 \text{ cm}^{-1}\); corresponding to the stretching vibration of the amide backbone in protein) may be correlated with changes in the cell also identified in parallel biochemical assays. Figure 1 (log plot; control at 0 Gy not shown) demonstrates the fluorescent changes in Alamar Blue observed with radiation dose, and indicates apparent selectivity of damage at 200 mGy. Figure 2 demonstrates the variation in Amide I band intensity with dose, and while only displays measurements from four dose points, exhibits a similar variation to that found in figure 1. It has therefore been concluded\textsuperscript{6} that the Amide I band intensity could represent an assay of cellular metabolism, indicating possible uses of Raman spectroscopy as a multiplexed assay of many cellular functions.

References
INHIBITION OF LEUCOCYTE PHAGOCYTOSIS BY ANTIDEPRESSANTS APPEARS UNRELATED TO MONOAMINE REUPTAKE INHIBITION

M. Diamond, O. Duignan, A. Heneghan, K. Purcell, J.P. Kelly
Department of Pharmacology, NUI, Galway

Evidence indicates that antidepressants have immunomodulatory properties, with a number of studies reporting suppressive actions on lymphocyte function. By contrast, the impact of antidepressants on cells of the innate immune system remains largely unexplored. In this regard, phagocytosing leucocytes (neutrophils, monocytes/macrophages) constitute an integral part of the innate immune response, and form a crucial defence against microbial infection. The aim of this study was to examine the \textit{in vitro} effects of antidepressants (imipramine, desipramine, clomipramine, trimipramine, protryptiline, dothiepin, Maprotiline, sertraline, fluoxetine, fluvoxamine and citalopram) on rat splenic leucocyte phagocytosis. Male Sprague-Dawley rats (n=4-6) were anesthetised (CO\textsubscript{2}) and their spleens isolated. The phagocyte fraction was enriched via histopaque density centrifugation, and adjusted to 1x10\textsuperscript{6} cells/ml in HBSS. Following 1 hour pre-incubation with a concentration gradient of each drug, phagocytosis was stimulated using either opsonized zymosan (OZ) or phorbol-myristate-acetate (PMA) and measured by luminol-amplified chemiluminescence. OZ activates phagocytosis through a receptor-coupled response, whilst PMA directly activates the oxidative burst in phagocytic cells via protein kinase C (PKC). All drugs inhibited splenic phagocytosis (IC\textsubscript{50}: OZ 2-42.1 mM; PMA 4-18.5 mM) despite differential monoamine re-uptake inhibiting properties. Interestingly, the tricyclic antidepressant trimipramine, which lacks monoamine re-uptake inhibiting properties, was found to be as effective as other antidepressants in inhibiting phagocytosis (IC\textsubscript{50}: OZ 17.6 mM; PMA 11.6 mM). Moreover, the antipsychotic chlorpromazine, which bears considerable structural similarity to the tricyclic antidepressant clomipramine, displayed a similar pattern of inhibition (IC\textsubscript{50}: OZ 6.5 vs. 5.1 mM; IC\textsubscript{50}: PMA 12.8 vs. 12.7 mM). Since the response to both OZ and PMA was similarly affected by the antidepressants, it is likely that they target the signal transduction at, or distal to, PKC. It is also noteworthy that cell viability as measured by the resazurin-reduction method, was not altered by the antidepressant concentrations employed in the present study. Studies have shown that antidepressants accumulate up to 30 times higher in tissue than in plasma with the consequence that these drugs have the potential to elicit profound immunosuppressive properties within their clinical therapeutic window. These data demonstrate that antidepressants possess potent immunomodulatory properties, which are independent of their monoamine reuptake abilities.

References
25. IMMUNOMODULATORY PROPERTIES OF HAEMODIALYSIS MEMBRANES, POLARISATION OF A TH$_2$ T-CELL CYTOKINE RESPONSE

M. Diamond$^1$, J.P. Kelly$^1$, T.J. Connor$^{1,2}$, A. Harkin$^{1,3}$

$^1$Department of Pharmacology, NUI, Galway, $^2$Department of Physiology, Trinity College Dublin, $^3$School of Pharmacy, University College Cork

Haemodialysis is used routinely to treat renal failure patients, and is employed for three or more hours, three times weekly. Haemodialysis patients express an altered immune phenotype; in particular, a suppression of TH$_1$ and an increase of TH$_2$ T-cell cytokines has been observed$^1$, which may be attributed to contact with haemodialysis membranes. Originally, haemodialysis membranes consisted primarily of cellulosic (cuprophone) or modified cellulose (hemophane, cellulose diacetate/triacetate) based materials, however in recent years, synthetic membranes (polyethersulphone, polyamide) have been introduced. We examine here the effects of a fixed equivalent surface area of these different membrane types on mitogen-stimulated lymphocyte proliferation, and TH$_1$ and TH$_2$ cytokine responses in both human and rat blood. Blood obtained from either male Sprague Dawley rats (n=8), or healthy human volunteers (5 male, 3 female) was diluted 1:5 in culture medium (RPMI 1640 + 1% penicillin/streptomycin), and incubated with the membranes for three hours on a gyrorocker in a 5% CO$_2$ atmosphere at 37°C. Blood was further diluted 1:2, and cells were stimulated with the T-cell mitogen concanavalin A (con A) for 72 hours. Lymphocyte proliferation was measured by determination of $[^3]$H] thymidine incorporation following stimulation with con A, and viability was assessed via transient $[^3]$H] thymidine incorporation. Sandwich ELISA’s were used to determine concentrations of lymphocyte-derived TH$_1$ (IFN-g) and TH$_2$ (IL-6) cytokines. Data were analysed using one-way ANOVA, and differences between groups were determined using a Dunnett’s post hoc comparison test. Changes were deemed significant when P<0.05. The data indicate that exposure to the haemodialysis membranes failed to alter cell viability. However all membrane types with the exception of polyethersulphone suppressed Con A-stimulated T-cell proliferation in rat blood (34-70% inhibition). By contrast, only cuprophan and cellulose triacetate membranes suppressed T-cell proliferation in human blood (54 and 41 % inhibition respectively). In addition, production of the TH$_1$ cytokine IFN-g was suppressed by all membrane types in rat blood (18-78% inhibition), but to a lesser extent in human blood (<30% inhibition). Conversely, IL-6 production was increased by all membranes in human (396-596% activation), and to a lesser extent in rat blood (<239% increase). However, both in human and rat blood, incubation with haemodialysis membranes promoted a consistent suppression of the IFN-g:IL-6 ratio, indicating a shift towards a TH$_2$ cytokine phenotype. This shift in cytokine phenotype occurred irrespective of whether the membrane was cellulosic (original or modified), or was synthetic, thereby indicating that immunological dysfunction associated with these materials is not confined to a particular class of membrane. In conclusion, the results of this study reveal that contact with haemodialysis membranes promotes a shift from a TH$_1$ to a TH$_2$ T-cell cytokine phenotype, irrespective of the material that the membrane is composed of.

References
CAFFEINE AND ITS MAJOR METABOLITE PARAXANTHINE SUPPRESS HUMAN LYMPHOCYTE FUNCTION

Louise A. Horrigan¹, John P. Kelly¹, Thomas J. Connor¹²
¹Department of Pharmacology, National University of Ireland, Galway, ²Department of Physiology, Trinity College Institute of Neuroscience, Trinity College, Dublin 2

The objective of this study was to investigate the effects of caffeine and its major metabolite paraxanthine on human lymphocyte function. Following a period of abstinence (> 15hrs) from caffeine-containing food and beverages, heparinized venous blood was taken from 8 healthy female volunteers. Written informed consent was obtained from all volunteers. Following a 2hr pre-incubation of diluted whole blood with caffeine or paraxanthine (0–500 mM), cultures were stimulated with the T-cell mitogen concanavalin A (Con A; 5 mg/ml) for 72hr. Con A-stimulated lymphocyte proliferation was assessed using the [³H]-thymidine uptake assay, and IFN-γ and IL-5 production were measured by ELISA as an index of Th₁/Th₂ polarisation. Results were analysed using a one-way repeated measures ANOVA followed by Newman-Keuls post-hoc test. Using the Alamar Blue assay, the drugs were shown to have no effect on cell viability, at the concentrations used.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Drug</th>
<th>Conc. (mM)</th>
<th>% control (mean ± s.e.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocyte Proliferation</td>
<td>Caffeine</td>
<td>50</td>
<td>71.5 ± 22.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>54.3 ± 13.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>21.7 ± 5.1</td>
</tr>
<tr>
<td></td>
<td>Paraxanthine</td>
<td>100</td>
<td>60.3 ± 9.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>34.0 ± 3.9</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Caffeine</td>
<td>100</td>
<td>91.7 ± 4.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>66.5 ± 5.4</td>
</tr>
<tr>
<td></td>
<td>Paraxanthine</td>
<td>500</td>
<td>67.0 ± 7.5</td>
</tr>
<tr>
<td>IL-5</td>
<td>Caffeine</td>
<td>100</td>
<td>64.4 ± 14.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>46.5 ± 8.2</td>
</tr>
<tr>
<td></td>
<td>Paraxanthine</td>
<td>500</td>
<td>37.2 ± 5.5</td>
</tr>
</tbody>
</table>

Table 1: The effects of caffeine and paraxanthine on immune parameters. Only significant changes are included (P < 0.05).

In conclusion, both caffeine and paraxanthine suppressed lymphocyte function. The fact that both IFN-γ and IL-5 were suppressed to a similar extent following caffeine/paraxanthine exposure, indicates that Th₁ and Th₂ lymphocytes were not differentially affected by these methylxanthines. As 100 mM is the highest reported concentration of caffeine measured in human plasma, the effects described in this study occur at the higher end of the concentration range relevant to normal caffeine consumption. Nonetheless these results may have health implications, particularly in individuals with impaired lymphocyte function, such as the elderly, or HIV+ individuals. However, further studies are required to determine the impact of caffeine consumption on host resistance to disease in such susceptible populations.

Reference
27.
**MDMA (“ECSTASY”) PROMOTES A SHIFT FROM A TH\(_1\) TO A TH\(_{2/3}\) CYTOKINE PHENOTYPE IN VIVO**

N.T. Boyle, T.J. Connor
Neuroimmunomodulation Research Group, Department of Physiology & Trinity College Institute of Neuroscience, Trinity College, Dublin 2

The widely abused amphetamine-derivative methylenedioxymethamphetamine (MDMA; “Ecstasy”) has immunosuppressive actions in both animal and humans\(^1\). We have previously demonstrated that MDMA promotes and anti-inflammatory cytokine phenotype in mice in response to an *in vivo* challenge with bacterial lipopolysaccharide (LPS); characterised by reduced production of the pro-inflammatory cytokine interleukin (IL)-12, and increased production of the anti-inflammatory cytokine IL-10. IL-12 is a pivotal cytokine in driving uncommitted T helper (T\(_h\)) cells towards a Th\(_1\) phenotype, primarily by inducing IFN-\(\gamma\) production. In contrast, IL-10 is known to inhibit IFN-\(\gamma\) production, thereby favouring a shift to a Th\(_2\) or a regulatory T-cell (Th\(_3\)) phenotype. Therefore, in this study we determined whether MDMA altered IFN-\(\gamma\) production in response to LPS *in vivo*. The results indicate that LPS (100 mg/kg; i.p.) induced a significant increase in circulating IFN-\(\gamma\) concentrations 8hrs following administration, (Control 0; LPS 91.1± 21.1 pg/ml) and this increase in IFN-\(\gamma\) was significantly suppressed by MDMA (20mg/kg) (11.2 ± 3.2pg/ml) (P<0.01), analysed by a one-way ANOVA and Student Newman Keuls test. Despite the fact that MDMA promoted a shift from IL-12/IFN-\(\gamma\) to IL-10 production in response to an *in vivo* LPS challenge, following an antigenic challenge with keyhole limpet haemocyanin (KLH; 100mg/kg; i.p.) MDMA induced a broad-spectrum suppression of T-cell cytokine responses. Specifically, MDMA suppressed production of the Th\(_1\) cytokine IFN-\(\gamma\), the Th\(_2\) cytokine IL-5 and the Th\(_{2/3}\) cytokine IL-10 following re-stimulation of splenocytes with antigen *ex vivo*. It is also noteworthy that LPS induced a similar suppression of T-cell cytokine production to MDMA. This was an unexpected finding, and may indicate that LPS induces a stress response in mice, which could account for the observed suppression of T-cell responses. In contrast to the suppressive effect of MDMA and LPS on antigen specific cytokine production, these treatments failed to alter cytokine production in response to the T-cell mitogen concanavalin A, indicating that both MDMA and LPS selectively suppress antigen-specific T-cell responses. Overall these data indicate that MDMA promotes a shift in the innate immune system from IL-12/IFN-\(\gamma\) production to IL-10 production. However the precise mechanism that underlies the ability of MDMA and LPS to induce a broad-spectrum suppression of T-cell responses remains to be elucidated.

*The authors acknowledge grant support from IRSECT*

**References**

28.

6-HYDROXYDOPAMINE-INDUCED DEGENERATION OF RAT NIGRAL DOPAMINERGIC NEURONS AND MICROGLIAL ACTIVATION IS REDUCED BY TREATMENT WITH A NOVEL PHOSPHOLIPID-BASED DRUG FORMULATION

P Fitzgerald1,2, DM Harris1,2, S Crotty1,2, A Fisher1,2, A Mandel3, AE Bolton1, AM Sullivan2, Y Nolan2
1Vasogen Ireland Ltd., Dublin, Ireland, 2Dept of Anatomy/Neuroscience, UCC, Cork, Ireland
3Vasogen Inc., Toronto, ON, Canada

We have recently demonstrated that VP025 (Vasogen Inc.), a preparation of phospholipid microparticles incorporating phosphatidylglycerol, confers a protective effect in the rat brain by inhibiting 6-hydroxydopamine (6-OHDA)-induced dopaminergic neuronal death, amphetamine-induced ipsilateral rotational behaviour, and decreased striatal dopamine levels1. Evidence suggests that the pathogenic effects of 6-OHDA on dopaminergic neurons residing in the substantia nigra are in part mediated via the sustained activation of microglia, the resident immune cells of the brain. Here we investigated the possibility that 6-OHDA-induced microglial activation may be inhibited by VP025.

Male Sprague Dawley rats (225-250g) were pre-treated with VP025 (150µl of a 1.2 x 10^7 particles/ml suspension in PBS; i.m.) or saline 14, 13 and 1 day before unilateral lesioning of the medial forebrain bundle with 6-hydroxydopamine (6-OHDA; 8µg/4µl). Prior to surgery rats were anaesthetised with a 1:1 mixture of xylazine hydrochloride and ketamine hydrochloride (2ml/kg, i.p.). Ten days later, rats were sacrificed, transcardially perfuse-fixed and cryosectioned (15mm) for immunocytochemical staining of dopaminergic neurons and activated microglia in the substantia nigra (n=4).

The number of TH-positive neurons was significantly decreased in ipsilateral nigral tissue prepared from 6-OHDA-treated rats (910±150/mm³) compared with controls (2362±408/mm³; p<0.05; ANOVA), with a concurrent increase in positive immunostaining for activated microglia. Treatment with VP025 abrogated these changes. These data suggest that VP025 may attenuate death of dopaminergic nigral neurons by preventing activation of microglia.

Reference


This work has been funded by Vasogen Ireland Limited
NGF PRETREATMENT INHIBITS 6-HYDROXYDOPAMINE-INDUCED TOXICITY AND PROMOTES SURVIVAL IN PC12 CELLS

E. Kavanagh, J.P. Loughlin, K.M. Doyle, A.M. Gorman

1Department of Physiology, National University of Ireland, Galway, 2Department of Biochemistry, National University of Ireland, Galway

This study investigated the neuroprotective effect of NGF against 6-OHDA-induced cell death in PC12 cells. Furthermore the ability of the cells to proliferate and differentiate following treatment was investigated.

6-OHDA treatment (200 µM) of PC12 cells caused significantly greater apoptotic (13.4%) and necrotic (12.9%) morphology than in the corresponding controls (0.8% and 1.5%, respectively, n=5). Caspase-3 activity, caspase-3 processing and PARP cleavage were significantly increased in 6-OHDA-treated cells, confirming induction of apoptosis (n=6). Release of the cytosolic marker, LDH, (61.4% of maximal release) was also significantly increased in 6-OHDA treated cells indicating necrosis had also occurred (n=3).

Pretreatment of cells with NGF (100ng/ml) for 24 h protected PC12 cells from apoptotic and necrotic cell death induced by 6-OHDA. A significant decrease in apoptosis was apparent when assessed by morphology (p<0.01, n=5), caspase-3 activity (p<0.01, n=6), caspase-3 processing, and PARP cleavage. LDH release was also reduced in the presence of NGF (p<0.001, n=3), indicating a reduction in necrosis. Statistical significance was assessed using one-way ANOVA.

Clonogenic assays were carried out on 6-OHDA-treated cells (50-200 µM) to investigate the ability of these cells to survive and proliferate over a 2 week period post treatment. NGF pretreated cells showed a pronounced improvement in their ability to survive and proliferate in comparison to cells treated with 6-OHDA alone (n=2). In addition, the ability of the cells surviving 6-OHDA treatment to differentiate into neuronal cells in the presence of NGF was also shown.

The authors acknowledge grant support from Health Research Board of Ireland, IRCSET/EMBARK, Higher Education Authority and the NUI Galway Millennium fund.
30. **UP-REGULATION OF ADRENOMEDULLIN AND ITS' RECEPTOR COMPONENTS DURING CARDIAC MYOCYTE HYPTERTROPHY INDUCED BY CHRONIC INHIBITION OF NITRIC OXIDE SYNTHESIS**

D Bell, EJ Kelso, YY Zhao, LM Rush, VM Lamont, DP Nicholls, BJ McDermott
Cardioendocrine Research Group, School of Medicine, Queen’s University Belfast, UK.

Adrenomedullin may provide a compensatory mechanism to attenuate left ventricular hypertrophy (LVH). NOS inhibition, induced by chronic administration of N\textsuperscript{ω}-nitro-L-arginine methyl ester (L-NAME) to rats, induces cardiac hypertrophy in some, but not all cases; there are few reports of direct assessment of cardiomyocyte parameters. The objective was to characterise hypertrophic parameters in left (LV) and for comparison, right ventricular (RV) cardiomyocytes, following administration of L-NAME to 8 week old rats for 8 weeks and to determine whether adrenomedullin and its receptor components were up-regulated. Following treatment with L-NAME (20 and 50mg/kg/day), compared to non-treated animals: (1) systolic blood pressure increased (\textsuperscript{↑}40.2, \textsuperscript{↑}110.9 mmHg); (2) heart weight: body weight ratio increased \textsuperscript{↑}24.1% at the higher dose only (P<0.05); (3) protein mass of cardiomyocytes was increased (p= ns) \textsuperscript{↑}22.4% LV, \textsuperscript{↑}59.0% RV at the higher dose); (4) cardiomyocyte protein synthesis (incorporation of \textsuperscript{14}C-phenylalanine) was greater (P<0.05) \textsuperscript{↑}93%, \textsuperscript{↑}66% LV; \textsuperscript{↑}137%, \textsuperscript{↑}137% RV); (5) expression of sk a-actin (x3.3, 2.6) , ANP (x1.2, 17.9) , BNP (x2.9, 3.1) and endothelin-1 (x2.7, 3.9) mRNAs was enhanced (P<0.05) in LV but not RV cardiomyocytes; (6) expression of adrenomedullin (x1.8), RAMP3 (x3.1) and RAMP2 (x1.8), (but not CL and RAMP1) mRNAs was increased by L-NAME (20mg/kg/day) in LV.

In conclusion, L-NAME enhances protein synthesis in both LV and RV cardiomyocytes but elicits a hypertrophic phenotype accompanied by altered expression of the counter-regulatory peptide, adrenomedullin, and its receptor components RAMP2 and RAMP3 in LV only, indicating that the former is due to impaired nitric oxide synthesis, while the phenotypic changes represent a response to pressure overload.
A COMPARISON OF TAIL-CUFF SPHYGNOMANOMETRY AND TELEMETRIC MEASUREMENT OF ARTERIAL BLOOD PRESSURE IN CONSCIOUS RATS

A. Lewis†, D. Bell†, C. Johnson‡
Therapeutics and Pharmacology† and Vascular Biology Group‡, Queen’s University of Belfast

In setting up telemetric monitoring of arterial blood pressure in conscious rats we carried out a comparative study with the more conventional and less technological method of tail-cuff sphygmomanometry to investigate accuracy and reliability and application of each method.

Experiments were conducted on male Sprague-Dawley rats. For tail-cuff measurements, an appropriately sized sphygmomanometer cuff was attached to a standard blood pressure monitor (Harvard Apparatus) amplified, and displayed on a computer. For telemetric measurements, a small cannula was implanted into the abdominal aorta under inhaled isoflourane anaesthesia which was attached to a small or medium sized transmitter device (Data Services International). This was closed in the abdominal cavity and animals allowed to recover. Data was gathered from a receiver under the rat cage and stored on computer.

During simultaneous measurements telemetry systolic values were slightly but not significantly higher than tail cuff (difference 5.5±3.10 mmHg, mean±SE, n=4 rats, student’s paired t-test). Coefficients of variation in systolic blood pressure measured simultaneously with tail cuff (5.6±0.60 %) and telemetry (4.65±0.45 %) were not significantly different (student’s paired t-test, mean of 6 consecutive measurements made on 20 occasions on each of 4 rats) when on 20 different occasions Simultaneous measurement of systolic blood pressure from a cannula inserted into femoral artery (under inhaled isoflourane anaesthesia) and telemetric measurement were no different (difference 2±0.2 mmHg, n=2). Telemetric measurement of systolic pressure during restraint for tail-cuff measurement in naive rats revealed a sharp increase for the first five minutes (15.5±4.6mmHg at peak, n=2) which usually recovered within 15 minutes. This was moderately reduced after training with restraint once a day for eight days (3.3±1mmHg, P<0.001, student’s paired t-test). Circadian rhythms were apparent in blood pressure and heart rate from telemetric recordings.

We conclude that telemetric blood pressure values are less liable to variation and allow continuous monitoring. With adequate procedures, tail cuff measurement can provide accurate single measurements.

Our thanks to DSI who supported this work.
32. THE USE OF A FRICTION-LOADED CYCLE ERGOMETER TO MEASURE THE EFFECT OF MUSCLE TEMPERATURE ON PEAK POWER AND PEAK VELOCITY IN ADULT MALES

A. Rossiter, P.M. Jakeman
Human Science Research Unit, University of Limerick.

The contractile properties of skeletal muscle are temperature dependent\(^1\). Studies in humans report a change of 4% in peak power output per °C change in muscle temperature (T\(_m\)) during isokinetic cycling\(^2\). The purpose of this study was to investigate whether similar changes in peak power output (PPO) and peak knee angular velocity (KAV\(_{\text{peak}}\)) with respect to T\(_m\) occur during cycling when the flywheel was allowed to freely accelerate and where PPO was corrected for the inertial effect of the ergometer flywheel\(^3\).

With ethical approval, eight healthy males (mean(SD): age 21(1.4)y, height 177.8(6.2)cm, mass 77.4(5.9)kg, lean upper leg volume (LULV) 4.79(0.7)L) volunteered for this study. Subjects performed a series of 7-s maximal sprints on the cycle ergometer at frictional loads (FL) equivalent to 2%, 6% and 10% of body mass, following immersion in water for 30min at 10±1°C (COLD) and 45±1°C (HOT). T\(_m\) of the vastus lateralis was measured at a depth of 3cm prior to and immediately after immersion using a needle thermocouple. Data are reported as mean (SEM) and analysed by repeated measures ANOVA.

Table 1: The effect of hot- and cold-water immersion on PPO and KAV\(_{\text{peak}}\) measured across various FL (2%, 6% and 10% body mass). Data are mean (SEM), n=8.

<table>
<thead>
<tr>
<th>FL</th>
<th>2%</th>
<th>6%</th>
<th>10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>222(8)</td>
<td>221(12)</td>
<td>218(14)</td>
</tr>
<tr>
<td>HOT</td>
<td>223(11)</td>
<td>228(17)</td>
<td>218(13)</td>
</tr>
<tr>
<td>COLD</td>
<td>177(13)</td>
<td>178(11)</td>
<td>172(12)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FL</th>
<th>2%</th>
<th>6%</th>
<th>10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>10.8(0.5)</td>
<td>8.9(0.4)</td>
<td>6.3(0.2)</td>
</tr>
<tr>
<td>HOT</td>
<td>11.0(0.5)</td>
<td>9.1(0.3)</td>
<td>6.5(0.3)</td>
</tr>
<tr>
<td>COLD</td>
<td>9.4(0.5)</td>
<td>7.4(0.3)</td>
<td>5.0(0.2)</td>
</tr>
</tbody>
</table>

T\(_m\) increased by 2.6°C in HOT and decreased by 3.8°C in COLD. PPO was found to be independent of FL. Compared to CONTROL (CON), cooling the muscle induced a mean reduction of 21% (p<0.001) in PPO but warming the muscle had no significant effect (Table 1). An inverse linear relationship between FL and KAV\(_{\text{peak}}\) was evident for all conditions, which was displaced downwards with a decrease in T\(_m\). These observations confirm a similar temperature dependence of the contractile properties of muscle measured under isokinetic and freely accelerating cycle ergometry.

References