

mRNA expression in the arcuate nucleus. In conclusion, these data demonstrated that there is a desensitization of hypophagic effect in response to repeated exposure to endotoxin. Circulating leptin and OB-Rb and MC4R in the hypothalamus are likely to be involved in the control of food intake during endotoxemia.

FAPESP, CNPq, FAEPa.

*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

## C111

### Low glucose availability induces metabolic transformation of primary rabbit muscle cells in culture

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Adult skeletal muscle cells are differentiated into various fibre types with distinct mechanical and biochemical properties. Nevertheless skeletal muscle fibres reveal an enormous potential to adapt to chronic physical load by an alteration of metabolic capacity and contractile properties up to a complete transformation of fibre type. Using primary skeletal muscles grown on microcarriers we could show that the transformation of fast to slow myosin depends on the signalling cascade calcium-calmodulin-calcineurin-NFATc1. However, the regulation of metabolic adaptation to exercise and chronic electrical stimulation seems not to be solely linked to calcium signalling and not to calcineurin activation. Therefore we investigated possible further mechanisms which might be involved in metabolic transformation typically associated with fast-to-slow transition of fibre type in rabbit skeletal muscle cells. We hypothesized that lowered intracellular ATP concentration or reduction of the glycogen stores could act as triggers of metabolic transformation. Three days of pharmacological reduction of cytosolic ATP concentration by 5 mM 3-guanidino propionic acid had no impact on the investigated metabolic markers, whereas incubation of the cells with little or no glucose leads to decreases in glycogen in conjunction with decreases in glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter activity, GAPDH mRNA and specific GAPDH enzyme activity (indicators of the anaerobic glycolytic pathway). Furthermore, reduction of glucose availability increased promoter activity of mitochondrial acetoacetyl-CoA thiolase (MAT, also known as ACAT), mRNA expression of peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$  (PGC-1 $\alpha$ ) and citrate synthase (CS) specific enzyme activity (all indicators of oxidative metabolic pathways). In experiments with two inhibitors of glycogen degradation 1,4-dideoxy-1,4-D-arabinol (DAB, Novo Nordisk, Bagsvaerd, Denmark) or N-Butyldeoxynojirimycin (BDN, Calbiochem/Merck, Darmstadt, Germany) we found that the observed metabolic transformation caused by low glucose was induced even when intracellular glycogen content was high.

These findings provide evidence that metabolic adaptation of skeletal muscle cells from rabbit in primary culture can be induced not only by elevation of intracellular calcium concentration or by a rise of AMPK activity, but also by reduction of glucose availability.

*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

## C112

### Ca<sup>2+</sup> influx pathways in white adipocytes from rat

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Ca<sup>2+</sup> is an important signaling molecule for many cell types. Changes in its intracellular concentration may involve alteration in Ca<sup>2+</sup> flux across the plasma membrane or/and Ca<sup>2+</sup> mobilization from intracellular Ca<sup>2+</sup> stores. Abnormal handling of intracellular Ca<sup>2+</sup> in white adipocytes, a major target for insulin, is thought to be associated with insulin resistance and diabetes mellitus. However, since little is known about the Ca<sup>2+</sup> pathways and their related functions in white adipocytes, we have explored this area with Ca<sup>2+</sup> imaging technology.

White adipocytes were isolated from rat epididymal fat pads by collagenase digestion and were attached to glass coverslips. Adipocytes were imaged at 32°C with constant perfusion of a HEPES-buffered salt solution. Changes in intracellular Ca<sup>2+</sup> were monitored as changes in the fluorescence intensity of the Ca<sup>2+</sup>-sensitive dye, Fluo-4. Fluorescence intensity is presented as the mean  $\pm$  SEM percentage of control value with n, the number of experiments. Unpaired Students T-test or ANOVA were used to determine statistical significance relative to control.

Oxytocin (1  $\mu$ M), but not insulin, elicited a transient increase in fluorescence ( $282 \pm 34\%$ , n=21), which then decayed back down to a plateau level ( $123 \pm 5\%$ , n=21) which was sustained for the duration of the peptide addition. Omission of extracellular Ca<sup>2+</sup> did not affect the peak response, but decreased the plateau level to value that was sub-basal ( $83 \pm 22\%$ , n=6; p<0.05). 2-APB (75  $\mu$ M), a selective blocker of store-operated Ca<sup>2+</sup> channels, did not affect the peak response to oxytocin but abolished the plateau phase ( $104 \pm 6\%$ , n=10; p<0.05). Elevation of extracellular K<sup>+</sup> from 5 to 50 mM, by isosmotic substitution with Na<sup>+</sup>, increased intracellular Ca<sup>2+</sup> with a peak fluorescence intensity of  $207 \pm 17\%$  (n=21). This response had an obligatory requirement for extracellular Ca<sup>2+</sup> and was significantly attenuated by either 20  $\mu$ M nifedipine ( $124 \pm 4\%$ , n=17; p<0.05) or 20  $\mu$ M (149  $\pm$  9%, n=12; p<0.05), inhibitors of the L-type voltage-gated Ca<sup>2+</sup> channel. Reduction of extracellular Na<sup>+</sup> from 145 to 98 mM, by isosmotic replacement with N-Methyl-D-glucamine H<sup>+</sup>, also increased intracellular Ca<sup>2+</sup> ( $130 \pm 4\%$ , n=11; p<0.05); an effect abolished by 10  $\mu$ M KB-R 7343, a putative Na<sup>+</sup>/Ca<sup>2+</sup> exchange inhibitor (p<0.05).

In summary, oxytocin, but not insulin, can increase intracellular Ca<sup>2+</sup> in rat adipocytes via mobilization of Ca<sup>2+</sup> from internal stores and influx through store-operated Ca<sup>2+</sup> channels.

Furthermore, the results of this study suggest that rat white adipocytes possess functional L-type  $\text{Ca}^{2+}$  channels and that they can also undergo reverse mode  $\text{Na}^+/\text{Ca}^{2+}$  exchange. The roles of these  $\text{Ca}^{2+}$  pathways in adipocyte physiology remain to be established.

We wish to thank Diabetes UK for support

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## C113

### **LH-induced depolarization of membrane potential in mice Leydig cells can function as a trigger for the subsequent intracellular calcium increase**

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Testosterone synthesis in Leydig cells is regulated by the Luteinizing Hormone (LH) and external  $\text{Ca}^{2+}$  is required for this process. Using fluo3 confocal microscopy we show that cAMP causes intracellular  $\text{Ca}^{2+}$  transients that depend crucially on the  $\text{Ca}^{2+}$  influx via a T-type calcium channel located at the plasma membrane of mice isolated Leydig cells. While the involvement of  $\text{Ca}^{2+}$  in LH induced steroidogenesis is well known, the actual mechanisms that determine the intracellular  $\text{Ca}^{2+}$  signaling processes are poorly understood. Similarly to cAMP, the LH dependent  $[\text{Ca}^{2+}]_i$  increase is inhibited by removal of the extracellular  $\text{Ca}^{2+}$  or by addition of 400  $\mu\text{M}$   $\text{NiSO}_4$ . The kinetic properties of the  $[\text{Ca}^{2+}]_i$  transients are, however, different. The mean rise time ( $t_p$ ) for LH (1  $\mu\text{g}/\text{ml}$ ) is  $64 \pm 4.2$  s ( $n = 60$  cells), whereas for 400  $\mu\text{M}$  dibutyl- $\text{cAMP}$  it is  $38.2 \pm 5.3$  s ( $n = 44$  cells), and the amplitude of the fluorescence signal ( $\Delta F$ ) is  $2.9 \pm 0.4$  times the baseline fluorescence for cAMP and  $3.3 \pm 0.2$  for LH. Ryanodine receptors are involved in the rise of  $[\text{Ca}^{2+}]_i$  since 100  $\mu\text{M}$  ryanodine blocks the  $[\text{Ca}^{2+}]_i$  increase subsequent to the addition of 1  $\mu\text{g}/\text{ml}$  of LH to the bath (4 experiments). Interestingly, removal of ryanodine under these conditions leads to  $[\text{Ca}^{2+}]_i$  oscillations. Three isoforms of IP3 receptors were also identified by immunocytochemistry with a highly heterogeneous spatial distribution. Despite the presence of IP3 receptors, addition of 100  $\mu\text{M}$  2-APB had no effect on the LH induced  $[\text{Ca}^{2+}]_i$  changes ( $t_p = 87.2 \pm 5.8$  s,  $\Delta F = 0.8 \pm 0.1$ ;  $n = 12$  cells, 4 experiments). The LH induced  $[\text{Ca}^{2+}]_i$  increase is also evident inside the mitochondria as revealed by experiments with mitotracker green (Invitrogen Corporation). Measurements of the membrane potential of Leydig cells with Di-4-ANEPPS (Invitrogen Corporation) show that treatment with 1  $\mu\text{g}/\text{ml}$  LH causes a depolarization around 30 mV ( $n = 3$  experiments). We argue that this change in membrane potential may function as the driving force for the  $\text{Ca}^{2+}$  influx through the T-type channels, constituting therefore the initial signal for subsequent  $\text{Ca}^{2+}$  release from the ER via the ryanodine and the IP3 receptors. The details on how the structure and the spatial distribution of the above mentioned molecules determines the observed  $\text{Ca}^{2+}$  dynamics still remains to be elucidated.

This research was supported by grants from FAPESP, CNPq and FAEPA.

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## C114

### **PKA mediates intracellular calcium increase induced by LH in mice Leydig cells by modulating Cav3.2 channels**

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Binding of Luteinizing hormone (LH) to its receptor, in mice Leydig cells, leads to the activation of both adenylyl cyclase and phospholipase C pathways. Action of the LH is also associated to an increase in the intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) mediated by calcium entry through a T-type  $\text{Ca}^{2+}$  channel present in the plasma membrane (Costa and Varanda, 2007). In order to study the possible pathway by which LH activates the previously reported  $\text{Ca}^{2+}$  channel, we investigated the effects of modulators of the PKC and PKA pathways on the  $\text{Ca}^{2+}$  currents stimulated by LH. Using confocal microscopy we also tested the effects of these agents on the overall LH induced  $[\text{Ca}^{2+}]_i$  increase. The amplitude of maximal current at -20 mV increases with the addition of 1  $\mu\text{g}/\text{ml}$  LH (from  $-2.4 \pm 0.3$  in control to  $-3.1 \pm 0.4$  pA/pF with LH;  $n = 8$ ,  $p < 0.00672$ , t-test) and 1  $\mu\text{M}$  phorbol 12-myristate 13-acetate (PMA) (from  $-3.6 \pm 0.4$  with PMA;  $n = 5$ ,  $p < 0.00009$ , t-test). Despite of this, no change in the voltage dependence of the activation, inactivation or deactivation was observed. The half voltage ( $V_{0.5}$ ) for activation at control conditions and 37 °C were  $-39.4 \pm 0.2$  mV and  $-40.1 \pm 0.1$  in the presence of LH. The  $V_{0.5}$  of steady state inactivation was  $-51.6 \pm 0.9$  mV and  $-52.2 \pm 0.9$  mV, respectively for control and LH. The  $V_{0.5}$  of activation with PMA was  $-37.7 \pm 0.6$  mV and for PMA+LH it was  $-39.9 \pm 0.1$  mV. For steady state inactivation the  $V_{0.5}$  values were  $-49.8 \pm 1.4$  mV and  $-51.6$  mV respectively. The deactivation time constant,  $\tau_{\text{deact}}$ , at control conditions was  $11.6 \pm 1.9$  ms. For LH, PMA and PMA+LH, the values of  $\tau_{\text{deact}}$  were  $15.3 \pm 0.6$  ms,  $\tau_{\text{deact}} = 24 \pm 6.5$  ms and  $\tau_{\text{deact}} = 16.17 \pm 3.7$  ms, respectively. Experiments carried out with the calcium sensitive dye fluo-3, show that inhibition of PKC and PKA with 400 nM staurosporine blocks the increase of  $[\text{Ca}^{2+}]_i$  induced by LH. Treatment of the cells with 10  $\mu\text{M}$  H89, an inhibitor of cAMP-dependent protein kinase, also abolishes the elevation of  $[\text{Ca}^{2+}]_i$  caused by LH ( $n = 5$ ). PMA slowly increases the fluorescence when the cells are incubated at 37 °C. Nevertheless, the subsequent addition of 1  $\mu\text{g}/\text{ml}$  LH induces the typical increase in  $[\text{Ca}^{2+}]_i$  showing that PKC does not inhibit the LH induced  $[\text{Ca}^{2+}]_i$  rise. Taken together, these results suggest that PKA plays a leading role in mediating the LH action on the  $[\text{Ca}^{2+}]_i$ , despite the fact that PKC is able to increase the amplitude of Cav3.2.

Costa RR & Varanda WA (2007). J Physiol 585, 339–349.

This work was supported by FAPESP, CNPq and FAEPA.

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## C115

### **Environmental enrichment facilitates long-term potentiation within striatal grafts transplanted into a mouse model of Huntington's disease**

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We have previously demonstrated that Embryonic striatal grafts express long-term potentiation (LTP) and long-term depression (LTD) consistent with the normal striatum (Mazzocchi-Jones, et al 2005). Previous analysis of environmental enrichment on graft function has demonstrated an increase in performance on a number of tasks involving motor learning (Dobrossy et al, 2005). In this present study we investigate the effect of enrichment on host-graft synaptic plasticity.

Mice were raised in two environments, 'standard' animals were housed in pairs, and 'enriched' animals were housed in groups of 6, with numerous environmental stimuli. Following suitable habitation time mice were anaesthetised in an induction chamber, via Isoflourane, and maintained on this anaesthesia throughout the entire, and all, surgical procedures. Mice receiving striatal grafts were subjected to quinolinic acid induced unilateral lesions of the striatum. Seven days later all lesioned mice received a unilateral transplant of embryonic striatal tissue, obtained from E17 PrP-L-EGFP-L transgenic mice. Beginning at four weeks post-transplantation mice were euthanased via cervical dislocation, and brain slices were produced for in vitro recording.

Environmental enrichment had no significant effect on the level of LTP and LTD observed in control mice (n=7) when compared to control mice housed in standard environments (n=8) (ANOVA, followed by Newman Kheuls Post-Hoc. Groups,  $F(1,13)=3.00$ ,  $p=0.10$ , n.s.). Similarly, there was no significant difference in the level of LTD observed in grafted mice from enriched environments (n=8) when compared to grafted mice housed in control environments (n=5) (ANOVA, followed by Newman Kheuls Post-Hoc. Groups,  $F(1,11)=0.10$ ,  $p=0.91$ , n.s.). However, there was a significant facilitation of LTP in grafted animals housed in enriched environments (n=12), when compared to those housed in standard environments (n=8) (ANOVA, followed by Newman Kheuls Post-Hoc. Groups,  $F(1,19)=8.41$ ,  $p<0.001$ ). Previous analysis of the effect of environmental enrichment on graft function has shown an increase in Brain derived neurotrophic factor (BDNF) levels (Dobrossy et al, 2005). Tissue samples of various brain regions were isolated and subjected to an ELISA to assess BDNF levels. Environmental enrichment resulted in an increase in BDNF levels across samples, with a significantly greater increase in levels of BDNF observed in the grafted striatum, compared to both the normal and grafted striatum from standard environments (ANOVA, Standard Graft n=3, Standard Control n=3, Standard Enriched n=3, Graft Enriched n=3. Groups,  $F(1,23)=47.43$ ,  $p<0.001$ ). Mazzocchi-Jones D, Dobrossy MD, & Dunnett SB (2005). Basal Ganglia VIII. Eds Bolam P, Ingham C, & Magill P. PP 313-320.

Dobrossy MD, Dunnett SB (2005) Rev Neurosci 16: 1-21.

This work was supported by the MRC.

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## C116

### **The long term potentiation in amygdalar BLA-CE pathway from MMP-9 overexpressed rats**

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Matrix metalloproteinase-9 (MMP-9) acts extracellularly to cleave components of extracellular matrix (Michaluk and Kaczmarek, 2007). MMP-9 is expressed in the brain, where it is produced especially by neurons. MMP-9 is crucial for remodeling of extracellular environment, essential for synaptic plasticity, learning and memory. The amygdala is a heterogenous brain structure important for regulation of emotional aspects of memory as well as autonomic and endocrine responses. It has been shown that the central nucleus (CE) of amygdala is involved in appetitive learning (Knapska et al., 2006). In the present study, we investigated the importance of MMP-9 in synaptic plasticity in the CE. MMP levels were analyzed by zymography and MMP-9 function was assessed by employing long term potentiation (LTP) model of synaptic plasticity. We studied slices obtained from the transgenic rats with neuron-specific MMP-9 overexpression driven by Synapsin I promoter (Wilczynski et al., 2008). The basolateral nucleus of amygdala (BLA) was tetra burst stimulated using a bipolar electrode and EPSPs were collected from CE. At 15 min. after achieving a stable baseline recording, theta-burst stimulation (TBS, three trains of high frequency stimuli applied every 5 min) protocol was used to evoke LTP that was recorded for the next 90 min. In both, MMP-9-overexpressing and control group, LTP persisted throughout the experiment. However, the level of LTP obtained in slices from MMP-9 overexpressing rats (6 slices from 4 animals) was lower than from the control ones (5 slices from 3 rats) by  $50\pm 3,19\%$  ( $p=0.015$ , ANOVA for repeated measures) and this effect was maintained throughout all the 90 min of recording. The present observation suggests that the proper level of MMP-9 expression and activity is essential for synaptic plasticity in the BLA-CE pathway, whereas MMP-9 overexpression may cause destabilization of neuronal environment and decreased activity-dependent strengthening of synaptic transmission.

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Knapska et al. (2006) Learn. Mem., 13: 192-200.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC126

**Tempol, a SOD-mimetic, improves muscle function in a rat model of sleep apnoea**

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Obstructive sleep apnoea (OSA) is a common disorder characterised by repeated occlusions of the upper airway during sleep. Upper airway muscle dysfunction is implicated in the pathophysiology of OSA. We have shown that intermittent hypoxia – a feature of OSA due to recurrent apnoea – impairs respiratory muscle function. In this study, we tested the hypothesis that antioxidant treatment following chronic IH exposure would improve muscle function in our rat model of OSA.

15 adult male Wistar rats were placed in chambers and exposed to alternating periods (90s) of normoxia and hypoxia. Control rats (n=15) were placed in identical chambers and were exposed to an air/air cycle continuously under identical conditions in paired studies. Exposures lasted 8 hours per day for 9 days. Following the treatments, animals were killed humanely. The paired sternohyoid muscles were dissected out and prepared for in vitro examination. Isometric contractile properties of isolated strips of sternohyoid muscle were examined in tissue baths under hyperoxic (95%O<sub>2</sub>/5%CO<sub>2</sub>) or hypoxic (95%N<sub>2</sub>/5%CO<sub>2</sub>) conditions in the absence (control) or presence of the superoxide dismutase (SOD) mimetic, Tempol (10mM). Specific force was measured in response to stimulus frequencies ranging from 10-100Hz.

Under in vitro hyperoxic conditions, IH caused a reduction in force at 60 to 100Hz [Peak tetanic force at 100Hz was 22.7±0.8 vs. 15.9±0.9\* N/cm<sup>2</sup>, control (n=8) vs. IH (n=8), \*P<0.001 ANOVA]. Tempol had a positive inotropic effect at high stimulus frequencies in both normoxic and IH-treated rats [Force at 100Hz in hyperoxia was 26.4±1.0 and 22.8±1.4 N/cm<sup>2</sup>, control+tempol (n=7) and IH+tempol (n=7); both significantly different from their respective controls, ANOVA. The relative increase in force was greater in the IH-treated rats compared to normoxia. Under in vitro hypoxic conditions, forces were significantly lower than hyperoxic values but there was no difference between normoxia and IH-treated rats.

This study illustrates that chronic IH decreases force production of rat sternohyoid muscle but has no effect on force generation during in vitro hypoxia. Furthermore, Tempol has a positive inotropic effect on sternohyoid muscle in both groups, but the relative increase in force was greater in IH-treated animals with a recovery of force to control values. We conclude that chronic IH causes maladaptive plasticity in a pharyngeal dilator muscle and as such may be implicated in the pathophysiology of OSA. We conclude that antioxidants may be beneficial as adjunct therapies in the treatment of OSA.

Supported by the Health Research Board. JRS is a UCD Ad Astra Research Scholar.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC127

**Patterns of Ca<sup>2+</sup> signals induced by purinergic and adrenergic stimulation of smooth muscle cells from rat renal resistance arteries**

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Renal vascular tone, which determines renal blood flow, is controlled by sympathetic nerves via activation of  $\alpha_1$ -adrenoceptors ( $\alpha_1$ -ARs) and P2X purinoceptors (P2X-Rs) [1]. Activation of these causes a robust increase of [Ca<sup>2+</sup>]<sub>i</sub>. The aim of this work was to visualise with high temporal resolution sub-cellular calcium events evoked by stimulation of  $\alpha_1$ -ARs or P2X-Rs in renal vascular smooth muscle cells (RVSMCs) and to analyse the dynamics of these events. Experiments were performed on RVSMCs freshly isolated from rat renal resistance arteries, which were separated from the kidney using an iron oxide sieving technique [2]. Analysis of gene expression in single RVSMCs (250 cells were collected with a glass micropipette) using RT-PCR technique confirmed expression of the genes encoding for  $\alpha_{1A}$ -ARs and P2X<sub>1</sub>-Rs, but not for  $\alpha_{1B}$ -ARs, P2X<sub>3</sub>-Rs and P2X<sub>5</sub>-Rs. Changes of [Ca<sup>2+</sup>]<sub>i</sub> induced by stimulation of  $\alpha_1$ -ARs or P2X-Rs in RVSMCs loaded with the Ca<sup>2+</sup> indicator Fluo-4AM were visualised using fast (33-40 Hz) x-y confocal imaging. Data are presented as mean ± S.E.M.; independent Student's t-test was performed to verify statistical significance of the results. Stimulation of  $\alpha_1$ -ARs with 10  $\mu$ M noradrenaline (NA) or P2X-Rs with 10  $\mu$ M  $\alpha,\beta$ -methylene adenosine 5'-triphosphate (AMP-CPP) evoked a rise in [Ca<sup>2+</sup>]<sub>i</sub>, which consisted of transient and sustained components and was initiated by a sub-plasmalemmal [Ca<sup>2+</sup>]<sub>i</sub> upstroke (SPCU) [3]. AMP-CPP-evoked SPCUs had a significantly (p<0.0001) faster upstroke (mean time-to-peak 0.72±0.06 s; n=13) than NA-induced ones (mean time-to-peak 1.56±0.15 s; n=12). Time-to-peak of the AMP-CPP-induced global [Ca<sup>2+</sup>]<sub>i</sub> rise (0.79±0.07 s; n=19) was also significantly (p<0.0001) shorter than the NA-induced one (1.89±0.18 s; n=17). Both AMP-CPP- and NA-induced [Ca<sup>2+</sup>]<sub>i</sub> transients, however, were of similar duration: the full duration at half-maximal amplitude of the transients were 4.08±0.30 s (n=19) and 4.49±0.35 s (n=17), respectively. Simultaneous imaging of AMP-CPP-induced [Ca<sup>2+</sup>]<sub>i</sub> changes in cytosol and mitochondria ([Ca<sup>2+</sup>]<sub>m</sub>) using Fluo-4 and Rhod-2, respectively, revealed that [Ca<sup>2+</sup>]<sub>m</sub> started to rise with a delay of 0.34±0.08 s following initiation of the [Ca<sup>2+</sup>]<sub>i</sub> transient and reached its peak 1.02±0.17 s later (n=14). Mitochondrial sequestration of Rhod-2 was confirmed by double staining with MitoTracker Green FM (MTG; a

molecule that covalently binds to the inner mitochondrial membrane and fluoresces independently of mitochondrial membrane potential and  $[Ca^{2+}]_m$ ). Double-staining of RVSMCs with Brefeldin A BODIPY 558/568 and MTG revealed a tight association between the sarcoplasmic reticulum (SR) elements and mitochondria. The spatial patterns of agonist-induced  $[Ca^{2+}]_i$  transients were related to the intracellular distribution of SR and mitochondria.

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Supported by WT (042293, 074724 and 075112) & BHF (FS/06/077).

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## PC128

### **SNAP-25-dependent exocytosis regulates plasma membrane insertion of Orai1 and contributes to store-operated $Ca^{2+}$ influx**

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$Ca^{2+}$  release-activated  $Ca^{2+}$  (CRAC) channels are activated when free  $Ca^{2+}$  concentration in the intracellular  $Ca^{2+}$  stores is substantially reduced and mediate sustained  $Ca^{2+}$  entry in a number of cell types. Although the molecular identity of CRAC channels has not been precisely determined, recent findings have identified Orai1 as a CRAC channel subunit. SNARE proteins have been shown to be important for the insertion of  $Ca^{2+}$  channels of the TRPC family in the plasma membrane, whose surface expression is determined by a recycling-type of trafficking mechanism in a number of cells (Singh et al., 2004). The aim of the present study was to investigate whether Orai1 surface expression is regulated by depletion of the intracellular  $Ca^{2+}$  stores and the possible involvement of the SNARE protein SNAP-25 in this process.

Human HeLa and embryonic kidney 293T (HEK-293T) cells were obtained from the American Type Culture Collection and cultured in Dulbecco's modified Eagle's medium. Cytosolic free  $Ca^{2+}$  concentration ( $[Ca^{2+}]_i$ ) measurement, Western blotting and biotinylation of cell-surface proteins were performed as previously described (Rosado et al., 2000a; Lopez et al., 2006). Passive  $Ca^{2+}$  store depletion using the inhibitor of the sarcoplasmic reticulum  $Ca^{2+}$ -ATPase (SERCA), thapsigargin (TG), enhances the surface expression of Orai1 to  $246 \pm 22\%$  and  $193 \pm 20\%$  of control (resting cells), in HeLa and HEK cells, respectively (mean  $\pm$  SEM;  $P < 0.05$ , Student's t-test;  $n = 4$ ). This effect depends on rises in  $[Ca^{2+}]_i$ , as demonstrated in cells loaded with dimethyl BAPTA, an intracellular  $Ca^{2+}$  chelator that prevented

TG-evoked elevation in  $[Ca^{2+}]_i$ . Cleavage of the soluble N-ethylmaleimide-sensitive-factor attachment protein receptor (SNARE) SNAP-25 with Botulinum neurotoxin A (BoNT A) impaired TG-induced increase in the surface expression of Orai1. BoNT A also decreased TG-induced  $Ca^{2+}$  entry by 60%. In aggregate, these findings demonstrate that store depletion enhances Orai1 plasma membrane expression in an exocytotic manner that involves the protein SNAP-25, a process that contributes to store-dependent  $Ca^{2+}$  entry.

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This work was supported by Junta de Extremadura and Spanish Ministry of Education and Science grant BFU2007-60104/BFI.

*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

## PC129

### **Oleuropein and cycloolivil from olive tree wood exert antiaggregant effects in platelets from patients with type 2 diabetes mellitus**

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Type 2 diabetes mellitus induces a number of cardiovascular disorders, including platelet hyperactivity and hyperaggregability, which are associated to an increased oxidant production and abnormal cytosolic  $Ca^{2+}$  handling (Redondo et al., 2005). Phenolic compounds have been reported to modulate several biological processes in platelets, such as cytosolic free  $Ca^{2+}$  concentration, endogenous reactive oxygen species generation, protein tyrosine phosphorylation and aggregation (Ben Amor et al., 2007). In the present study, we have investigated the effect of two phenolic compounds obtained from the olive tree, oleuropein and cycloolivil, on  $Ca^{2+}$  mobilization, protein tyrosine phosphorylation and aggregation in platelets from type 2 diabetic and healthy donors.

Blood was drawn from volunteers with local ethical committee approval. Cytosolic free  $Ca^{2+}$  concentration ( $[Ca^{2+}]_i$ ) measurement, Western blotting and platelet aggregation were performed as previously described (Bouaziz et al., 2007). Pre-treatment of platelets with 100  $\mu$ M oleuropein or cycloolivil reduced  $Ca^{2+}$  release and  $Ca^{2+}$  entry induced by the physiological agonist thrombin (1 U/mL) or by treatment with the SERCA inhibitor thapsigargin (1  $\mu$ M) in combination with a low concentration of ionomycin (50 nM) in platelets from diabetic donors. As a result, in the presence of oleuropein or cycloolivil

Ca<sup>2+</sup> mobilisation was similar in platelets from healthy and diabetic subjects. In addition, oleuropein and cyclooolivil reduced thrombin-induced aggregation in platelets from type 2 diabetic subjects and controls in a concentration-dependent manner. The effect of oleuropein and cyclooolivil at the concentration 100 µM on platelet aggregation was comparable to that induced by 100 µM hydroxytyrosol, a well known oxygen radical scavenger. Finally, both oleuropein and cyclooolivil significantly reduced thrombin-evoked protein tyrosine phosphorylation in platelets from diabetic donors and controls. We conclude that oleuropein and cyclooolivil exert an effective anti-aggregant effect in platelets from patients with type 2 diabetes mellitus and reverses the enhanced Ca<sup>2+</sup> mobilization and hyperaggregability described for this pathology.

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This work was supported by Tunisian-Spanish PCI project (A/8203/07) and Junta de Extremadura.

*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

## PC130

### Acidic Ca<sup>2+</sup> store refilling by SERCA3 is regulated by STIM1 in human platelets

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Ca<sup>2+</sup> mobilization regulates a wide variety of cellular functions. Platelets possess agonist-releasable Ca<sup>2+</sup> stores in acidic organelles where SERCA3 pump is involved in store refilling. STIM1, which has been presented as a central regulator of platelet function, is a Ca<sup>2+</sup> sensor of the intracellular Ca<sup>2+</sup> stores (Roos et al., 2005). The aim of the present study was to explore the possible involvement of STIM1 in acidic Ca<sup>2+</sup> store refilling in human platelets. Blood was drawn from volunteers with local ethical committee approval and in accordance with the Declaration of Helsinki. Cytosolic Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>i</sub>) measurement, immunoprecipitation and Western blotting were performed as previously described (Redondo et al., 2004). Electroporation and incorporation (electrotransfection) of anti-STIM1 antibody (or a mouse IgG of the same nature of the anti-STIM1 antibody) into cells was performed following a previously reported procedure (Lopez et al., 2006). To compare the rate of decay of [Ca<sup>2+</sup>]<sub>i</sub> to resting values after stimulation with thrombin we used the constant of the exponential decay as previously shown (Rosado & Sage, 2000). Acidic store refilling was investigated by remobilizing Ca<sup>2+</sup> from the acidic stores using

2,5-di-(t-butyl)-1,4-hydroquinone, a specific SERCA3 inhibitor, after a brief refilling period that followed thrombin stimulation. Electrotransfection of cells with anti-STIM1 (Y231-K243) antibody, directed towards a cytoplasmic sequence of STIM1, significantly reduced acidic store refilling by 70%. In addition, the anti-STIM1 antibody reduced the rate of decay of the [Ca<sup>2+</sup>]<sub>i</sub> to resting levels after stimulation with 1 U/mL thrombin (the decay constants were 0.0094 ± 0.0004 and 0.0078 ± 0.0004 in electroporated cells incubated with mouse IgG or anti-STIM1, respectively; P<0.05 Students t-test; n=7). Platelet treatment with thrombin or with 1 µM thapsigargin in combination with 50 nM ionomycin, to induce extensive Ca<sup>2+</sup> store depletion, resulted in a transient increase in the interaction between STIM1 and SERCA3, reaching a maximum 30 s after stimulation and then decreased. The coupling between STIM1 and SERCA3 was abolished by electrotransfection with anti-STIM1 antibody. The interaction between STIM1 and SERCA3 induced by thrombin or by treatment with thapsigargin plus ionomycin is reduced in platelets from type 2 diabetic patients, as well as Ca<sup>2+</sup> reuptake into the acidic Ca<sup>2+</sup> stores. These findings provide evidence for a role of STIM1 in acidic store refilling in platelets probably acting as a Ca<sup>2+</sup> sensor and regulating the activity of SERCA3. This action is impaired in platelets from type 2 diabetics, which might lead to the enhanced cytosolic Ca<sup>2+</sup> concentration observed (Saavedra et al., 2004) and, therefore, in platelet hyperactivity.

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Supported by M.E.C.-FEDER (BFU2007-60104/BFI) and Junta de Extremadura

*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

## PC131

### Activation and translocation of Bid and Bax to the mitochondria in response to thrombin in human platelets

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Apoptosis or programmed cell death is a well-conserved physiological pathway where the pro-apoptotic proteins Bid and Bax play an essential role. Bid or Bax translocate to mitochondria in response to apoptotic stimuli to initiate the release of cytochrome c. (Luo et al., 1998). Thrombin is a physiological platelet agonist that activates a number of cell functions, as well as apoptotic events, including cytochrome c release and subsequent phosphatidylserine exposure (Lopez et al., 2007). The present study is aimed to investigate whether thrombin

induces activation and mitochondrial translocation of Bid and Bax and its relevance in thrombin-evoked apoptotic events. Blood was drawn from volunteers with local ethical committee approval and in accordance with the Declaration of Helsinki. Changes in the mitochondrial membrane potential were registered using the dye JC-1. Bid and Bax activation and translocation was detected by immunoprecipitation and Western blotting in whole cell lysates and samples from the mitochondrial and cytosolic fractions. Phosphatidylserine externalization was estimated using annexin V-FITC as previously shown (Lopez et al., 2007). Treatment of platelets with 1 U/mL thrombin induces concentration- and time-dependent activation and mitochondrial association of active Bid and Bax (thrombin enhanced the amount of active Bid and Bax in the mitochondria by  $212 \pm 25\%$  and  $200 \pm 22\%$  of control, respectively, mean  $\pm$  S.E.M.;  $P < 0.05$ , Student's t-test;  $n = 6$ ). Translocation of Bid and Bax to the mitochondria was reduced by treatment for 40 min with the actin polymerization inhibitor cytochalasin D ( $10 \mu\text{M}$ ) to  $131 \pm 9\%$  and  $115 \pm 11\%$  of control, respectively, ( $P < 0.05$ , Student's t-test;  $n = 6$ ). Blockage of the Bax channel significantly reduced thrombin-evoked mitochondrial membrane depolarization and phosphatidylserine exposure without having any effect on Bax activity. Our results indicate that thrombin induces activation and mitochondrial translocation of Bid and Bax, a process that is dependent on actin filament polymerization. Thrombin-evoked Bax channel formation is likely one of the initial apoptotic events in human platelets.

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Supported by MEC-DGI grant BFU2007-60091, BFU2007-60104 and Junta de Extremadura.

*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

## PC132

### NAADP-induced $\text{Ca}^{2+}$ release is an important regulator of platelet activation

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Although platelets have an essential role in haemostasis, inappropriate platelet activation is a major factor in pathological thrombosis, which can lead to myocardial infarction and stroke. A rise in intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) is a key step in platelet activation, regulating granule secretion, integrin activation and procoagulant activity. Platelet activators, such as collagen, thrombin and ADP, induce  $\text{Ca}^{2+}$  release from intracellular stores. Although inositol trisphosphate ( $\text{IP}_3$ ) has previously been thought to be the major  $\text{Ca}^{2+}$ -releasing intracellular secondary messenger other second messengers such as

nicotinic acid adenine dinucleotide phosphate (NAADP) have important and necessary roles in  $\text{Ca}^{2+}$  release.

Here we have investigated the role of NAADP-induced  $\text{Ca}^{2+}$  release in platelet activation, using NED-14, a recently-developed inhibitor of the NAADP receptor. First,  $[\text{Ca}^{2+}]_i$  was measured in fura-2-loaded human platelets.  $\text{Ca}^{2+}$  release induced by the physiological agonist ADP ( $10 \mu\text{M}$ ) was partially decreased by NED-14 (peak height above basal was  $48.9 \pm 5.1\%$  of control; mean  $\pm$  SEM;  $n = 4$ ;  $p = 0.002$ , Student's t test). Similarly, CRP (collagen-related peptide, a GPVI agonist;  $5 \mu\text{g/ml}$ ) -induced  $\text{Ca}^{2+}$  release was also decreased by NED-14 (peak height above basal was  $77.8 \pm 7.8\%$  of control;  $n = 3$ ;  $p = 0.018$ ). NED-14 also potently inhibited platelet granule secretion. CRP-induced dense granule secretion, was reduced to  $27.1 \pm 7.6\%$  of control ( $n = 3$ ;  $p = 0.011$ ) and  $\alpha$ -granule secretion, measured as surface P-selectin expression, was reduced to  $45.2 \pm 3.4\%$  ( $n = 3$ ,  $p = 0.035$ ). Platelet aggregation in response to CRP and ADP was also inhibited. ADP-induced aggregation was completely abolished ( $n = 3$ ), and CRP-induced aggregation was reduced to  $44.8 \pm 4.1\%$  of control ( $n = 4$ ;  $p < 0.001$ ). This was not wholly due to inhibition of ADP secretion from dense granules and subsequent stimulation of P2Y receptors, since CRP-induced aggregation was also inhibited by NED-14 in the presence of P2Y receptor antagonists. Consistent with this, CRP-induced Rap1b activation (required for integrin activation and so for aggregation), was inhibited by both NED-14 and by P2Y receptor antagonists, and completely abolished by a combination of these treatments ( $n = 3$ ).

These data suggest that NAADP is an important second messenger in platelet activation, and that NAADP-induced  $\text{Ca}^{2+}$  release is a key regulator, both of platelet granule secretion and also of secretion-independent aggregation. Inhibition of NAADP signalling may provide may offer an alternative therapeutic target in treatment of thrombosis.

This work was supported by the British Heart Foundation.

*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

## PC133

### Peroxynitrite prevents $\text{Zn}^{2+}$ -induced cell death by reactivating glutathione reductase

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Peroxynitrite is a nitric oxide-derived highly reactive cytotoxic molecule. Recently, however, we found that 3-morpholininosydnonimine (SIN-1), a peroxynitrite donor, prevented  $\text{Zn}^{2+}$ -induced cell death in differentiated PC12 cells. In the present study, therefore, we have investigated the mechanism responsible for the protective effect of SIN-1 on the  $\text{Zn}^{2+}$ -induced cell death.

Treatment of PC12 cells with zinc chloride ( $10 \mu\text{M}$ ) and pyridoxine ( $5 \mu\text{M}$ ), a  $\text{Zn}^{2+}$  ionophore, caused cell death in differentiated PC12 cells. The  $\text{Zn}^{2+}$ -induced cell death was inhibited by the pretreatment with SIN-1 over the concentration

range 100 - 500  $\mu$ M. Because SIN-1 produces both nitric oxide (NO) and peroxynitrite, we examined whether the protective effect of SIN-1 against PC12 cell death was attributed to NO or peroxynitrite. Pretreatment of cells with various NO donors, such as SNP and SNAP, was not able to attenuate the  $Zn^{2+}$ -induced cell death. In addition to this, peroxynitrite scavengers, such as uric acid, trolox and L-methionine, abolished the protective effect of SIN-1, suggesting that peroxynitrite was responsible for the neuroprotective effect of SIN-1 in differentiated PC12 cells. To investigate the mechanism by which peroxynitrite exerted its protective effect, the intracellular glutathione (GSH) and oxidized GSH (GSSG) levels were measured. An exposure of cells to  $Zn^{2+}$ -pyrithione increased the GSSG/GSH ratio.  $Zn^{2+}$  was also shown to inhibit the GSH reductase (GR) activity, which catalyzes the reduction of GSSG to the thiol form of GSH. However, the addition of 300  $\mu$ M SIN-1 almost completely restored the GR activity and the GSSG/GSH ratio. Furthermore, exogenously applied GSH rescued the PC12 cells from  $Zn^{2+}$ -induced cell death, indicating that disruption of GSH homeostasis plays a critical role in the  $Zn^{2+}$ -induced cell death. Therefore, we suggest that  $Zn^{2+}$  induces cell death, at least in part, by increasing the GSSG/GSH ratio through the inhibition of GR activity, and peroxynitrite attenuates the  $Zn^{2+}$ -induced cell death by re-activating the GR activity.

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This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD).(KRF-2005-041-E00100)

*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

#### PC134

##### **1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one induces neurite retraction by depolymerization of microtubules**

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The effect of the potent soluble guanylyl cyclase (sGC) inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), on neurite outgrowth and retraction was investigated in PC12 cells. ODQ inhibited NGF-, Y-27632- and staurosporine-induced neurite outgrowth in a concentration-dependent manner and triggered neurite retraction. The nitric oxide (NO) scavenger, 2-(4-carboxyphenyl)-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide (PTIO), had little effect on neurite outgrowth. In the presence of ODQ, treatment of PC12 cells with the cell permeable cGMP analogue, 8-bromo-cGMP, failed to re-trigger neurite outgrowth. Furthermore, the depletion of sGC by RNA interference failed to prevent Y-27632- and staurosporine-induced neurite

outgrowth. These results indicate that the NO/sGC/cGMP signaling cascade is not critically involved in ODQ-induced neurite remodeling.

We next investigated the effect of ODQ on microtubule network. Western blot analysis revealed that treatment of PC12 cells with ODQ increased the proportion of unpolymerized tubulin and decreased that of polymerized tubulin, suggesting that ODQ caused to disassemble the microtubule network. The effect of ODQ on microtubule network and neurite retraction was reversed by dithionite. In addition, ODQ was shown to depolymerize purified tubulin *in vitro* and the addition of reducing agents, such as dithionite and DTT, restored the polymerization activity of tubulin.

These results suggest that ODQ induces neurite retraction probably by direct depolymerization of microtubules resulting from tubulin oxidation.

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This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea Government (MOST). (F01-2005-000-10355-0)

*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

#### PC135

##### **The effect of *Aggregatibacter actinomycetemcomitans* on the expression of IL-8 in periodontal ligament cells**

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TITLE ONLY

*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

#### PC136

##### **Atomic force microscopic analysis of structural reorganization of peroxynitrite-stimulated neutrophils**

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TITLE ONLY



*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

PC137

#### 4-Aminopyridine mobilises $\text{Ca}^{2+}$ in human sperm

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4-Aminopyridine (4-AP; 2mM), a blocker of voltage-activated  $\text{K}^+$  channels, is one of the most potent inducers of human sperm hyperactivation yet described. The proportion of hyperactivated cells (Sort 7 assessed by CASA) in a population incubated in capacitating medium increases rapidly from 3-5% to 40-50% (Gu et al, 2004). Since data from our own studies and those of other laboratories have shown that  $[\text{Ca}^{2+}]_i$  plays a central role in regulating activity of the flagellum, we have investigated the effect of 4-AP on  $[\text{Ca}^{2+}]_i$  in human sperm using Oregon Green BAPTA. The application of 2mM 4-AP to human sperm caused a dose dependent, tonic increase in  $[\text{Ca}^{2+}]_i$  fluorescence of  $34.5 \pm 1.1$  s.e.m. in more than 90% of cells. Application of 4-AP to cells bathed in medium with no added  $\text{Ca}^{2+}$  ( $\approx 5 \times 10^{-6}$  M) caused, in most cells, a similar response to that of standard medium, though a proportion of cells (10-30%) showed no change in  $[\text{Ca}^{2+}]_i$ . Application of 4-AP in EGTA-buffered saline ( $<10^{-8}$ M) induced a clear response in  $\sim 25\%$  of cells but  $[\text{Ca}^{2+}]_i$  elevation was transient and was of diminished amplitude. This response was abolished after 10-15 min incubation under these conditions.

We used CASA to examine whether mobilisation of stored  $\text{Ca}^{2+}$  might contribute to the strong hyperactivating action of 4-AP on human sperm. The ability of 4-AP to increase the proportion of hyperactivated cells was not inhibited by simple omission of  $\text{Ca}^{2+}$  from the medium (standard medium  $43.3 \pm 2.9$  s.e.m., 'nominally  $\text{Ca}^{2+}$  free' medium  $48.0 \pm 8.5$  s.e.m.). Furthermore, following brief re-suspension in EGTA buffered saline, application of 2mM 4-AP induced hyperactivation in a proportion of cells only slightly lower than controls ( $39.7 \pm 8.25$  s.e.m.). However, after incubation in EGTA-buffered medium for 20 mins the proportion of motile cells was decreased and the hyperactivation effect of 4-AP was significantly reduced ( $19.8 \pm 5.3$  s.e.m.). Treatment of human sperm with 4-AP did not increase the occurrence of acrosome reaction in cells incubated under capacitating conditions.

4-AP is a weak base and our findings thus might reflect activation of the pH-sensitive  $\text{Ca}^{2+}$  current  $I_{\text{CatSper}}$ . We superfused sperm with saline adjusted to pH8.5 (replacing standard medium at pH 7.4). This induced a sustained increase in  $[\text{Ca}^{2+}]_i$ , consistent with tonic activation of CatSper channels. This pretreatment did not occlude the response to subsequent application of 4-aminopyridine.

We propose that 4-AP induced elevation of  $[\text{Ca}^{2+}]_i$  and hyperactivation of human sperm reflects the mobilisation of labile,

EGTA-sensitive store and also a sustained influx of  $\text{Ca}^{2+}$ . Our results suggest that the 4-aminopyridine induced  $\text{Ca}^{2+}$ -influx may not be solely pH/CatSper dependent and that modulation of capacitative  $\text{Ca}^{2+}$  influx may play a role in this effect.

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*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

PC138

#### Role of immunophilin family of protein in calcium homeostasis in human platelets

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Immunophilins are a large family of chaperones that binds to the N-terminus domain of protein containing proline residues. Two immunophilins groups have been described depending of their negative regulation by binding cyclosporin A (CsA, called cyclophilins) or FK506 (called immunophilins)(Adams et al.,2005; Kumar et al., 2005). Both types has been describes regulating calcium homeostasis through its bind to channel receptors, like RYR or IP3R, and calcium-dependent phosphatase, calcineurin (Cameron et al.,1995). The aim of this work is to elucidate the possible regulatory role of this family of protein on calcium homeostasis in platelets.

Human platelets were isolated of blood drawn from healthy donor according to Declaration of Helsinki, and treated as previously described(Rosado et al., 2000).

Incubation of fura-2 loaded platelet with CsA (10  $\mu\text{M}$ ) for 5 min. modified the rate of calcium release induced by the physiological agonist thrombin (0.1 U/mL) and the SERCA modulator, thapsigargin (TG, 200 nM). This modification on intracellular calcium homeostasis induced by CsA may be explained by inhibiting SERCA2b activity, while others  $\text{Ca}^{2+}$ -ATPases, such as PMCA and SERCA3a remain unaltered.

Store operated calcium entry (SOCE) evoked by thrombin or TG were significantly enhanced by incubation of platelets with FK506. Moreover treatment with CsA reported contradictory results depending which agent was used to induce SOCE activation. All together these results shown evidences for an important role of immunophilin in the regulation of calcium levels in human platelets.

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This work was supported by Ministerio de Educación y Ciencia (RYC-2007-00349).

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC139

**Disruption of an integrin-containing muscle adhesion complex causes muscle protein degradation in *Caenorhabditis elegans***

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Continuous transmission of mechanical signals (mechanotransduction) is required for maintenance of muscle protein mass (1). Of the many factors that cause muscle wasting in human beings, spaceflight and immobilization are thought to induce wasting via decreased use, possibly via decreased mechanotransduction. However, the nature and scope of the mechanical linkage to intracellular signalling pathways regulating protein mass are largely unknown. Proteins making up worm focal adhesions (known as dense bodies) are present in decreased amounts following spaceflight (2-3); these changes correlate with a post-flight movement defect (2). We conducted a series of experiments on the ground in order to examine the significance of this correlation.

We find that acute treatment of adult *C. elegans* with RNAi against one of these genes, *unc-97* (PINCH/LIM-domain), causes degradation of a reporter protein in muscle cytosol. Acute RNAi treatment against any of another eight genes, whose products are likewise conserved members of an integrin-containing muscle adhesion complex, also causes degradation. Suggesting specificity in the regulation of degradation, we find that RNAi against the gene for another complex member, *unc-95* (LIM-domain), fails to cause muscle protein degradation even though it causes a movement defect. Experiments using temperature-sensitive mutations in two of these genes, either *unc-112* (MIG-2) or *unc-52* (Perlican), confirm that disruption of this complex causes degradation, and further show that the extramuscular ligand (UNC-52/Perlican) is required to prevent degradation. In these mutants movement becomes uncoordinated and muscle structure is disrupted following temperature shift. In *C. elegans*, Acetylcholine Receptor and opposed Insulin Growth Factor Receptor-Fibroblast Growth Factor Receptor signalling networks control muscle protein degradation via proteasome and non-proteasome dependent mechanisms, respectively (4-5). Drugs, mutations, and RNAi treatments targeted at these networks, and known to block degradation in *C. elegans* muscle, fail to block degradation triggered by acute loss of members of the muscle attachment complex. Current goals include determining the identity and regulation of the relevant protease(s).

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This work has been funded by the NIH-NIAMS (AR054342), a Pennsylvania Space Grant studentship and University of Pittsburgh support to EOA, and by University of Nottingham and Derby Hospitals NHS Trust support to NJS.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

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**Generalized disruption to relaxation by Cumene Hydroperoxide in rat aorta**

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Increased oxidative stress is a common finding in diseases such as diabetes (1). Cumene hydroperoxide is an organic compound commonly used to induce free radical formation in vitro to mimic oxidative stress (2). We examined the effect of this compound on the relaxation induced by a variety of agonists in the rat aorta. Segments of the aorta from Sprague Dawley rats mounted in organ baths were incubated for 30 or 120 minutes in physiological salt solution containing 100 or 300  $\mu$ M of Cumene hydroperoxide (CHP) before concentration-response curves were constructed for acetylcholine (Ach, endothelium-dependent relaxant), sodium nitropruside (SNP, non endothelium-dependent nitric oxide donor), Levromakalim (ATP-sensitive potassium channel opener), Naringenin (large conductance potassium channel opener) and forskolin (activator of adenylyl cyclase-Protein kinase A pathway). Relaxations to all agonists were severely impaired by CHP (100 or 300  $\mu$ M) following exposure for as little as 30 minutes. The concentration-response curves for Ach, SNP, levromakalim, Naringenin and forskolin were all significantly ( $p < 0.05$ , t-test and ANOVA) shifted to the right of respective controls. PD<sub>2</sub> for levromakalim and forskolin were significantly ( $p < 0.05$ , unpaired t-test and ANOVA) increased, while the E<sub>max</sub> for all the agonists except for forskolin were significantly ( $p < 0.05$ , unpaired t-test and ANOVA) reduced.

The results show that CHP non-selectively alters the mechanisms of relaxation of the rat aorta resulting in generalized impairment in responses. This suggests that CHP may not be an ideal agent for induction of oxidative stress intended to target a specific cellular pathway.

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Supported by the Commonwealth Scholarship Commission UK

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.