

information. Further studies are now required to determine the DH cell type expressing this Cx45 protein.

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This work was supported by The Wellcome Trust.

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

PC106

Human cone photopigment regeneration assessed using the electroretinogram: slower recovery following intense bleaches

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Photopigment regeneration after bleaching can reveal much about retinal function in health and disease. Recently a "rate-limited" model has been proposed, whereby 11-*cis* retinal diffuses into photoreceptor outer segments through a resistive barrier from a constant pool in the pigment epithelium, resulting in regeneration proceeding linearly with time rather than as an exponential (Lamb & Pugh, 2004; Mahroo & Lamb, 2004). We tested this hypothesis for cone pigment regeneration following very intense bleaches, posing two questions. Does regeneration follow a linear rate after intense bleaches? If so, is the rate the same as for smaller bleaches, as the model predicts? We used a conductive fibre electrode to record electroretinogram photopic *a*-wave responses to red flashes (0.4 photopic $\text{cd m}^{-2} \text{s}$) following one-minute bleaching exposures (11 000 - 130 000 photopic cd m^{-2}) in two normal subjects with dilated pupils. A blue background (40 scotopic cd m^{-2}), present throughout, eliminated rod signals. Post-bleach response amplitudes were used to estimate pigment regeneration using a transformation published previously (Mahroo & Lamb 2004). Recoveries proceeded according to a linear rate: first order exponentials gave strikingly poorer fits than the "rate-limited" model (see Fig. 1). However, the rate was around 30% slower than that obtained previously from the same subjects following less intense bleaches (Mahroo & Lamb, 2004), suggesting that the model needs modification.

Cone pigment appears to regenerate more slowly following very intense bleaches. This may indicate a reduction in the 11-*cis* retinal pool available, shedding new light on retinal mechanisms after exposure to intense illumination.

Figure 1. Cone pigment recovery following intense bleach Fifteen dim red flashes (0.40 $\text{cd m}^{-2} \text{s}$ at 0.5 s intervals) were presented every 10 s after 1 min exposure to yellow light of 40,000 photopic cd m^{-2} . Response amplitudes, measured 14-15 ms after each flash, were used to estimate pigment level (Mahroo & Lamb, 2004, Eqn (9) and (10)). Points plot mean \pm S.E.M, over 20 s windows, with results from six exposures, so

each point averages c.180 flash presentations. Panels fit recovery for a 100% bleach with different models: points differ slightly between panels as fits give different estimated dark-adapted amplitudes, affecting normalization.

A, Curve shows best-fitting form of recovery as a single exponential, with time constant of 2.2 min.

B, Solid curve: best-fitting recovery according to rate-limited model, giving parameters $K_m = 0.2$ and rate $v = 0.33 \text{ min}^{-1}$. Dashed curve: expected recovery if v is 0.5 min^{-1} , which fitted recoveries in this subject from less intense exposures (Mahroo & Lamb, 2004).

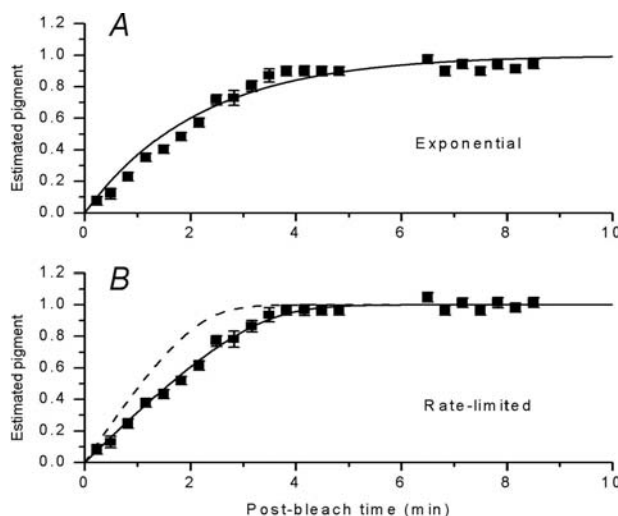


Figure 1

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MSD Studentship to OARM, ARC Federation Fellowship FF0344672 to TDL.

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

PC107

Levetiracetam inhibits calcium signalling in cultured dorsal root ganglia neurons from neonatal rats

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Although the novel antiepileptic drug levetiracetam (LEV) have been shown to be effective in the treatment of pain, the mechanisms mediating its antinociceptive actions are still not well understood. The aim of this study was to investigate the effects of LEV on Ca^{2+} transients, evoked by high- K^{+} (30 mM) in cultured rat dorsal root ganglion (DRG) neurons. DRG neuronal cultures were loaded with 5 μmol Fura-2 AM and Ca^{2+} responses to stimulation with high- K^{+} were assessed by using the fluorescent ratiometry. Fura-2 loaded DRG cultures were excited at 340 and 380 nm, and emission was recorded at 510

nm by using imaging system consisting of CCD camera coupled to an inverted microscope with a 40x (1.30 NA S Fluor, Oil) objective. High-K⁺ responses were determined by the change in 340/380 ratio (basal-peak) and the area under the fluorescence ratio-time curve (AUC) was also calculated for individual DRG neurons in selected microscopic fields. All data were analyzed by using an unpaired t test, with a 2-tailed P level of <.05 defining statistical significance. LEV dose-dependently reduced the [Ca²⁺]_i increase, elicited by 30 mM KCl, in a reversible manner. The mean 340/380 nm ratio was 1.18±0.06 (baseline, n=17), 1.15±0.06 (30 μM LEV, P>0.05, n=17) and 1.17±0.05 (recovery, n=17); 1.28±0.04 (baseline, n=17), 1.14±0.03 (100 μM LEV, P>0.05, n=17) and 1.28±0.03 (recovery, n=17); 1.21±0.03 (baseline, n=18), 1.08±0.02 (300 μM LEV, P>0.05, n=18), and 1.21±0.02 (recovery, n=18), respectively. The AUC changes were consistent with the mean ratio results; the effects of 100 and 300 μM LEV being significant. Our results indicate that LEV significantly suppressed depolarisation-induced intracellular calcium changes in a dose-dependent fashion in dorsal root ganglion neurons. The inhibition of calcium signals in these sensory neurons by levetiracetam might contribute to the antinociceptive effects of the drug.

Keywords: Levetiracetam; dorsal root ganglia, fluorescence calcium imaging, pain, sensory neurons

The authors wish to thank to UCB Pharma for generously providing Levetiracetam (ucb LO59).

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

PC108

Effects of essential hypertension on short latency human somatosensory evoked potentials

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Reduced sensitivity to peripheral nerve stimulation in hypertension may be explained by subclinical axonal neuropathy of sensory afferents (Edwards *et al.* 2008). The current study aimed to further explore this phenomenon by investigating whether the ascending somatosensory pathway is affected by hypertension. Following ethical approval and in accordance with the Declaration of Helsinki, we examined the peripheral median nerve N9, spinal N13 and cortical N20 short latency somatosensory evoked potentials (sSEPs) in 14 patients with unmedicated essential hypertension (9 men, 40 ± 6 years; mean ± sd) and 22 normotensive volunteers (10 men, 37 ± 6 years). The sSEPs were elicited by 100 μs electrocutaneous stimulation of the median nerve at the wrist for 2000 trials (Mauguiere *et al.* 1999). A series of 2 Group (hypertensive, normotensive) ANCOVAs were performed on sSEP amplitudes and latencies, with age and arm length as covariates. N9 amplitudes were significantly reduced (P<.01) in hypertensives (3.60 ± 1.26 μV) compared to normotensives (5.71 ± 2.24 μV). In contrast, N20 amplitudes

were not different between hypertensives (4.38 ± 2.35 μV) and normotensives (3.87 ± 2.20 μV). Furthermore, none of the sSEP latencies differed between groups: N9 (hypertensives: 10.21 ± 0.78 ms, normotensives: 10.36 ± 0.76 ms), N13 (hypertensives: 13.33 ± 0.99 ms, normotensives: 13.57 ± 0.98 ms) and N20 (hypertensives: 19.23 ± 1.26 ms, normotensives: 19.35 ± 0.95 ms). In addition, a 2 Group (hypertensive, normotensive) ANCOVA, with age as a covariate, performed on the sensory median nerve conduction velocity, revealed no differences between hypertensives (61.46 ± 3.77 m/s) and normotensives (61.27 ± 3.63 m/s). Two hierarchical regression analyses were conducted to determine the association between N9 amplitude and 24-hour ambulatory systolic and diastolic blood pressures while accounting for confounding by age and stimulation-to-recording distance. N9 amplitudes were inversely associated with systolic (P<.01) and diastolic (P<.05) blood pressure. As the amplitude of a sensory action potential reflects the number of large diameter myelinated fibres synchronously depolarised in the vicinity of the active recording electrode (Buchthal & Rosenfalck, 1966), a reduction may indicate axonal loss (Gilliat, 1978). As N9 amplitudes, generated by peripheral sensory nerve fibres at the brachial plexus, were 37% smaller in hypertensives than normotensives these data suggest that hypertension affects the peripheral nervous system by reducing the number of active sensory nerve fibres without affecting myelination. However, hypertension does not seem to affect the afferent somatosensory pathway within the central nervous system. In sum, hypertension may represent a risk factor for peripheral neuropathy of the sensory nerves.

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This research and LE was funded by the British Heart Foundation (FS/03/128).

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

PC109

Role of Transient Receptor Potential Vanilloid 1 receptors in C- vs Aδ-fibre-evoked spinal nociception in naïve rats and in a model of post-operative pain

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Transient Receptor Potential Vanilloid 1 (TRPV1) is a cation channel gated by noxious heat, H⁺ ions and capsaicin. TRPV1 is sensitised and upregulated in inflammation, and contributes to

the development and maintenance of chronic pain. TRPV1 receptors are synthesised in the cell bodies of C-fibre primary afferents, and transported to both spinal and peripheral terminals. Much is known of their role in the periphery but less of their role at central terminals. The aim of this *in vivo* study was to investigate the effects of spinal TRPV1 receptor antagonism on the processing of C- vs A δ -fibre-evoked spinal nociception; and to subsequently investigate the contribution of spinal TRPV1 receptors to central sensitisation in a rat model of post-operative pain.

All experiments were carried out on male Wistar rats. Anaesthesia was induced by inhalation of halothane (2-3% in O₂) and maintained using constant intravenous alfaxalone (16-30mg.kg⁻¹hr⁻¹). A heating lamp was evenly placed on the dorsal aspect of the hindpaw. Slow (1.7-2.5°Cs⁻¹) and fast (6.5-7.5°Cs⁻¹) surface heating rates were used to preferentially activate C- and A δ -nociceptors respectively (McMullan *et al.*, 2004). Withdrawal thresholds to noxious heating were recorded as EMG activity from the biceps femoris before and after intrathecal administration of the TRPV1 antagonist SB-366791 (Gunthorpe *et al.*, 2004; 10 μ l, 100 μ M; n=3) or vehicle solution (n=1). For the post-operative pain model, rats were anaesthetised by inhalation of isoflurane (2-3% in O₂) and a 1 cm longitudinal incision was made through skin, fascia and muscle of the plantar hindpaw (Brennan *et al.*, 1996). 24 hours post-surgery animals (n=3) were tested for the effects of spinal TRPV1 antagonism as described above.

In naïve rats, SB-366791 administration significantly increased (P<0.05; Kruskal-Wallis test) withdrawal thresholds to slow but not fast rates of heating. In the model of post-operative pain SB-366791 increased withdrawal thresholds to both slow and fast rates. Comparison of the change in EMG threshold from pre-drug baseline between naïve and surgical model rats reveals a greater effect of the antagonist after surgical incision for both C- and A δ -fibre activation.

These results provide novel evidence that TRPV1 receptors in the spinal cord play a role in the central processing of C- but not A δ -fibre nociceptive inputs in naïve rats. In the post-operative model, the augmented effect of SB-366791 on C- and A δ -nociceptive processing suggests that post-incision, there is a sensitisation or upregulation of spinal TRPV1 receptors in both C- and A δ -fibres. This provides evidence that spinal TRPV1 receptors play a role in central sensitisation in a model of post-operative pain and, as such, may prove a novel target for analgesic drugs.

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BBSRC

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

Descending control produced by cyclooxygenase-1 inhibition in the periaqueductal grey targets dorsal horn neurones with strong C-fibre inputs

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Descending control of spinal nociception that originates from the midbrain periaqueductal grey (PAG) is an important determinant of the pain experience. We have recently shown that cyclooxygenase-1 (COX-1) regulates activity at the level of the PAG and that COX-1 inhibition exerts a preferential effect on C- versus A-nociceptor-evoked withdrawal reflexes (Leith *et al.*, 2007). The current study investigated whether this differential control of C- versus A-nociceptor-evoked activity may be mediated in the spinal dorsal horn.

Extracellular recordings were made from wide dynamic range deep dorsal horn neurones (n=18) with receptive fields on the hindpaw dorsum in alphadolone/alphaxalone-anaesthetised (~20mg.kg⁻¹.hr⁻¹, i.v.) male Wistar rats (280-300g; n=18). At 8 minute intervals, either fast (7.5°C.s⁻¹, 30-57°C) or slow (2.5°C.s⁻¹, 30-55°C) rates of heating were applied to the receptive field to preferentially activate A δ - or C-heat nociceptors respectively (Yeomans *et al.*, 1996a; 1996b; McMullan *et al.*, 2004). Neuronal responses were recorded for 30min before and 65min after administration of the COX-1 inhibitor SC560 (50nM; 300nl volume; n=14) or vehicle (phosphate-buffered saline; n=4) into the ventrolateral-PAG. Afferent input to each cell was characterised by percutaneous electrical stimulation of the receptive field at suprathreshold (1.5 and 3.0 times threshold) intensity for C-fibre activation and the degree of C-fibre input was quantified.

SC560 significantly increased the firing threshold of neurones to both fast and slow heat ramps (to a peak of 127 \pm 3% and 145 \pm 11% of control threshold respectively, mean \pm S.E.M., ANOVA, both p<0.01, n=8-9; overall effect on firing threshold (measured as area under the curve (AUC) over the timecourse 0-65min) 186 \pm 25min. $^{\circ}$ C and 211 \pm 36min. $^{\circ}$ C respectively, mean \pm S.E.M., ANOVA, both p<0.01) compared to vehicle. Peak change in firing threshold post-SC560 and overall effect on firing threshold were not significantly different between fast and slow heat ramps (p=0.0911 and p=0.5791 respectively, t-test, n=8-9). A significant positive correlation was found between the change in firing threshold (both peak threshold and overall effect on firing threshold) produced by SC560 and the degree of C-fibre afferent input to the neurones (r=0.5795, p<0.05 and r=0.6625, p<0.01 respectively, Spearman's rank correlation). The data show that COX-1 inhibition in the ventrolateral-PAG inhibits the responses of wide dynamic range dorsal horn neurones to A- and C-heat nociceptor stimulation and suggests that the degree of descending control from the PAG on individual neurones may be dependent on the extent of their C-fibre innervation.

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JLL is supported by a BBSRC CASE Studentship with GSK.

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

PC111

The use of viral vectors to examine projections from the periaqueductal grey to pontine noradrenergic neurones

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The periaqueductal grey (PAG) is a key midbrain site involved in the modulation of nociception at the level of the spinal cord. However, projections from the PAG to the spinal cord are sparse and it is believed to control nociception, in part, by activating pontospinal noradrenergic (NA) neurones. To further understand the descending pathways from the PAG we have employed viral vectors to investigate the connections between the dorsolateral/lateral (DL/L-) PAG, and pontine NA neurones. In anaesthetised (Ketamine 60mg.kg⁻¹/medetomidine 25µg.kg⁻¹ i.p.) male Wistar rats (n=5) injections of the adeno-associated viral vector AAV-CMV-eGFP (400nl) were made into the dorsolateral/lateral (DL/L) column of the PAG at sites at which prior injection of an excitatory amino acid (DL-homocysteic acid; 50Mm; 80nl) evoked 'pressor' responses. Animals were recovered for 8 days to allow time for anterograde transport of the viral vector to the pons. They were then terminally anaesthetised (sodium pentobarbital 70mg.kg⁻¹ i.p.) and perfusion-fixed with 4% formalin. Brains were removed, post-fixed, and 40µm sections cut through the midbrain and pons. Sections were processed immunocytochemically to visualise terminals containing Green Fluorescent Protein (GFP) and to identify dopamine β-hydroxylase expressing NA neurones. Injection sites, terminal labelling and localisation of NA neurones were determined using conventional and confocal imaging.

AAV-CMV-eGFP produced strong GFP labelling of PAG neurones (>92% NeuN +ve) and transfection extended within the PAG column in the rostrocaudal axis. GFP positive axons and terminals were seen in the pons with a predominantly ipsilateral distribution. Following injection into the DL/L-PAG the greatest number of terminals were seen in the pontine reticular area. Within the NA cell groups the strongest terminal labelling was seen in the rostral locus coeruleus (LC). There were also moderate projections to caudal LC and A7 regions with a low number of projections also noted in the A5 territory. Using both

confocal microscopy and 3D imaging software (Velocity), many GFP labelled terminals in the LC and A7 territories were seen to closely appose both the somata and dendrites of NA neurones. In conclusion, using an adeno-associated viral vector, which has the advantage of being transported in the anterograde direction alone, we have been able to examine the connections of a functionally identified column of the PAG to regions of immunocytochemically identified NA neurones in the pons. The data support the view that neurones in the DL/L-PAG may exert their effects at the level of the spinal cord after engaging pontine NA centres, including LC.

Supported by a BBSRC case studentship with Eli Lilly.

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PC112

Medial prefrontal cortex influences the control of normal and abnormal urinary bladder function in rats

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Brain imaging studies have implicated the medial prefrontal cortex (mPFC) in the control of micturition and urinary continence in both humans and animals, although the exact role is still not fully understood. The present electrophysiological experiments investigated the contribution of the mPFC in the mediation of normal and abnormal bladder contractions in the anaesthetised rat.

Female Sprague Dawley rats (250-300g; n=6) were anaesthetised with isoflurane (50%:50% N₂O:O₂ mixture) and maintained with urethane (1.2 g kg⁻¹, i.v.). The bladder was infused (0.1 ml min⁻¹) continuously with saline or citric acid (10 mg ml⁻¹; pH 4) to evoke normal or abnormal bladder contractions respectively. Simultaneous recording of multiple single-unit and local field potential (LFP) activity using microelectrode arrays placed in the anterior cingulate gyrus of the mPFC measured bladder contraction-evoked neuronal activity. Single-unit and LFP activity, pre-voiding was compared with during/post-voiding-evoked activity using one-way ANOVA; p<0.05 was considered to be significant.

Single-units (n = 13/28 neurones) correlating to voiding were identified in the anterior cingulate gyrus. Activity in these responsive units was suppressed (≥30%) shortly after saline-infusion-evoked bladder contractions. This was paralleled by an increase in LFP signal amplitude (~ 2 fold increase in the LFP signal amplitude; basal mean peak-to-peak amplitude = 0.7 mV). LFP frequency power was significantly (p<0.001) increased in the delta (1-4 Hz) band, and decreased (p<0.001) in the theta (4-8 Hz) band during/post-voiding compared to pre-voiding. Continuous infusion of citric acid produced abnormal bladder contractions and abolished bladder-evoked single-unit activity in 40% of previously responsive neurones. In contrast to

ER- β but does not appear to involve direct activation on smooth muscle of Ca^{2+} -desensitising pathways. It remains to be seen if similar actions are evident in other human arterial vascular beds but nonetheless places oestradiol as a potential endocrine regulator of human placental vascular tone.

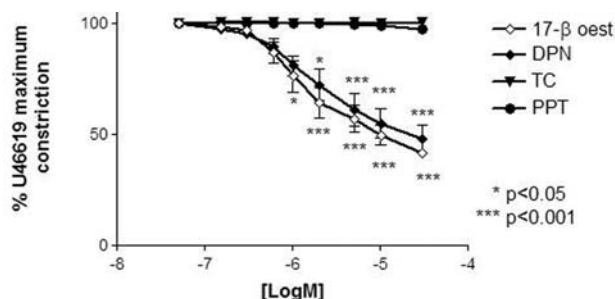


Figure 1: Dose-response curves to 17- β oestradiol, PPT and DPN with matched time controls. Significant relaxation (*) was achieved when exposed to 17- β oestradiol or DPN.

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PC145

Mathematical modelling of electrical action potentials in a uterine smooth muscle cell

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Uterine smooth muscle cells (USMCs) undergo ionic channel remodelling during gestation to facilitate spontaneous action potential (AP)-driven contractions during labour. Electrical activity of USMCs is related to the intracellular Ca^{2+} dynamics which in turn controls their contraction. Simple models of $[\text{Ca}^{2+}]_i$ dynamics and mechanics of USMCs are available but a rigorous model for the membrane excitation is lacking. The aim of this study was to construct a mathematical description of an USMC at the late pregnant stage with biophysically detailed membrane electrophysiology.

Mathematical models for thirteen ionic currents were developed based on voltage-clamp experimental data of late pregnant rat and human tissues in the literature. Several inward currents were considered: L-type Ca^{2+} current, attributed to be the major inward current, fast Na^+ current, T-type Ca^{2+} current and an hyperpolarisation-activated current. The outward currents include: fast A-type transient K^+ current, two voltage-gated K^+ currents (I_{K1} and I_{K2}), Ca^{2+} -activated K^+ current and a sustained background current. Ca^{2+} -activated Cl^- current and a non-specific cation current, each have reversal potentials within the reported AP amplitude range, are also included. All currents are modelled as ohmic resistors and their conductance kinetics described by Hodgkin-Huxley-type first order ordinary differential equations. Currents of a Na^+ - Ca^{2+} exchanger and a Na^+ - K^+ pump are also included to allow incorporation of $[\text{Ca}^{2+}]_i$ dynamics (via the membrane channels, the Na^+ - Ca^{2+} exchanger and the plasma membrane Ca^{2+} -ATPase) modified from a simple uterine excitation-contraction model [1].

The model was initially validated by the ability to produce bursting APs by an external stimulus. In Figure 1(a), repetitive APs were evoked by a current clamp consistent with experimental recordings from myometrium of pregnant rats [2]. Further model parameterisation was evidenced by the ability to closely simulate the effects of oestradiol, which modulates the amplitude and activation properties of individual Ca^{2+} and K^+ currents, to alter AP configuration to a tonic-like plateau [2-4]. In conclusion, a novel mathematical model of the electrical action potential of USMCs has been developed. This provides a powerful tool to investigate the ionic mechanisms underlying the physiological genesis of electrical excitation governing labouring contractions.

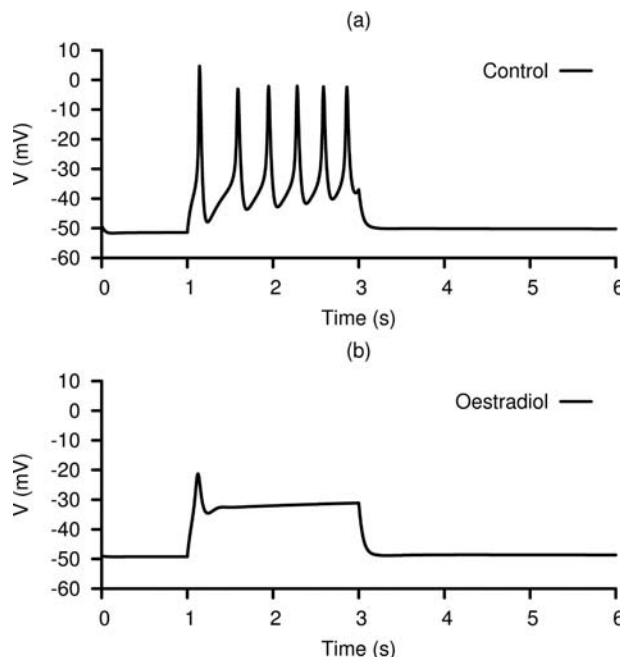


Figure 1. Simulated membrane action potential (V) of a USMC of pregnant rat in the absence (a) or presence (b) of oestradiol [2]. Current clamp applied at time = 1 – 3 s.

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The work was supported by Tommy's, the baby charity.

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PC146

Free radicals on dopaminergic neurons in medial preoptic area and sexual behavior of streptozotocin-induced diabetic male rat

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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.

PC147

Systemic leptin administration increases the electrical activity of supraoptic oxytocin neurones in urethane-anaesthetized female rats

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Leptin, an anorexigenic hormone synthesized in the adipose tissue, acts centrally to control food intake and body weight. Oxytocin acts as an anorectic peptide centrally and is involved in the regulation of electrolyte balance peripherally. Most supraoptic nucleus (SON) neurones are strongly immunoreactive for leptin receptor (1). Leptin interacts with acute satiety signals such as cholecystokinin (CCK) to regulate appetite (2). Here we investigated whether leptin can excite oxytocin neurones, and whether there are interactions between leptin and CCK, since systemic CCK excites oxytocin neurones. Leptin potentiates the anorectic and Fos expression responses to CCK (3,4). Hence, we hypothesized that leptin also potentiates the CCK-induced excitatory electrophysiological responses of SON oxytocin neurones. The electrical activity of single identified oxytocin neurones in the SON was recorded extracellularly through a glass 0.9% NaCl-filled microelectrode, via a ventral surgical approach in urethane-anaesthetised (1.25g/kg, i.p.) unfasted virgin female rats (n=19). SON oxytocin neurones (n=19) were identified antidromically, by stimulating the neurohypophyseal stalk, and by their excitatory response to CCK (20 µg/kg; i.v). The effect of systemic leptin (100µg; i.v) was predominantly excitatory. The firing rate (3.40 ± 0.37 spikes/s, group mean + s.e.m.) was increased by 0.31 ± 0.09 spikes/s two minutes after leptin injection (100µg; i.v). The rate returned to basal within 10min. The mean change in firing rate during the 10min after leptin injection was significantly greater ($P=0.013$, t-test) than in the 10 min before leptin injection. In the next experiment, an initial CCK response was recorded and then leptin (100µg; i.v) was injected followed by CCK after 20min. Hence, CCK responses were analysed with and without leptin treatment in the same neurones (n=8). The first injection of CCK increased the firing rate from 2.64 ± 0.59 spikes/s by 0.24 ± 0.07 spikes/s in the following 10 min, and after leptin a repeat injection of CCK raised the firing rate from 2.61 ± 0.58 spikes/s by 0.4 ± 0.1 spikes/s; a significant enhancement ($P<0.05$, one-tailed paired t-test). Hence, in contrast with a report of inhibitory leptin actions on SON neurones in vitro (5), in vivo leptin excited oxytocin neurones. This could result from leptin actions on inputs to oxytocin neurones. Excitation of oxytocin neurones by CCK was slightly enhanced by prior leptin injection. Oxytocin may be involved in central appetite regulation by CCK and leptin.

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BBSRC and CMVM PhD Studentship and ORS award from the University of Edinburgh.

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

PC148

Regulation of fat metabolism by central nervous system during physical exercise

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To acquire smooth performance in endurance exercise, the function of whole body organs, such as skeletal muscle, liver, adipose tissue, etc. should be fully coordinated. These integrated coordination seem to be achieved by the function of central nervous system. The hypothalamus is known to play an important role in whole body energy metabolism, however, its involvement to physical exercise was not fully understood. In this study, its contribution to fat metabolism during endurance exercise was investigated.

Experiments

Under the isoflurane anesthesia, cannulae for microinjection or a guide cannula for microdialysis was implanted into the ventromedial hypothalamus (VMH) of a rat. After at least 7 days of recovery period, rats were subjected to treadmill running at exercise intensity corresponding to 55% of maximum oxygen consumption (15 m/min, 10° incline). During treadmill running, the changes in respiratory exchange ratio (RER) and oxygen consumption (respiratory gas analysis) and the extracellular concentration of noradrenaline in VMH (microdialysis analysis) was determined. 10 ~ 15 min before onset of running, lidocaine (10 µg in 0.5 µl), phentolamine (4.2 µg in 1.0 µl), timolol (4.2 µg in 1.0 µl) and propranolol (8.0 µg in 1.0 µl) was injected bilaterally into VMH. 6-hydroxydopamine treatment and microdialysis analysis were done according to Fujikawa et al (2007). Respiratory gas analysis was executed according to Ishikawa et al. (2006).

Results and discussion

Bilateral microinjection of lidocaine into VMH caused suppression of the decrease in the RER during treadmill running when compared to those administered artificial cerebrospinal fluid (aCSF). This means that the attenuation of VMH function inhibits enhancement of fatty acid oxidation during exercise. The increase in the extracellular concentration of noradrenaline in VMH was observed during running by microdialysis analysis. This demonstrates the activation of noradrenergic neurons projecting to VMH during exercise. The destruction of noradrenergic neurons in VMH by prior administration of 6-OHDA showed similar suppression of fatty acid oxidation as the lidocaine treatment during running. This suggests the involvement

of noradrenergic neurons which are projecting to VMH and activated during running play an important role in enhancement of fatty acid oxidation during exercise. Furthermore, among microinjection of adrenoceptor antagonists into VMH, only beta-blockers (timolol and propranolol) caused suppression of fatty acid oxidation during running. These treatments had no effect on RER and oxygen consumption in sedentary state. These results indicate the importance of noradrenergic neurons projecting to and the pivotal role of beta-adrenoceptor in VMH on the regulation of energy metabolism during endurance exercise.

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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.

PC149

Glucocorticoid sensitivity in patients with metabolic syndrome

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The metabolic syndrome, a worldwide health problem, represents a cluster of metabolic abnormalities that are well established risk factors of cardiovascular disease. There is increasing evidence that physiological variations in glucocorticoid (GC) action could contribute to the development of these risk factors. Disturbances of the hypothalamic-pituitary-adrenal (HPA) axis might underlie the metabolic syndrome and its association with obesity and insulin resistance. In the present study we evaluated the sensibility to GC, in a series of patients with metabolic syndrome, compared to healthy individuals. Forty patients with metabolic syndrome (24 female, 16 male, 24-71 years old) were included in this study. The negative feedback action of GCs on the HPA axis was evaluated in vivo through salivary (SC) and plasma (PC) cortisol levels determined pre and post 0.25, 0.5 and 1 mg of dexamethasone (DEX) given at 11:00 pm. Forty healthy individuals previously studied were also submitted to the DEX tests. Wilcoxon rank-sum test and Wilcoxon matched pairs signed rank sum test were used when appropriate. Data are shown as mean \pm SEM. In patients with metabolic syndrome the administration of increasing doses of DEX (0.25, 0.5 and 1.0 mg) resulted in a dose-dependent increase in mean circulating DEX levels (0.24 ± 0.03 ; 0.40 ± 0.04 and 0.90 ± 0.08 nmol/L) and a dose-dependent decrease of plasma cortisol (208.3 ± 23.0 ; 83.8 ± 18.4 and 44.9 ± 7.1 nmol/L) and salivary cortisol (19.4 ± 2.7 ; 5.0 ± 1.4 and 2.1 ± 0.3 nmol/L). No significant difference of plasma and salivary cortisol levels (basal and post DEX) was observed between healthy individuals and patients with metabolic syndrome. Using plasma and salivary cortisol levels of <50 nmol/L and <2.6 nmol/L, respectively, as criteria of HPA axis suppression, patients with metabolic syndrome showed PC (79.5%) and SC (82.0%) suppression post 1.0 mg; 62.0% (PC) and 65.0% (SC) post 0.5 mg and

5.0% (PC) and 5.0% (SC) post 0.25 mg of DEX. Patients with metabolic syndrome showed a tendency of lower cortisol DEX suppression compared to controls. In conclusion, the present data suggest that decreased sensitivity to GC feedback is more prevalent in metabolic syndrome patients, confirming that the HPA axis is activated in this condition. These results give support to the emerging concept that disruption of feedback regulation of the HPA axis might be involved in the pathogenesis of metabolic syndrome.

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.

PC150

Central nitric oxide reduce vasopressin, oxytocin release and arterial blood pressure induced by central angiotensin-II stimulation

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Nitric oxide (NO) is produced and released in the hypothalamus-neurohypophyseal system (HNS) and modulates a wide variety of physiological functions. Angiotensin-II (ANG-II) acts in the HNS to control vasopressin (AVP), oxytocin (OT) release and mean arterial pressure (MAP). We evaluated the central effects of the nitrergic system on the hormonal release and MAP induced by central ANG-II stimulation. Male Wistar rats (± 280 g) anaesthetized with 2.5% tribromoethanol (1ml/100g body weight, i.p.) had a stainless guide cannula placed into the right lateral ventricle. Six days after surgery, they received an intracerebroventricular (icv) injection of Nw-Nitro-L-arginine methyl ester (L-NAME, 40 μ g), an inhibitor of NO synthase, L-arginine (20 μ g), a precursor of NO, or vehicle (0.15M NaCl) and 10 min later they received an icv injection of ANG-II (26ng) or vehicle. Blood samples were collected 5 min after injection of ANG-II for determination of plasma neurohypophyseal hormones. MAP was determined during 30 min after ANG-II in another set of rats. The results are reported as means \pm SEM. The data were analysed by two way ANOVA followed by Newman-Keuls post hoc test. Differences were considered significant at $P < 0.05$. Rats treated with L-NAME followed by vehicle ($n=6$) and vehicle followed by ANG-II ($n=7$) showed, respectively, an increase of plasma AVP (3.1 ± 0.2 pg $P < 0.01$ and 4.6 ± 0.4 pg $P < 0.001$), OT (12.3 ± 1.5 pg $P < 0.05$ and 31.5 ± 5.8 pg $P < 0.001$) levels and MAP (14.2 ± 0.7 mmHg $P < 0.001$ and 14.6 ± 0.8 mmHg $P < 0.001$), compared to the control group (vehicle followed by vehicle $n=9$, AVP: 1.9 ± 0.2 pg, OT: 4.6 ± 0.6 pg and MAP: 5.9 ± 0.4 mmHg). L-arginine followed by vehicle ($n=7$) did not modify the hormonal concentrations and MAP. Pre-treatment with L-NAME enhanced the AVP (5.7 ± 0.4 pg $P < 0.01$), OT (43.4 ± 5.7 pg $P < 0.05$) release and MAP (18.2 ± 0.8 mmHg $P < 0.001$) induced by ANG-II ($n=6$). On the other hand, L-arginine reduced the increase of AVP

(3.1 ± 0.3 pg $P < 0.001$), OT (17.5 ± 5.2 pg $P < 0.05$) secretion and MAP (5.9 ± 0.6 mmHg $P < 0.001$) induced by ANG-II ($n=7$). In conclusion, the present data demonstrate that the inhibition of central nitric system facilitates AVP and OT release and the increase of MAP in response to ANG-II, suggesting an inhibitory modulation of NO in the control of neurohypophyseal hormone secretion and blood pressure regulation.

Financial Support: FAPESP, CNPq and Capes.

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

PC151

Ghrelin and GHRP-6 treatment prevent caspase-8-mediated apoptosis in the pituitary of diabetic rats

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Poorly controlled type I diabetes mellitus is associated with hormonal imbalances and increased cell death in different tissues and organs such as retina, kidney, cardiovascular tissue, neurons, oligodendrocytes, epithelial tissue, and pituitary. Ghrelin and GH secretagogues have been reported to prevent apoptosis in different tissues and to have neuroprotective actions. The aim of this study was to analyze the effect of ghrelin and growth hormone-releasing peptide-6 (GHRP-6) in diabetes-induced apoptosis in the pituitary of diabetic rats. Diabetes was induced in male Wistar rats by a single injection of streptozotocin (70 mg/kg, ip). Six weeks after diabetes onset mini-pumps were implanted in the jugular vein of the animals (under iso-fluorane anaesthesia (2.5% inhalation in O₂ at 0.5 ml/min)). Diabetic and control rats were infused with saline (12 µl/day), ghrelin (24 nmol/day) or GHRP-6 (150 µg/day) for two weeks. Eight weeks after diabetes induction all animals were sacrificed by decapitation. Total cell death was increased in the pituitary of diabetic rats, and as previously reported, the majority of cells undergoing apoptosis, as detected by TUNEL, were lactotrophs, and both ghrelin and GHRP-6 decreased this effect. Cleaved caspase-8 and X-chromosome linked inhibitor of apoptosis protein (XIAP) were elevated in diabetic rats infused with saline ($P < 0.05$) whereas caspase-3 was unchanged. Ghrelin and GHRP-6 treatment decreased both caspase-8 and XIAP levels in the pituitary of diabetic rats ($P < 0.05$). According to these results we can conclude that delayed treatment with either ghrelin or GHRP-6 prevents further caspase-8 mediated apoptosis (extrinsic cell-death pathway) in the pituitary of diabetic rats.

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

PC152

Kisspeptins as inotropic agents in human and mouse heart

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Kisspeptin, a 54-amino acid peptide (KP-54), has recently been found to act as a regulator of the gonadotrophic axis (Seminara *et al.*, 2003) in addition to having roles as a metastasis suppressor and in trophoblast invasion. KP-54 and the C terminal fragments KP-13 and KP-10 bind to the recently de-orphanised KISS1 receptor, previously known as GPR54. Kisspeptin inhibits matrix metalloproteases-2 and -9, both of which are produced by cardiomyocytes. Additionally, we have reported vasoconstrictor activity of the kisspeptins in human umbilical vein and atherosclerosis-prone vessels (Mead *et al.*, 2007) and have detected kisspeptin and KISS1 immunoreactivity in endocardial endothelial cells and cardiomyocytes of the human heart (Mead, unpublished data). We hypothesise that kisspeptin is an inotropic agent in both human and mouse heart, acting through the KISS1 receptor.

<p>4mm human atrial appendage strips and atria from male 129S6/SvEv mice (20-30g; euthanised by CO₂ inhalation) were set up in organ baths and paced using field stimulation (<4V). Tension on the tissue was adjusted to 50% of optimum resting tension before construction of cumulative concentration response curves to KP-54, -13 and -10 and termination by CaCl₂ addition (6.7 mM). Agonist responses were expressed as %CaCl₂ and data are mean \pm s.e.mean. <p>In human paced atrial appendage, KP-10, KP-13 and KP-54 induced positive inotropic effects with comparable potency (pD_2 : 10.60 ± 0.73 , 10.38 ± 0.57 , 10.10 ± 0.44) and maximal response (E_{max} : 75.7 ± 8.0 , 85.2 ± 14.0 , 89.8 ± 13.9) respectively ($n=5$; $P > 0.05$, ANOVA). In mouse atria, KP-54 also induced positive inotropic effects (pD_2 : 10.19 ± 0.51 , E_{max} : 27.3 ± 5.8 ; $n=4$). <p>In conclusion, kisspeptins appear to act as inotropic agents in both the human and mouse heart. All three peptides had comparable potency, indicating that the activity is retained in the final 10 amino acids.

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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.

PC153

Peroxisome proliferator-activated receptor gamma stimulation by rosiglitazone and hypercatabolic state in sepsis

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Peroxisome proliferator-activated receptor gamma (PPARgamma) is a ligand-activated transcription factor belonging to the nuclear hormone receptor superfamily. PPARgamma

regulates several metabolic pathways by binding to sequence-specific PPAR response elements in the promoter region of target genes, including lipid biosynthesis and glucose metabolism. It has also been reported that has an anti-inflammatory effect. Sepsis is an acute inflammation process associated with an hypercatabolic state that include a decrease in serum concentrations of insulin-like growth factor I (IGF-I), liver IGF-I gene expression, and alterations in adipose tissue metabolism. The aim of this study was to analyse the effect of the PPARgamma agonist rosiglitazone in the hypercatabolic state in sepsis induced by lipopolysaccharide (LPS). Adult male Wistar rats were simultaneously intraperitoneally injected with LPS (1mg/kg) and/or rosiglitazone (3mg/kg) 19 and 4 hours before decapitation. Food was removed to avoid possible effects of food intake. Serum IGF-I was measured using radioimmunoassay (RIA). Gene expression of leptin and hormone-sensitive lipase (HSL) in white adipose tissue, TNF-alpha, IGF-I and PPARgamma in liver and white adipose tissue were quantified using RT-PCR. In LPS-injected animals, hepatic expression of PPARgamma decreased ($P<0.01$), as well as circulating IGF-I and its hepatic gene expression ($P<0.05$). In white adipose tissue leptin and HSL were increased ($P<0.05$) after LPS injections. Rosiglitazone administration decreased white adipose tissue leptin and TNF-alpha mRNA in vehicle rats ($P<0.05$), but it did not modify any of the parameters studied in LPS-injected rats either in the liver or white adipose tissue. These data suggest that the administration of the PPAR gamma agonist rosiglitazone don't seem to modify the catabolic response studied in sepsis.

This work has been supported by a grant from Fundación de Investigación Médica Mutua Madrileña, by a fellowship from Gobierno Vasco to E Castillero (BFI06.31), and from Ministerio de Educación y Ciencia to M López-Menduiña (BES-2007-16001).

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

PC154

Central administrations of melatonin and ghrelin have different effects on catecholamine concentrations in rat hippocampus

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Dopamine and noradrenaline (NA) are involved in generation of long term potentiation (LTP) which play critical role in learning and memory processes. It is known that the pineal hormone melatonin inhibits LTP in hippocampal neurones involving different mechanisms. Gastric hormone ghrelin is also suggested to involve in enhancement of hippocampal memory processes, but the involved mechanism of this effect is not clear yet. The electrophysiological studies involving LTP are in vitro nature and there is no data on the possible effects of melatonin and ghrelin on NA release and thereby in this process. The purpose

of this study was to assess the effects of exogenous melatonin and ghrelin on catecholamine levels in the rat hippocampus. Male Wistar rats (280-300 g) were anaesthetized by administering intraperitoneally choral hydrate (500 mg/kg) before being placed on a stereotaxic frame. Melatonin (n=7) and ghrelin (n=7) were intracerebroventricularly (icv) administered at dose of 1 µg/5µL separately. The animals in the control group received artificial cerebrospinal fluid (icv, n=7) and vehicle of melatonin was administered to the vehicle group (icv, n=7). Following 20 minutes of injections, all animals were sacrificed by decapitation and brains were promptly removed. Left hippocampus tissues were obtained by guidance of rat stereotaxic atlas. The whole hippocampus tissues were homogenised for catecholamine analysis. NA, dihydroxyphenylglycol (noradrenaline metabolite), dopamine and dihydroxyphenylacetic acid (dopamine metabolite) content of samples were analysed in high performance liquid chromatography with electrochemical detector. Mann Whitney-U test was used for statistical evaluation. Melatonin group had significantly lower NA level (33.61 ± 0.72 pg/wet tissue) than the vehicle group (6.97 ± 0.77 pg/wet tissue, $p<0.01$). Whereas NA level in ghrelin group (10.92 ± 1.05 pg/wet tissue) was significantly higher in ghrelin group compared to control group (6.75 ± 1.48 pg/wet tissue, $p<0.05$). Dihydroxyphenylacetic level in melatonin group was significantly lower compared to vehicle group ($P<0.05$). There were no significant differences between the levels of both dopamine and dihydroxyphenylglycol in any groups. The results of this study demonstrate that melatonin has decreasing effect on NA concentration, whereas ghrelin has elevating effect on NA level in the hippocampus. These results may ensure additional evidence for the tested catecholamine on memory, evidenced from electrophysiological studies of these hormones on hippocampal neurons.

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

PC155

Circadian rhythms in the supraoptic nucleus

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Measures of single unit activity that quantify the irregularity of spike activity (log interval entropy; ENT) and pattern (mutual information between adjacent log intervals; MUT) as well as mean spike frequency (MSF) were applied to recordings from the supraoptic nucleus (SON) at all stages of the day/night cycle in urethane-anaesthetised rats. C-association methods were developed to assess the significance of the rhythms of activity revealed. Continuous cells in intact animals showed significant rhythms for MSF ($P=0.001$) and ENT ($P=0.001$) but not MUT. After osmotic stimulation a significant rhythm was seen in all three parameters as it was after pinealectomy (carried out under halothane anaesthesia > 4 weeks before recording) and

after osmotic stimulation in pinealectomised animals. Phasic cells also showed statistically significant daily rhythms in MSF ($P=0.001$), ENT ($P=0.016$) and (MUT $P=0.001$). After osmotic stimulation significant rhythms ($P=0.001$) remained for all three parameters. Rhythms remained after pinealectomy for MSF and ENT (but not MUT) although all three parameters showed significant rhythms after osmotic stimulation.

Cosinor analysis showed that, for ENT, the amplitude of the rhythm was significantly reduced in continuous (but not phasic) cells after osmotic stimulation ($P=0.002$, $t=3.1021$). A reduction was also seen after pinealectomy ($P=0.001$, $t=3.7004$). There was no difference between the mesor (the value midway between the highest and the lowest values of the cosine function best fitting the data.) values for these groups.

Similar cosinor analysis showed that while there were no differences in the amplitude of the rhythms in phasic cells there were significant differences in the mesor values between intact and osmotically stimulated animals ($P=0.001$, $t=3.763$) and after pinealectomy ($p=0.001$, $t=5.3005$). No such differences were seen in continuous cells.

Cosinor analysis of the ENT values also revealed that the amplitude of the rhythms was significantly greater ($P=0.002$, $t=3.837$) in continuous than in phasic cells. A significant difference was also seen between the timing of the peak in continuous cells following pinealectomy (from ZT 2249 to ZT 02.30, $P=0.034$, $t=2.1274$).

The Mann-Witney test showed that after pinealectomy the MSF of both phasic and continuous cells was significantly increased ($P=0.001$).

C-association methods also revealed daily rhythms of MSF and ENT ($P=0.001$) in the suprachiasmatic nucleus (SCN) in vivo. Cosinor analysis of the entropy values in intact and pinealectomised animals showed that the amplitude of the rhythm was significantly greater ($t=1.9901$; $P=0.047$) in intact (mesor 0.3525 bits) than in pinealectomised animals (mesor 0.1786 bits).

We have shown for the first time that there is a daily rhythm of electrical activity in the SON in vivo and that pinealectomy profoundly affected both SON and SCN. Particular attention should thus be given to the time of day when interpreting recording studies in the hypothalamus.

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

PC156

Effects of *Zingiber officinale* on reproductive functions in male rats

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AIM: To investigate the effects of *Zingiber Officinale* on male reproductive functions and study the mechanisms underlying these effects.

METHODS: Aqueous extract of *Zingiber Officinale* were administered orally to two groups of male rats at 500mg/kg b.w. (Group 2) and 1000mg/kg b.w. (Group 3). Group 1 served as control and received distilled water which is the treatment vehicle. Treatment lasted for 14 and 28 days before sacrifice. Organ weight, epididymal sperm counts, motility, viability and morphology, seminal fructose, testicular malonhydialdehyde, and serum testosterone were determined.

RESULTS: The treatment caused a significant increase ($P<0.05$) in the weight of the testis and epididymis. There were dose and duration dependent increases in sperm count and motility ($P<0.05$). There was also a significant increase ($P<0.05$) in serum testosterone level. Malonhydialdehyde levels were significantly reduced ($P<0.05$).

CONCLUSION: Our results indicated that extract of *Zingiber Officinale* possesses pro-fertility properties in male rats which might be a product of both its potent antioxidant properties and androgenic activities.

Effects of *Z. Officinale* on sperm count, motility, viability and abnormal morphology after 14 days and 28 days treatment.

Groups	Count (106/ml)	Motility (%)	Viability (%)	Abnormal Morphology
14 Days				
Control	8.10±0.14	85.40±1.07	96.00±1.52	2.18±0.38
500mg/kg	8.93±0.72	89.50±2.14*	95.20±1.77	2.23±0.24
1000mg/kg	9.08±0.17*	91.70±1.68*	95.70±1.45	2.17±0.47
28 Days				
Control	8.17±0.25	84.60±1.31	93.80±1.72	2.63±0.41
500mg/kg	9.26±0.28*	89.40±2.23*	95.30±2.11	2.59±0.36
1000mg/kg	9.53±0.42*	92.50±1.84*	95.50±1.87	2.67±0.33

* $P<0.05$, values expressed as mean±sem, n= 6, abnormal morphology in %.

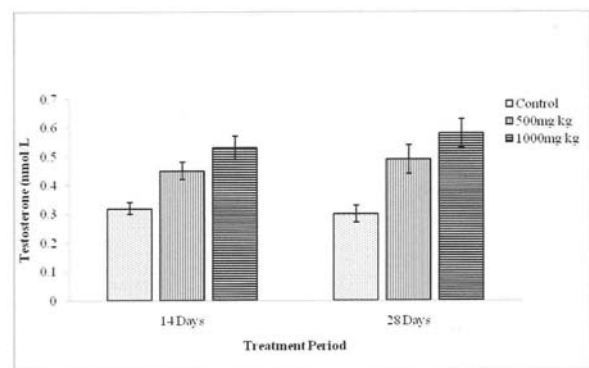


Figure 2: Effects of *Z. Officinale* on serum testosterone levels in rats after 14 days and 28 days treatment.

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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.

PC157

Experimental arthritis-induced skeletal muscle wasting is associated with increased proteolysis but not with decreased myogenesis

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Experimental arthritis is an animal model of chronic inflammation that induces cachexia and skeletal muscle atrophy. It has been previously reported that during acute and chronic phases of the disease there is an increase of muscular protein degradation by the ubiquitin-proteasome pathway. Potential changes in myogenesis during arthritis-induced cachexia have not been studied. The aim of this work was to analyze the effect of adjuvant-induced arthritis on markers of muscle regeneration. For this purpose, arthritis was induced in male Wistar rats by an intradermal injection of Freund's adjuvant in the sole of the paw. The clinical symptoms of arthritis began 10 days after the injection, and the highest arthritis index was reached on day 22. Arthritic and control rats were humanely killed on days 10, 15 and 22 after the adjuvant injection. In the arthritic rats gastrocnemius relative weight was not modified on day 10, it decreased on day 15 ($P < 0.01$), and it was markedly lower on day 22 ($P < 0.01$). Arthritis induced a marked increase in serum interleukin-6 concentrations and in gastrocnemius ubiquitin ligase muscle-RING-finger protein 1 (MuRF-1) gene expression on all days studied, specially on day 15 ($P < 0.01$). Neither of muscular differentiation markers myogenin or myogenic differentiator 1 (MyoD) were decreased in arthritic rats. On the contrary, arthritis increased myogenin expression on days 15 and 22 ($P < 0.01$), and MyoD expression on all days studied ($P < 0.05$). Muscular proliferating cell nuclear antigen (PCNA) expression was also increased on days 15 and 22 in arthritic rats. These data suggest that arthritis-induced muscle wasting is mediated by increased proteolysis, but not by decreased myogenesis.

This work was supported by CYCYT (Ref: BEF2003-02149), a grant to E. Castillero (Gobierno Vasco, BFI06.31) and a grant to M. Lopez-Menduiña (BFU, 2006-11899)

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

PC158

Involvement of ENaC in the initiation of signaling events leading to decidualization/implantation in Mice

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The endometrium undergoes an indispensable differentiation process – decidualization in embryo-implantation. While the

maternal-embryo cross-talk in initiating decidualization remains largely unknown, it has been cleared that the endometrial epithelium is required. Our previous studies have demonstrated that the endometrial epithelial expression of ENaC is enhanced during implantation and that intrauterine injection of ENaC blocker, amiloride, inhibits the implantation rate in mice, indicating the potential role of the epithelial ENaC in implantation. Interestingly, serine proteases, the reported ENaC activity-modulating factors, are known to be required for implantation and also, have been shown to promote the release of PGE2 from many epithelial cells, which has been demonstrated to play crucial roles in decidualization. Thus, we hypothesized that ENaC may be involved in the initiation of PGE2 release from endometrial epithelial cells leading to decidualization. In the present study, endometrial epithelial cells of mice were primarily cultured and grown on semipermeable membranes for forming polarized monolayers. Trypsin, a serine protease, aprotinin, the protease inhibitor and amiloride, the ENaC blocker, were added to the culture medium, and the release of PGE2 to basolateral compartment was detected using an EIA kit. The results showed that, incubating with trypsin (20 µg/mL) for 10 min could significantly enhance the PGE2 level in the treated cells as compared to the untreated control. While pretreated the cells with amiloride (10 µM) or aprotinin (20 µg/ml) for 30 min reversed the effect of trypsin. These results have demonstrated the involvement of ENaC in the release of PGE2 from endometrial epithelial cells, indicating its potential role in decidualization. Thus, ENaC appears to be essential to implantation.

The work was supported by the National 973 projects (2006CB504002 and 2006CB944002), the Strategic Investment and Li Ka Shing Institute of Health Sciences of The Chinese University of Hong Kong, and the Morningside Foundation

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

PC159

Functional characterisation of novel aquaporins from the European Eel *Anguilla Anguilla*

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The European Eel is a euryhaline teleost, which is able to adapt to survive in a fresh or seawater environment. The aquaporin water channels play important roles in osmoregulation in both mammals and fish. Recently it was shown that seawater adaptation leads to changes in the expression levels of the aquaporins eAQP1 and eAQP3 (Lignot *et al.*, 2002, Martinez *et al.*, 2005). Screening of eel cDNA libraries highlighted two putative clones with high homology to mammalian AQP8, which we have labelled eAQP8 and eAQP8b. In the current study we have investigated the permeability properties of the eel aquaporins, eAQP1, 3, 8 and 8b. The cDNA encoding the eel proteins eAQP1, eAQP3, eAQP8 and eAQP8b was subcloned into the *Xenopus* expression vector