for 10 to 12 weeks post weaning, to give four dietary study groups (C/C, C/HF, HF/C and HF/HF) comprising 10 animals per group. Blood pressure (tail cuff plethysmography, SBP average 6 measures) and body weight were recorded at weekly intervals. Femoral artery vasorelaxation to ACh (1nM-10μM) was assessed using wire myography and basal NO production using 4,5-diaminofluoresceine diacetate (DAF-2 DA), an NO-sensitive fluorescent dye. Vascular segments were incubated for 45 min at 37°C with 5 μM DAF-2 DA in HEPES buffer (pH 7.4) and digital images collected using confocal microscopy (excitation at 490 nm; emission 535 nm). The images were analyzed using image software by measuring the mean OD of the fluorescence observed in the endothelium.

Offspring fed the HF diet post weaning (HF/HF and C/HF groups) gained more weight than the C/C and HF/C animals. Blood pressure was also significantly higher in the HF/HF and C/HF. Arteries from HF/HF and C/HF animals showed an impaired endothelium-dependent relaxation to ACh compared with C/C and HF/C animals ($P<0.05$). Basal NO production was greater in CC arteries compared with HF/HF, with staining most evident in the inter-junctional regions of the endothelium and in the underlying intima.

Our preliminary data demonstrate the impact of an in utero and postnatal HF diet on vascular function and suggest that the endothelial dysfunction observed is a result of impairment of NO production and/or bioavailability. It further suggests that both predisposition to disease, acquired in early life, and later lifestyle may contribute to the development of cardiovascular disease which are sustained into adulthood.

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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.

C72

Endothelial dysfunction induced by maternal protein restriction is present at 5 weeks of age in male rat offspring

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Epidemiological studies demonstrate that low birth weight is associated with an increased risk of cardiovascular disease in adult life (Osmond *et al*., 1993). In the rat, the restriction of dietary protein during gestation leads to raised systolic blood pressure and endothelial dysfunction in the offspring (Langley & Jackson, 1994; Brawley *et al*., 2003). Yet whilst blood pressure has been shown to be elevated by 4 weeks of age, endothelial function has not been assessed before 80 days (Brawley *et al*., 2003). The aim of the present study was to determine if endothelial dysfunction was present with the onset of raised blood pressure.

Pregnant Wistar rats were fed a control (C; 18% casein) or protein restricted (PR; 9% casein) diet throughout pregnancy and returned to standard chow postpartum. Pups were weaned from their mothers at 21 days. At approximately 36 days blood pressure in male offspring was recorded using tail cuff plethysmography, before animals were sacrificed and thoracic aorta dissected and mounted in a wire myograph. Aorta segments were bathed in PSS heated to 37° C and continually gassed with 95% O_2 and 5% CO_2. Concentration response curves were conducted to phenylephrine (PE), the thromboxane mimetic U46619, acetylcholine (ACh), bradykinin (BK) and sodium nitroprusside (SNP). Responses to ACh were repeated in the presence of L-NAME (100 μM). Data is given as mean ± S.E.M. and differences were assessed by one-way ANOVA with Bonferroni *post hoc* correction. Significance was accepted at $p<0.05$.

Blood pressure at 5 weeks was similar between the groups (mmHg: C, 86.61 ± 4.15, n=9; PR, 88.50 ± 6.18, n=8; *p*=ns). Contractile responses to PE were similar between the groups, yet vasoconstriction to U46619 was significantly enhanced in the PR group compared to controls (pEC_{50}: C, 7.67 ± 0.05, n=7; PR, 8.01 ± 0.05, n=6, $p<0.01$). Endothelial-dependent vasodilatation to both ACh (pEC_{50}: C, 7.82 ± 0.10, n=7; PR, 7.52 ± 0.07, n=6; $p<0.05$) and BK (% max response: C, 29.2 ± 4.5, n=6; PR, 9.0 ± 3.4, n=5; $p<0.01$) was significantly impaired in the PR group compared to controls. Incubation with L-NAME completely abolished the ACh response in all groups and responses to the NO-donor SNP were similar in both groups (*p*=ns).

The data demonstrates that protein restriction during gestation leads to vascular dysfunction in isolated thoracic aorta segments, which is present from 5 weeks of age and is independent of any increase in blood pressure.

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Langley & Jackson (1994) Clinical Science **86**: 217-222.

Osmond *et al*. (1993) BMJ. **307**: 1519-1524

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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.

C73

Characterisation of epidermal primary afferents in the mouse

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The epidermis is richly innervated with sensory afferents most of which are of the "non-peptidergic" group (Zylka *et al.,* 2005; Belle *et al.,* 2007) and in the present study some of the characteristics of these afferents have been determined.

Studies were conducted on C57/Bl6 mice (SA36 strain) carrying the *thy1.2-egfp* gene expressed in non-peptide epidermal afferents (Belle *et al.,* 2007). For anatomy, animals were terminally anaesthetised with sodium pentobarbitone i.p. (80mg/kg) prior to whole body perfusion fixation with 4% paraformaldehyde and for electrophysiology they were anaesthetised with halothane and killed by decapitation. Dorsal root ganglion

(DRG) neurones were either studied shortly following acute dissociation (AD) within 2-9 hours of plating or after maintaining in short-term culture (SC) between 1-3 days post-plating. The responses of eGFP positive neurones recorded in whole-cell current-clamp mode to depolarising and hyperpolarising current pulses were investigated. All results given are mean ± S.E.M and statistical analysis between cell types was done by one-way ANOVA with Tukey post test unless otherwise stated.

Immunoreactivity for $P2X_3$ (mean surface area (m.s.a.) 444.3 ±4.43μm^2, *n*=800) was observed in 85.1% of eGFP-positive neurones (m.s.a. 509.9 ± 7.32μm^2, *n*=737), whilst $TRPV_1$ immunoreactivity (m.s.a. 358.1 ± 15.7μm^2, *n*=179) marked a separate, smaller population of neurones only 5.02% of which expressed eGFP (1.62% of eGFP population). The mean size of the AD eGFP-positive neurones (*n*=32) (mean soma diameter 26.1 ± 0.76μm) was larger than those in SC (*n*=52) (mean soma diameter 21.31 ± 0.42μm, $p < 0.001$). This could be due to shrinkage following loss of axonal arborisation or be due to selective loss of small neurones that may not have settled in the acutely dissociated preparations. Their firing patterns to depolarising pulses could also be separated into five distinct groups burst (AD 12.5%, SC 3.8%), delayed (AD 3.1%, SC 0%), phasic (AD 9.3%, SC 1.9%), transient (AD 68.7%, SC 50%) and tonic firing (AD 6.2%, SC 44.2%). Action potential overshoot magnitudes were similar under the two conditions (AD 28.14 ± 2.12mV, SC 28.96 ± 1.39mV, $p > 0.05$) but action potentials were broader in SC neurones (AD 6.59 ± 1.1ms, SC 9.88 ± 0.85ms, $p < 0.05$) and more frequently had inflections on their repolarising slope. Approximately half of the AD neurones (53.13%) displayed delayed rectification and a rebound spike when hyperpolarised suggesting the HCN mediated current, I_h, is present.

These results are consistent with the literature, showing that non-peptidergic primary afferents innervating the epidermis in the mouse are sensitive to ATP but not to capsaicin. Whilst some display characteristics of nociceptors (broad, inflected action potentials) others share properties that have been reported in cold sensitive neurones (I_h).

Belle M.D. *et al.* (2007). *Genesis* **45**, 679-688

Zylka M.J. *et al.* (2005). *Neuron* **45**, 17-25

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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.

C74

Quantitative characterization of low-threshold mechanoreceptor inputs to wide dynamic range lamina I spinoparabrachial neurons

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The cell types that comprise ascending projections from the superficial dorsal horn of the spinal cord are typically modality specific *e.g.* nociceptive-specific, polymodal nociceptive, thermoreceptive (Craig *et al*. 2001). However, in monkey and rat a small proportion of projection neurons receive convergent inputs from both low-threshold mechanoreceptors and nociceptors (Bester *et al*. 2000, Ferrington *et al*. 1987). These 'wide dynamic range' neurons comprise the majority of neurons in pathways from spinal laminae IV and V, but they are rare (*ca.* 10%) in ascending projections from lamina I. It has been assumed that rapidly-conducting A-fibre (myelinated) afferents provide the low-threshold inputs to wide dynamic range neurons, as these cells typically receive inputs from primary afferents with Aβ conduction velocities. However, myelinated low-threshold mechanoreceptors do not terminate in lamina I (Brown, 1981). One potential source of low-threshold mechanoreceptor input to lamina I projection neurons is C-fibre mechanoreceptors. These fibres terminate heavily in the superficial dorsal horn, and they are preferentially activated by slowly moving stimuli (Valbo et al. 1999), a feature that distinguishes them from myelinated mechanoreceptors. The aim of the present study was to quantitatively characterize the low-threshold inputs to wide dynamic range projection neurons in lamina I of the spinal cord to investigate their source.

Experiments were performed on male Sprague-Dawley rats that were anaesthetized with Urethane (1.2g/kg I.P.) and neuromuscularly blocked with Tubocurarine (150μg I.V.). During neuromuscular blockade, anaesthetic depth was considered sufficient if blood pressure and heart rate were stable during noxious stimulation. The activity of single, antidromically identified lamina I spinoparabrachial neurons with hindlimb receptive fields was recorded extracellularly. Wide dynamic range neurons were characterized with graded velocity brushing (6.6 – 126 cm/s) stimuli. Cells were also tested with graded thermal and mechanical stimulation as well as electrical stimulation of peripheral nerve fibres.

Nine of 95 lamina I spinoparabrachial neurons were responsive to low- and high-threshold stimuli. All of the neurons had receptive fields that included both glabrous and hairy skin, but low-threshold responses were only evoked from hairy skin. Most neurons showed decreasing responses to increasing brush velocity (*n*=7), one cell had a flat stimulus-response curve and another showed a U-shaped relationship between discharge and brush velocity. None of the cells studied received inputs from Aβ axons.

The present findings suggest that C-fibre mechanoreceptors provide the dominant low-threshold inputs to wide dynamic range neurons in the lamina I spinoparabrachial pathway. As C-fibre mechanoreceptors are thought to be important in affective touch, the lamina I spinoparabrachial pathway may be the projection that relays this information to the brain.

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Vallbo ÅB *et al*. (1999) J Neurophysiol **81**, 2753-2763.

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C75

Primary afferent neuropeptide mRNA regulation in experimental periodontitis in the rat

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Previously we have shown that chronic periodontal bone loss in rats is associated with an alteration in the expression of neuropeptide mRNAs in both ipsi- and contralateral trigeminal ganglia (TG) (1), indicating a possible neurogenic component to periodontitis. The aim of this study was to determine whether denervation of the periodontium prior to induction of periodontitis would affect the expression of neuropeptide mRNA in TG.

Periodontitis was induced in adult male rats (250-300g) by unilateral buccal intragingival injection of lipopolysaccharide (LPS, 10mg/ml; 1μl) between the first and second mandibular molars (1) under recoverable anaesthesia (Hypnorm 0.3 mg/kg (fentanyl citrate 0.1 mg/kg and fluanisone 3 mg/kg) i.m. injection + Diazepam 2.5 mg/kg i.p.). The effect of denervation ± periodontal inflammation was studied using inferior alveolar nerve (IAN) section either ipsilateral or contralateral to periodontitis. Animals with IAN section alone, and surgical exposure with no section served as control groups. Animals were killed by decapitation under halothane anaesthesia 7 days after periodontitis induction, and TG removed, rapidly frozen, sectioned and processed for in situ hybridisation for preprotachykinin (PPT) and calcitonin gene-related peptide (CGRP) mRNA expression. Levels of mRNA expression were determined by silver grain counting in small (<30μm diameter) neurons in both TG. Data were compared using one way ANOVA followed by Tukey-Kramer multiple comparisons test comparing the expression levels in the experimental groups to those of the controls.

LPS injection, and subsequent unilateral periodontitis resulted in significant up-regulation in small neurons in the ipsilateral ($p<0.05$) contralateral TG ($p<0.05$) as found previously (1). IAN section alone resulted in significant ($p<0.03$) down-regulation of both PPT and CGRP mRNA in the ipsilateral TG, with no changes on the contralateral side. Surprisingly, unilateral periodontitis with contralateral IAN section was associated with a significant ($p<0.03$) down-regulation ipsilateral, and up-regulation ($p<0.04$) of PPT and CGRP contralateral to the periodontitis. Periodontitis + ipsilateral IAN section resulted in significant ipsilateral down-regulation ($p<0.05$) of PPT and CGRP, with no contralateral changes. Sham IAN section did not result in any significant changes in mRNA expression.

While unilateral experimental periodontitis was associated with increased expression of PPT and CGRP in TG neurons ipsilateral and contralateral to the inflammation, denervation prior to induction of periodontitis resulted in an alteration in these changes. This suggests that mRNA changes are regulated in TG by the presence of inflammation. We speculate that neurogenic inflammation could be a contributing factor to periodontal disease.

Abd El-Aleem et al.(2004). Eur J Neurosci. 19(3: 650-8.

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C76

The effect of spinal nerve axotomy on I_h in dorsal root ganglion (DRG) neurons with Aα/β-fibres in rats *in vivo*

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It has been suggested that I_h drives neuropathic pain (Chaplan et al. 2003) partly because rat isolated large diameter L5 DRG neuronal somata *in vitro*, showed increased I_h density and depolarised $V_{0.5}$ (half-activation potential) after L5 spinal nerve ligation. In this study we examined whether such changes occur *in vivo* in Aα/β-neurons after L5 spinal nerve axotomy (SNA).

Young female Wistar rats were anaesthetised (pentobarbital 80mg/kg i.p.) and underwent neuromuscular blockade (pancuronium 0.5mg/kg i.v.). The muscle relaxant was giving hourly and always accompanied by additional dosage of anesthetic (10mg/kg), a level that in the absence of muscle relaxant keeps the animals deeply anaesthetised. The arterial blood pressure was monitored throughout the experiment. By using discontinuous single electrode voltage clamp (dSEVC) technique (3M KCl, 40~80MΩ, 30 deg), I_h was recorded in somata of L4-L6 DRG neurons in normal and in axotomised L5 DRG neurons 7 days after SNA. I_h at -100 mV was determined from the difference between instantaneous and steady state current. I_h density is I_h divided by the cell capacitance. Action potentials (APs) were evoked by stimulating the dorsal root with a bipolar electrode. Neurons were classed as having C-, Aδ- or Aα/β conduction velocities. Animals were killed by a pentobarbital overdose. Medians were compared by non-parametric Kruskall-Wallis or Mann-Whitney test.

I_h was recorded from 98 neurons in normal rats (n=27), and 68 in SNA rats (n=13). I_h was identified on the basis of its activation properties, time-dependant rectification and reversal potential (V_{rev}) which were consistent with previous findings (Tu et al. 2004; Yao et al. 2003).

Some properties of I_h did not change in axotomised Aα/β-fibre neurons. V_{rev} was similar (-30.3±1.0mV n=35 for normal, -31.1±2.0mV n=12 for axotomy). The slope of the I-V curve derived from tail currents was reduced (~0.09 nA/mV for normal, ~0.07 nA/mV for axotomy, $p<0.05$). However, after dividing the currents by cell capacitance this difference vanished (~0.82 ns/pF for normal, ~0.71 ns/pF for axotomy) suggesting a shrinkage of neurons after axotomy.

Changes in I_h occurred in axotomised L5 Aα/β-fiber neurons compared with in normal neurons. Both I_h amplitude and density were significantly reduced ($P<0.01$) after axotomy. A higher proportion of axotomised neurons expressed I_h (100% n=68 for axotomy, 89% n=98 for normal; $p<0.05$, Chi2 test). $V_{0.5}$ for axotomised Aα/β-fibre neurons was shifted negatively from -84.1±0.8mV (n=36) to -87.4±1.5mV (n=27) ($p<0.01$).

Our findings suggest that I_h is likely to be both harder to activate and smaller in axotomised Aα/β-fibre neurons. Therefore,

I_h is an unlikely primary cause of increased excitability in axotomised Aα/β-fibre neuronal somata.

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Tu H et al. (2004) J Neurosci Res 76: 713-722.

Yao H et al. (2003) J Neurosci 23: 2069-2074.

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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.

C77

Background light modulates activated rhodopsin lifetime in mouse rods

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Retinal rods adapt to steady background light by acceleration of response decay and a decrease in sensitivity. Recent experiments (1) have shown that in mouse, response decay quickens largely from modulation of turn-off of cyclic GMP phosphodiesterase (PDE). This process also decreases sensitivity, but experiments on salamander rods suggest that a Ca-dependent change in activated rhodopsin (R*) lifetime may also make an important contribution (2). Changes in R* lifetime are difficult to study directly, since it is normally so short that PDE turnoff is rate limiting for the decay of the light response. We therefore made suction-electrode recordings from isolated rods (as in ref. 1) of mice genetically engineered to make PDE turnoff much more rapid than normal, and R* turnoff slower, so that rod responses would decay only as R* activity was extinguished. We used R9AP95 mice in which the GTPase activating (GAP) proteins are over-expressed by about 6-fold. Since the GAP proteins are obligate activators of transducin alpha GTP hydrolysis, they regulate the rate of PDE turnoff, and overexpression of these proteins greatly speeds the kinetics of PDE deactivation (3). We then mated the R9AP95 mice with animals in which rhodopsin kinase (RK) activity had been reduced either to about 40% (in RK+/-) or about 15% (in RKux), in order to slow the rate of rhodopsin phosphorylation and turnoff of R*. We quantified the rate of turnoff by fitting the waveform of response recovery to a single exponential with time constant τ_REC. Previous experiments showed that τ_REC in animals that are R9AP95 alone is less than 80 ms and much more rapid than in WT animals (3), indicating that R9AP95 alone greatly accelerates PDE turnoff, which is normally rate-limiting. The value of τ_REC, however, was progressively slowed to 112 ± 16 (SE, n = 14) in R9AP95;RK+/- and 415 ± 70 (n = 7) in R9AP95;RKux. This shows that decreasing rhodopsin kinase activity with RK+/- and RKux slows the rate of rhodopsin phosphorylation sufficiently, so that R* lifetime becomes rate-limiting for response decay. When these rods were then exposed to background light, flash response recovery was accelerated. This could only have occurred if the R* lifetime was shortened by the background. This is the first direct physiological demonstration that R* lifetime is modulated during light adaptation. Our results also indicate that response recovery can be accelerated even in the absence of a background simply by increasing the flash intensity. Since increasing intensities produce progressively larger and longer reductions in circulating current and decreases in outer segment Ca, our results are consistent with a mechanism in which background light lowers Ca, which in turn decreases R* lifetime probably by modulating the rate of rhodopsin phosphorylation.

Woodruff ML et al. (2008). J Neurosci 28, 2064 2074.

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C78

Odor learning under anaesthesia: behavioural, neurochemical and electrophysiological effects

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Carbon disulphide (CS_2) in the exhaled breath of rodents increases the attractiveness of food odours. Thus information about foods which are safe to eat is transmitted between individuals. When one individual smells a food odour on the breath of another, it eats more of that food than it would otherwise. Here we investigate this system of odour learning in anaesthetised mice.

Food odours were prepared by mixing the ingredient with normal feed, and introducing the altered food in non-airtight capsules into gas-sampling bags containing N_2. Bags were also prepared with CS_2 (10μM), or the unaltered feed, both in N_2. Mice were anaesthetised with isofluorane in 21% N_2 and 79% O_2. Stimuli were delivered by switching from this supply to a matched supply carrying a food odour and/or CS_2. In experiment 1, Mice were trained by exposure to a novel food odour combined with CS_2 for 60s, then allowed to recover from anaesthesia. Controls were exposed to a novel food odour alone, or to CS_2 alone. When tested 24h after odour exposure, food preference was biased towards an odour that had been presented with CS_2 during anaesthesia.

In experiment 2, mice were anaesthetised and a microelectrode array (6x4 tungsten electrodes, tip separation 350μ) was positioned in the OB. Action potentials (spikes) were sampled from neurons in the mitral cell layer of the OB (≤8 neu-

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

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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 (Gilliatt, 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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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 Aδ-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 O_2) and maintained using constant intravenous alfaxalone (16-30mg.kg^{-1}hr^{-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 Aδ-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; 10μl, 100μM; n=3) or vehicle solution (n=1). For the post-operative pain model, rats were anaesthetised by inhalation of isoflurane (2-3% in O_2) and a 1cm 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 Aδ-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 Aδ-fibre nociceptive inputs in naïve rats. In the post-operative model, the augmented effect of SB-366791 on C- *and* Aδ-nociceptive processing suggests that post-incision, there is a sensitisation or upregulation of spinal TRPV1 receptors in both C- and Aδ-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.

PC110

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 Aδ- 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±3% and 145±11% of control threshold respectively, mean±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±25min.°C and 211±36min.°C respectively, mean±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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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/meditomidine 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.

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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% N_2O:O_2 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

saline infusion, citric acid altered the LFP power under pre-voiding, but not during/post-voiding conditions, by causing a significant increase ($p<0.01$) in the delta band and a significant decrease ($p<0.05$) in the theta band.
These data provide evidence for a neuronal response to normal and abnormal bladder contractions in the anterior cingulate gyrus of the mPFC. The lag in response to voiding may imply a sensory involvement of the mPFC following normal/abnormal bladder contractions rather than a 'motor drive' role.

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

PC113

Two unrelated subjects with 'congenital' analgesia, retention of innocuous somatic cutaneous sensations and hyperhidrosis

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Subject 1 (who has one sister) was born in 1968, and suffered many childhood injuries, including fractures to his left arm such that he now has peripheral motor and sensory nerve damage in this appendage, and spinal scoliosis. He sweats unconscionably. Subject 2 was born in 1923, one of 5 siblings; painlessness was noted from childhood onwards. Neither subject has any family history of "congenital" analgesia. Both subjects have hyperhidrosis, with a skin production 3-8 greater than normal..Pharmacological manipulation in Subject 1 (stellate ganglion block, hyoscine) failed to influence the hyperhidrosis, and neither did hyoscine in Subject 2.
Both had normal tactile and sharpness thresholds, and only slightly elevated vibration and innocuous thermal thresholds in comparison to normal controls. No nerve fibres could be detected in sural nerve biopsies in subject 1; and in subject 2, sural biopsy showed the C:Aδ:Aβ ratio to be 33: 6.5: 1 (270 myelinated, 178 unmyelinatd), compared with a normal ratio of 21:3:1 (4628 myelinated, 22427 unmyelinated; Jacobs and Love, 1985).

Genetic analysis of Subject 1 showed no change in gene SCN9A, thus ruling out channelopathy-associated insensitivity to pain.

Punch biopsies of skin in Subject 1 showed that nerve supply to the cutaneous vasculature, sweat glands and pilo-erector muscles was mostly intact, whereas virtually all of the other cutaneous innervation was absent or severely depleted, including all types of C, Aδ and Aβ-fiber endings. Importantly, the results revealed a previously unknown cholinergic innervation to the resistance arteries and arteriovenous shunts in the hand, which was also seen in normal subjects. These results suggest that humans may use their vascular afferents for purposes of conscious, albeit reduced, non-noxious somatic sensation.

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

PC114

Direct modulatory effect of inflammatory mediators on cold- and menthol-sensitive neurons from rat dorsal root ganglia

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It has recently been shown that inflammatory mediators (bradykinin and prostaglandin E2) inhibit responses to cooling in cold-and menthol-sensitive neurons from dorsal root ganglia (DRGs), which probably express the cold and menthol receptor TRPM8. This inhibition may be involved in inflammatory pain conditions (1). However, the high concentration of inflammatory mediators (10 μM) used in that study increased [Ca2+]i immediately on application, leading us to question whether the inhibition of TRPM8 was due to a direct effect of inflammatory mediators or simply to desensitization following activation of the neurons.
We have now examined the effects of an inflammatory soup of bradykinin, prostaglandin E2 and serotonin (each at 1 μM) on cold responsiveness of rat DRG neurons. DRG neurons from adult Sprague Dawley rats were cultured with 100 ng/ml nerve growth factor for 12-24 hours and responsiveness of individual neurons was evaluated using calcium microfluorimetry (ΔF/F0). As previously described (1), neurons were classified as cold- and menthol-sensitive (CSMS) or cold-sensitive and menthol-insensitive (CSMI).
Of 647 neurons, 151 (23.3 %) were cold sensitive, and of these 38 neurons (29 %) were also sensitive to menthol (CSMS neurons). Of these 38 CSMS neurons, 14 (37 %) showed a decrease in the amplitude of the response to cooling (ΔF/F0 decreased from 0.23 +/- 0.04 to 0.10 +/- 0.04, mean ± SD, n= 14; P=0.0008, Student's paired t test). Of the remaining 113 cold-sensitive neurons which did not respond to menthol (CSMI neurons), 28 (25 %) showed a decrease in the amplitude of the response to cooling (ΔF/F0 decreased from 0.29 +/-0.04 to 0.14 +/-0.03, n=28, P<0.0001, Student's paired t test).
The lower concentrations of inflammatory mediators used here avoided direct activation of neurons at base temperature of 32°C. Dampening of the response to cooling at these low concentrations is thus indicative of a direct modulatory effect of inflammatory mediators on cold sensitive neurons, rather than simply a desensitization following activation.

Linte RM, Ciobanu C, Reid G & Babes A (2007). Exp Brain Res 178, 89-98.

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PC115

A comparison of the effects of capsazepine on type I and type II slowly adapting mechanoreceptors in the rat sinus hair follicle

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There is currently significant interest in determining the molecular bases for mechanotransduction in the vertebrate (Lumpkin & Caterina, 2007). We therefore tested a broad spectrum transient receptor potential (TRP) channel antagonist in an isolated sinus hair follicle preparation in which slowly adapting type I (St I) and type II (St II) could be distinguished.
28 adult Wistar-derived rats (mean weight 335 g) were used. Sinus hair follicles with a 10 mm length of deep vibrissal nerve attached were microdissected from the whisker pad of animals killed by I.P. and I.C. 3g/kg urethane). Follicles were kept in carbogenated (bubbled medical 95% oxygen, 5% carbon dioxide) synthetic interstitial fluid (SIF). They were slit open lengthways and fixed with insect pins to a silicone elastomer Sylgard platform in a custom-made tissue bath. Capsazepine was made up in SIF and used between 10 – 200 μM. Doses were applied to the preparation at the rate of 1 ml/min for up to 20 min. The two types of units were distinguished by their characteristic static phase firing as previously described (Senok & Baumann, 1997). Statistical analyses included t tests.
Capsazepine was tested on a total of 13 St I units. Between 30 – 200 μM caused a transient increase in activity (mean±SEM 52±5 Hz to 80±8 Hz, $p < 0.01$), notably of the static component. Repeated doses resulted in clear habituation to this excitatory effect. With high concentrations 100 – 200 μM the excitatory effect was followed by a long-lasting and profound depression of all activity ($p < 0.01$). A total of 15 St II units were studied. Only doses above 50 μM had any effect, and this consisted of a uniform and long-lasting depression of all activity components (dynamic, static and spontaneous) ($p < 0.01$). In about 25% of St II units which were spontaneously active, a delayed drug effect produced an inversion of response such that ongoing firing was interrupted during the mechanical ramp stimulus. The same effect was only seen in one St I unit.
The depression by capsazepine of all activity in both St I and St II units suggest a non-selective effect on mechanoreceptor nerve endings. The excitatory effect of capsazepine that was seen only in St I units may represent an activation of Merkel cells. In another in vitro cell system, capsazepine has been found to increase intracellular calcium (Huang et al, 2006). Pharmacological manipulation (e.g. caffeine (Senok & Baumann, 1997)) which cause calcium influx in Merkel cells results in a selective increase in the static component, as seen here. In conclusion, these results using capsazepine provide weak support for a TRP channel role in St I and St II mechanotransduction. However, the excitatory effect seen in St I mechanoreceptors emphasises their difference from St II mechanoreceptors.

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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.

PC116

Orexin/hypocretin-A induces intracellular calcium transients in rat cultured dorsal root ganglia neurones

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The hypothalamic peptides orexin A/hypocretin-1 and Orexin B/hypocretin-2 are involved in a range of physiological functions including control of feeding and energy metabolism, sleep and arousal. Orexin fibers innervate many regions of the brain and spinal cord which include areas involved in pain processing and emerging evidence suggests that orexins modulate pain transmission. This study investigate the effects of Orexin A on intracellular calcium, ([Ca2+]i), signals in cultured rat dorsal root ganglion (DRG) neurons, with the aim of exploring possible involvement of this agent in nociceptive transmission. DRG neuronal cultures were loaded with 1 μmol Fura-2 AM and Ca2+ responses 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. [Ca2+]i changes were determined by the change in 340/380 ratio (basal-peak) was also calculated for individual DRG neurons in selected microscopic fields. All data were analyzed by using unpaired t test, with a 2-tailed P level of <.05 defining statistical significance. ORX-A caused increase in [Ca2+]i in a dose dependent manner. The ORX-A-induced [Ca2+]i responses were similar to those observed with high-K+ (30 mM). The mean 340/380 nm ratios were (baseline vs OrX-A): 0.90±0.01 vs 1.34±0.03 (1 nM OrX-A, $P<0.001$, n=21); 1.10±0.03 vs. 2.15±0.09 (10 nM OrX-A, $P<0.001$, n=21); 0.82±0.02 vs. 1.65±0.09 (100 nM OrX-A, $P<0.001$, n=18) and 0.72±0.02 vs. 1.69±0.08 (200 nM OrX-A, $P<0.001$, n=34), respectively. These results, in line with previous findings in different preparations, show that Orexin A increases intracellular calcium levels in a dose-dependent fashion in DRG neurons. We conclude that orexin-A has excitatory effects on DRG neurones, consistent with the perspective that orexin/hypocretins have a role in orchestrating reactions related to nociception, pain and temperature sense.
Keywords: Orexin A; fluorescence calcium imaging, nociception, sensory neurones

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PC117

Epac activation, altered calcium homeostasis and ventricular arrhythmogenesis in Langendorff-perfused mouse hearts

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The recently described cAMP sensor, Exchange protein directly activated by cAMP (Epac), has been implicated in distinct cAMP-dependent, protein kinase A-independent cellular signalling pathways (Bos JL, 2006). We investigated effects of Epac activation in catecholamine-induced ventricular arrhythmogenesis. In contrast to control findings (n = 20), monophasic action potentials showed spontaneous triggered activity in 2 out of 10 intrinsically beating and 5 out of 20 extrinsically-paced Langendorff-perfused murine hearts perfused with the specific Epac activator 8-pCPT-2'-O-Me-cAMP (8-CPT, 1 μM) (Christensen AE *et al.* 2003). During steady extrinsic pacing at 8 Hz, 3 out of 20 such hearts showed spontaneous ventricular tachycardia (VT). Programmed electrical stimulation provoked VT in 10 of 20 similarly treated hearts ($P < 0.001$; $n = 20$, Fisher's Exact Test). However, no statistically significant changes ($P > 0.05$, ANOVA) in left ventricular epicardial (40.7 ± 1.2 versus 44.0 ± 1.7 ms; n = 10), or endocardial action potential durations (APD_{90}) (51.8 ± 2.3 versus 51.9 ± 2.2 ms; n = 10), transmural (ΔAPD_{90}) (11.1 ± 2.6 versus 7.9 ± 2.8 ms; n = 10) or apico-basal gradients of repolarization, ventricular effective refractory periods (29.1 ± 1.7 versus 31.2 ± 2.4 ms in control and 8-CPT-treated hearts, respectively; n = 10) and APD_{90} restitution characteristics accompanied these arrhythmogenic effects. However, fluo-3 fluorescence imaging of cytosolic Ca^{2+} demonstrated alterations in Ca^{2+} homeostasis in the form of increased Ca^{2+} wave generation in both paced and resting isolated 8-CPT-treated ventricular myocytes. An independent method of Epac activation that applied 100 nM isoproterenol to stimulate beta-adrenoreceptors in parallel with protein kinase A inhibition by 2 μM H-89, was also arrhythmogenic in the whole heart and similarly altered cytosolic Ca^{2+} homeostasis. The Epac-dependent effects at both the whole heart and cellular levels were reduced by inhibition of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) with 1 μM KN-93. These findings associate VT in an intact cardiac preparation with altered cellular Ca^{2+} homeostasis and Epac activation through a CaMKII-dependent mechanism for the first time, in the absence of the altered repolarization gradients previously implicated in re-entrant arrhythmogenesis (Killeen MJ *et al.* 2007; Thomas G *et al.* 2007).

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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.

PC118

Is nitric oxide (NO) important in the adenosine A_{2A}-receptor-mediated vasodilatation of skeletal muscle contraction?

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During systemic hypoxia the contribution of adenosine to skeletal muscle vasodilatation is dependent on the presence of NO; NO is required for the release of adenosine from the endothelium (Edmunds *et al*. 2003) and mediates dilatation via endothelial A_1-receptors (Ray & Marshall, 2005). By contrast, skeletal muscle vasodilatation accompanying muscle contraction (exercise hyperaemia) is mediated by adenosine acting at A_{2A}-, but not A_1-receptors (Ray & Marshall, 2008). Adenosine can release NO from endothelium by acting at A_{2A}-receptors (Ray *et al*. 2002). Thus, we investigated the role of NO in exercise hyperaemia.

In three groups of rats, anaesthetized with Saffan (7-12 $mgkg^{-1}hr^{-1}$ I.V.), we recorded arterial blood pressure (ABP), femoral blood flow (FBF) and tension in the extensor digitorum longus. Isometric twitch contractions were evoked by stimulation of the sciatic nerve at 4Hz. Integral femoral vascular conductance (IntFVC) was calculated off-line. Group 1 (n=7) was the time control for, Group 2 (n=10), which received NOS inhibitor L-NAME before the third, and A_{2A}-receptor antagonist ZM241385, before the fourth contraction. Group 3 (n=12) received L-NAME before the third, the NO-donor SNAP to restore baseline FVC during the fourth and fifth contraction and ZM241385 before the fifth.

Time controls showed consistent tension and hyperaemic responses. In Group 2, baseline IntFVC was reduced by L-NAME (0.555±0.04 (mean±SEM) to 0.297±0.02CU*, ANOVA for repeated measures, p<0.001) but not by ZM241385. L-NAME reduced exercise hyperaemia (13.91±1.31 to 9.52±1.09CU*), and it was further attenuated by ZM241385 (to 5.46±1.12CU*). In Group 3, SNAP after L-NAME restored baseline IntFVC to control levels (Control: 0.702±0.09, L-NAME: 0.377±0.05*, L-NAME + SNAP: 0.616±0.09CU); ZM241385 had no further effect. Exercise hyperaemia was also restored to control levels after L-NAME by SNAP (Control: 17.10±1.18, L-NAME: 10.87±1.09*, L-NAME + SNAP: 16.99±1.38CU), and this response was further attenuated by ZM241385 (12.75±0.98CU*).

These results confirm that adenosine acting via A_{2A}-receptors contributes to exercise hyperaemia. However, they indicate