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.

British Heart Foundation for funding the study.

Dr. David Johnston for his invaluable assistance with confocal microscopy

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

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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., 2007). 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% O2 and 5% CO2. 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 (pEC50: C, 7.67 ± 0.05, n=7; PR, 8.01 ± 0.05, n=6, p<0.01). Endothelial-dependent vasodilation to both ACh (pEC50: 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.


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.

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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 (Zylnka 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 C57BL6 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
(DRC) 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 P2X3 (mean surface area (m.s.a.) 444.3 ± 4.43μm², n=800) was observed in 85.1% of eGFP-positive neurones (m.s.a. 509.9 ± 7.32μm², n=737), whilst TRPV1 immunoreactivity (m.s.a. 358.1 ± 15.7μm², 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, Ih, 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 (Ih).

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

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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 neurones 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 neurones 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 neurones 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 neurones 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 neuro-muscularly 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 neurones with hindlimb receptive fields was recorded extracellularly. Wide dynamic range neurones 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 neurones were responsive to low- and high-threshold stimuli. All of the neurones had receptive fields that included both glabrous and hairy skin, but low-threshold responses were only evoked from hairy skin. Most neurones 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 neurones 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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Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.
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 infragingival 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 after 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 + 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.


We are very grateful to professor Bruce Mathews

The effect of spinal nerve axotomy on I\textsubscript{h} in dorsal root ganglion (DRG) neurons with A\textalpha/\beta-fibres in rats in vivo

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It has been suggested that I\textsubscript{h} drives neuropathic pain (Chapman et al. 2003) partly because rat isolated large diameter L5 DRG neuronal soma in vitro, showed increased I\textsubscript{h} density and depolarised V\textsubscript{rev} (half-activation potential) after L5 spinal nerve ligation. In this study we examined whether such changes occur in vivo in A\textalpha/\beta-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\textsubscript{h} was recorded in somata of L4-L6 DRG neurons in normal and in axotomised L5 DRG neurons 7 days after SNA. I\textsubscript{h} at -100 mV was determined from the difference between instantaneous and steady state current. I\textsubscript{h} density is I\textsubscript{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-, A\textalpha/\beta- or A\textalpha/\beta-conduction velocities. Animals were killed by a pentobarbital overdose. Medians were compared by non-parametric Kruskall-Wallis or Mann-Whitney test.

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

Some properties of I\textsubscript{h} did not change in axotomised A\textalpha/\beta-fibres. V\textsubscript{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\textsubscript{h} occurred in axotomised L5 A\textalpha/\beta-fiber neurons compared with in normal neurons. Both I\textsubscript{h} amplitude and density were significantly reduced (P<0.01) after axotomy. A higher proportion of axotomised neurons expressed I\textsubscript{h} (100% n=68 for axotomy, 89% n=98 for normal; p<0.05, Chi2 test). V\textsubscript{rev} for axotomised A\textalpha/\beta-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\textsubscript{h} is likely to be both harder to activate and smaller in axotomised A\textalpha/\beta-fibre neurons. Therefore,
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* turnover 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 over-expression 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 τREC. Previous experiments showed that τREC 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 τREC, 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.

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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.
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: Leviteracetam; dorsal root ganglia, fluorescence calcium imaging, pain, sensory neurons

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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.

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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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PC109

Role of Transient Receptor Potential Vanilloid 1 receptors in C- vs Aδ-fibre-evoked spinal noiception 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 O2) 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°C.s⁻¹) and fast (6.5-7.5°C.s⁻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 (58x477) and a 1cm longitudinal incision was made on the hindpaw dorsum in alphadolone/alphaxalone-anaesthetised 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±13°C and 145±11°C 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.


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


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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.

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

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
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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 vibrisal nerve attached were microdissected from the whisker pad of animals killed by I.P. and I.C. 3g/kg urethane). Follicles were kept in carbonated (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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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+), signals in cultured rat dor- sal root ganglion (DRG) neurones, 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+] 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+] in a dose dependent manner. The ORX-A-induced [Ca2+] 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 (10 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 neurones. 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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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 ± 1.7 ms; n = 10), or endocardial action potential durations (APD90) (51.8 ± 2.3 versus 51.9 ± 2.2 ms; n = 10), transmural (∆APD90) (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 APD90 restitution characteristics accompanied these arrhythmogenic effects. However, fluo-3 fluorescence imaging of cytosolic Ca2+ demonstrated alterations in Ca2+ homeostasis in the form of increased Ca2+ 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-adrenergoreceptors in parallel with protein kinase A inhibition by 2 μM H-89, was also arrhythmogenic in the whole heart and similarly altered cytosolic Ca2+ homeostasis. The Epac-dependent effects at both the whole heart and cellular levels were reduced by inhibition of Ca2+/calmodulin-dependent protein kinase II (CaMII) with 1 μM KN-93. These findings associate VT in an intact cardiac preparation with altered cellular Ca2+ homeostasis and Epac activation through a CaMII-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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Is nitric oxide (NO) important in the adenosine A2A-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 A1-receptors (Ray & Marshall, 2005). By contrast, skeletal muscle vasodilatation accompanying muscle contraction (exercise hyperaemia) is mediated by adenosine acting at A2A receptors, but not A1-receptors (Ray & Marshall, 2008). Adenosine can release NO from endothelium by acting at A2A-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-1hr-1 i.v.), we recorded arterial blood pressure (ABP), femoral blood flow (FFB) 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-NNAME before the third, and A2A-receptor antagonist ZM241385, before the fourth contraction. Group 3 (n=12) received L-NNAME 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-NNAME (0.555±0.04 (mean±SEM) to 0.297±0.02CU*, ANOVA for repeated measures, p<0.001) but not by ZM241385. L-NNAME 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-NNAME restored baseline IntFVC to control levels (Control: 17.10±0.02CU*; L-NAME + SNAP: 0.616±0.09CU*; ZM241385 had no further effect. Exercise hyperaemia was also restored to control levels after L-NNAME by SNAP (Control: 17.10±1.18, L-NNAME: 10.87±1.09*, L-NNAME + SNAP: 16.99±1.38CU*), and this response was further attenuated by ZM241385 (12.75±1.98CU*).

These results confirm that adenosine acting via A2A-receptors contributes to exercise hyperaemia. However, they indicate...