## C58

**The inducible isoform of nitric oxide synthase (iNOS) has an important peripheral role in the development of inflammatory and neuropathic pain**

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We have investigated the role of the inducible isoform (iNOS) in models of inflammatory and neuropathic hypersensitivity using immunohistochemistry, chemiluminescence and the selective iNOS inhibitor GW274150 (Alderton et al, 2005).

Male random hooded rats (200-250g) were used. Freund's complete adjuvant (FCA): animals received 0.1ml of FCA (1mg/ml) or saline intraplantar into the left hind paw. The hypersensitivity was assessed at different time points (6h, 24h, 48h, 1 week and 3 weeks) after the FCA. The hypersensitivity was determined using the dual channel weight averager. At each time point animals were humanely killed, the paws, dorsal root ganglion (DRG), spinal cord and brain were removed and snap frozen. In the chronic constriction injury (CCI) model, under anaesthesia (isofluorane) the common left sciatic nerve was exposed and four loose ligatures of chromic gut (4.0) tied around it. The rats were allowed a period of 3 days to recover from the surgery before the hypersensitivity was determined using the algesymeter (Randall & Selitto, 1957) from day 3 to days 23 post surgery. Again after testing, different groups of animals at each time point were humanely killed and the sciatic nerves, DRG, spinal cords and brains were taken. In the FCA model GW274150 (1-30mg/kg p.o.) was dosed 24h post FCA and the effect on the hypersensitivity was determined at 1 and 6h post dose. In the CCI model GW274150 (3-30mg/kg s.c.) was dosed 23h post surgery and the effect on the hypersensitivity was determined 1h post dose. Nitrate/nitrite levels were determined using chemiluminescence, iNOS expression was determined using immunohistochemistry. Results are expressed as mean ±S.E.M. (n=7-10) and statistical analysis was carried out using one-way ANOVA followed by Dunnett's test where p<0.05 is considered significant.

iNOS was detected locally in the paw 6h after FCA injection and was associated with macrophages. The expression plateaued 24-72h post FCA and then slowly declined. This was associated with the development of the hypersensitivity. GW274150 (1-30mg/kg p.o.) suppressed the accumulation of nitrite (ED50=1.9±0.74mg/kg.) and partially reversed the hypersensitivity (max effect:52±5% at 30mg/kg). In the CCI model iNOS was only detected around the sciatic nerve after 3 days, appeared to concentrate proximal to the ligatures and was associated with inflammatory cells (macrophages). Between 7 and 26 days iNOS expression had spread away from the ligatures and surrounded the nerve fibres. GW274150 (3-30mg/kg s.c.) on day 23 produced a dose-related inhibition of the CCI-induced hypersensitivity 1h post dose (max effect: 105±29% at 30mg/kg s.c.; ED50=3.02 (0.5-17)mg/kg).

In conclusion, the study supports an important role for peripherally expressed iNOS in both inflammatory and neuropathic pain, and therefore GW274150 may have clinically utility in the treatment of these pain states.

Alderton WK et al. (2000). In Biology of Nitric Oxide, ed. Moncada S, Gustaffson LE, Wiklund NP & Higgs E, p. 22. Portland Press.

Randall LO & Selitto JJ. (1957). Archives Internationale de Pharmacodyyamie et de Therapy 111, 409-419.

*Where applicable, the experiments described here conform with Physiological Society ethical requirements.*

## C59

**Evidence for a role of peripheral prostaglandins in determining the mechanical sensitivity of primary afferent C-fibres of the rat knee joint**

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Prostaglandins (PGs), products of cyclooxygenase (COX) enzymes, activate and sensitise articular mechanonociceptors to nociceptive stimuli (Birrell *et al.* 1991). Evidence suggests that there is basal release of PGs in the rat knee joint (Egan *et al.* 2000). The analgesic dipyrone inhibits PG production in peripheral tissues and is anti-nociceptive when administered peripherally. However, the site of action of dipyrone remains controversial. We investigated the effects of peripheral administration of dipyrone on noxious mechanically evoked responses of knee joint afferents in non-inflamed rats and rats with Freund's Complete Adjuvant (FCA)-induced monoarthritis (100μg/100μl injected intra-articularly under brief halothane (3% in $O_2$) anaesthesia; n=7 each group). FCA-injected rats exhibited a significant reduction in weight bearing on ipsilateral hind-limbs (p<0.01, 1-way ANOVA), and ipsilateral knee joints were significantly swollen (p<0.01 vs contralateral, paired t test). For electrophysiological studies, rats were anaesthetised (60mg/kg pentobarbital i.p.) and the external jugular vein and trachea cannulated. Mechanically evoked responses (~170 g mechanical indent, 5 s duration every 5 min) of teased filaments of the medial articular nerve were recorded ipsilateral to either a non-inflamed joint or a FCA-inflamed joint. Once stable control evoked responses were obtained, 100μl of saline or dipyrone (50 and 100μM = 1.5 and 3μg/100μl) was injected intra-articularly, and effects on mechanically evoked responses of primary afferent C-fibres (conduction velocities = 0.7-2.4 m/s) were followed for 50-60 min. The frequency of evoked response was quantified and expressed as % of control response. Data are means ± S.E.M. Saline had no significant effect on mechanically evoked responses in non-inflamed (81 ± 13%) and inflamed (80 ± 6%) rats. However, dipyrone inhibited mechanically evoked responses in non-inflamed (50μM: 54 ± 10%; 100μM: 42 ± 12%, p<0.05 vs control, paired t test) and inflamed (50μM: 60 ± 11%; 100μM: 37 ± 10%, p<0.05 vs control, paired t test) rats.

Inhibitory effects of dipyrone were similar in the two groups and were dose related. These data suggest that PGs released into the rat knee joint under basal conditions and during inflammation are involved in determining the mechanical sensitivity of C-fibre afferents. This study provides support for a peripheral site of action for dipyrone. As low concentrations of dipyrone were used, we postulate that dipyrone may inhibit mechanically

evoked activity by inhibition of COX1 or an as yet unidentified COX isoform.

Birrell GJ *et al.* (1991). *Neurosci Lett* **124**, 5-8.

Egan CG *et al.* (2000). Expt Physiol **86**, 191-197.

## C60

### Relationship of trkA expression to electrophysiology of Aα/β nociceptive DRG neurones: evidence linking this to Nav1.8 expression

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Title only.

## C61

### Spontaneous neuropathic pain behaviour is associated with increased spontaneous activity frequency in uninjured nociceptive C-fibre neurones adjacent to axotomised nerve fibres

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Spinal nerve (SN) lesion models of neuropathic pain, including the L5 SN axotomy (SNA) model, cause allodynia and hyperalgesia, but spontaneous foot lifting (SFL) thought to indicate spontaneous pain (e.g. Bennett & Xie, 1988) is rarely reported. The causes of spontaneous pain, an important aspect of human neuropathic pain, are poorly understood. To test the hypothesis that spontaneous activity (SA) in dorsal root ganglion (DRG) neurones may cause SFL in a modified SNA model (mSNA) that shows significant SFL (Koutiskou & Lawson, 2002), we examined SFL and SA in both this (mSNA) and SNA models. The mSNA model involves SNA plus loose-ligation of the adjacent L4 SN with chromic gut which causes inflammation.

For electrophysiology, female rats (150-180g) were anaesthetised with pentobarbitone (60 mg kg$^{-1}$, i.p.), neuromuscularly blocked with pancuronium (0.5 mg kg$^{-1}$, i.a.) and artificially ventilated. The end-tidal $CO_2$ was monitored and maintained between 3-4% by adjusting the volume and stroke of the respiratory pump and blood pressure was monitored and maintained at >80 mmHg. Axotomy was carried out under deep anaesthesia (sodium pentobarbitone 50 mg kg$^{-1}$, i.p.). Intracellular recordings of somatic action potentials evoked by dorsal root stimulation and any spontaneously occurring spikes were made from: a) normal L4/L5 DRG neurones in control rats (n=48), b) axotomised L5 neurones in mSNA rats (n=18), c) adjacent L4 neurones in both mSNA and SNA rats (n=10) and d) L4/L5 neurones in rats (n=38) 4 days after hindlimb inflammation induced by intradermal injections of Complete Freunds Adjuvant (100 μl). Neurones were classified from their dorsal root conduction velocities as C, Aδ or Aα/B units and according to their responses to natural stimuli as non-nociceptive or nociceptive-type units.

One week after SN injury, significantly increased percentages of nociceptive-type C-fibre neurones showed SA (from 7% to about 35%) in L4 but not L5 DRGs in both SNA and mSNA rats (Fisher's test, P<0.01). Thus this increased percentage of SA could not have been the cause of SFL seen only in mSNA rats. It may result from inflammation (perhaps from degeneration of L5 fibres) since a similar increase in percentages of C-nociceptors with SA was seen 4 days after hindlimb inflammation (Fisher's test, P<0.05). Interestingly, L4 nociceptive-type C-fibre neurones showed faster SA rates in mSNA (1.8 Hz) than SNA (0.02 Hz) rats (Mann Whitney test, P<0.001) implicating the L4 in SFL. Thus mSNA rats had both greater SA rates and SFL (Fisher's test, P<0.001). We propose that faster firing rates in intact C-nociceptive neurones are required for SFL/spontaneous neuropathic pain and that these result from added inflammation/damage, caused by chromic gut in mSNA rats.

Bennett GJ & Xie YK (1988). Pain 33, 87-107.

Koutisikou S & Lawson SN (2002). J Physiol 543, 113P.

## SA27

### Interplay of opioid-producing inflammatory cells and nociceptors

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We have shown that opioid receptors are present and upregulated on peripheral sensory nerves and that opioid peptides are expressed in immune cells within peripheral inflamed tissue (Nat Med. 9:1003-8, 2003). Environmental stimuli (stress) and releasing agents (corticotropin releasing factor, cytokines) can liberate these peptides to elicit local analgesia, while suppression of the immune system abolishes these effects (J Clin Invest. 100:142-8, 1997). These findings have led to the concept that opioid peptides can be secreted from immunocytes, occupy opioid receptors on sensory nerves and produce analgesia by inhibiting the excitability of these nerves and/or the release of proinflammatory neuropeptides. Our recent investigations have examined G-protein coupling in sensory neurons innervating injured tissue (Mol Pharmacol. 64:202-10, 2003; J Pharmacol Exp Ther. 308:712-8, 2004), subcellular pathways of opioid peptide processing and release in immune cells (Endocrinology. 145:1331-41, 2004) and adhesion molecules, chemokines and growth factors governing the migration of opioid containing immune cells to injured tissue (Nat Med. 4:1425-8, 1998; J Neurosci. 22:5588-96, 2002; Anesthesiology. 100:149-57, 2004; Pain. 108:67-75, 2004). Clinical studies have now shown that small doses of opioids (e.g. morphine) applied into arthritic joints can not only produce long lasting pain relief but also decrease synovial inflammation (Nat Med. 9:1003-8, 2003; Pain. 83:525-32., 1999).

Stein C et al. (2003) Nat Med 9, 1003-1008.

Brack A et al. Anesthesiology (2004) 100, 149-157.

Zoellner C et al. (2003) Mol Pharmacol 64, 202-210.

Brack A et al. (2004) Pain 108, 67-75.

Mousa SA et al. (2004) Endocrinology 145, 1331-1341.

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## SA28

### The neutrophil as an essential link in mediating hyperalgesia in skin

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Nerve growth factor (NGF), in addition to acting as a growth factor for nerves that include capsaicin-sensitive fibres, is a potent mediator of inflammatory hyperalgesia. Work in this laboratory has demonstrated that the thermal hyperalgesia mediated by NGF is neutrophil-dependent (Bennett et al. 1998). These studies have now been extended to investigate the mechanisms via which neutrophils accumulate in response to NGF in the skin (Foster et al. 2003).

Rats were injected either intraplantarly or intradermally with test agents under short term anaesthesia (2% isofluorane). At the end of the experiments myeloperoxide was obtained from the skin of humanely killed rats and assayed as an index of neutrophil accumulation. The mechanism by which NGF (40 pmol/site) induces neutrophil accumulation in the rat is protein synthesis-dependent in that actinomycin D (1μmol/site) inhibited neutrophil accumulation measured over 5h. This indicates that the neutrophil accumulation is secondary to upregulation of endothelial cell adhesion molecules. Intracellular adhesion molecule-1 (ICAM-1) plays an important role in neutrophil emigration. Thermal hyperalgesia was measured by the Hargreaves method where the reaction time to an automated heat source was determined. A monoclonal antibody to endothelial-derived ICAM-1 inhibited thermal hyperalgesia, in addition to neutrophil accumulation. In separate experiments, a similar response of neutrophil accumulation and thermal hyperalgesia was observed in response to intraplantar NGF in both wildtype and tachykinin NK1 receptor knockout mice, indicative that the NK1 receptor is neither involved in the neutrophil accumulation nor the hyperalgesic response.

The results provide evidence that an important step in NGF-induced hyperalgesia is the activation of endothelial cells to express adhesion molecules that then act to promote neutrophil accumulation and in turn thermal hyperalgesia. The relevance of this to inflammatory hyperalgesia in disease is not yet known.

Bennett G et al.(1998). Pain 77, 315-322.

Foster PA et al. (2003). FASEB J 17, 1703-1705.

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