C41

Blockade of metabotropic glutamate receptors enhances responses of rat slowly adapting type I cutaneous mechanoreceptors *in vitro*

P.M. Cahusac[1] and S.S. Senok[2]

[1]*Department of Psychology, University of Stirling, Stirling, UK and* [2]*Department of Physiology, Arabian Gulf University, Manama, Bahrain*

The nerve endings of type I units make synapse-like contacts with Merkel cells in the basal epidermis of the skin. Recent work demonstrated the existence of vesicular glutamate transporters in Merkel cells (Hitchcock et al. 2004), and metabotropic glutamate (mGlu) receptors on Merkel cells and associated nerve terminals (Tachibana et al. 2003). It is therefore possible that these receptors play a modulatory role in neurotransmission at this junction. We examined the effects of two different mGlu receptor antagonists on the evoked responses of type I units.

Whisker pads were taken from nine humanely killed adult male Wistar-derived rats. Extracellular single unit recordings were made from sinus hair slowly adapting mechanoreceptor units using methods previously described (Fagan & Cahusac, 2001). The vibrissa hair shaft was deflected mechanically for 5 s (0.5 s onset and offset ramps, 4 s plateau). The preparation was superfused with oxygenated synthetic interstitial fluid, by which drugs could be administered. Means±S.D. are given.

During the application of broad spectrum mGlu receptor antagonist LY341495 (10 - 100μM) the static component of the response was significantly increased to 119-162% of control ($t(4) = 4.95$, $p = 0.008$). The dynamic response component was more variable and generally less affected (95-194% of control, $t(4) = 2.009$, $p = 0.104$). The effect on spontaneous firing was highly variable, although an increase was often seen (mean firing before drug was 1.3±1.7 Hz, mean during drug was 3.5±4.4 Hz). The effects were relatively short lasting after wash; mean duration was 19±14 min.

The more selective mGlu receptor antagonist (RS)-4C3HPG (10 - 100μM) was also tested. Again, the static component was significantly affected (86-207% of control, $t(4) = 3.051$, $p = 0.038$), while the dynamic was less so (94-133% of control, $t(4) = 2.29$, $p = 0.084$). Spontaneous firing, although variable, tended to increase during the drug application (before, 0.9±1.0 Hz; during drug, 3.1±4.2 Hz). The mean duration of the effects was 34±25 min. The effects observed were clearly dose-dependent.

Slowly adapting type II units were studied as a control, since lanceolate endings do not make synaptic contacts. In two units using LY341495 (10μM) there was no apparent effect (static = 101%, dynamic = 108% of control). A further two units using (RS)-4C3HPG (100μM), was again without effect (static = 97%, dynamic = 97% of control).

These experiments show that mGlu receptor antagonists selectively increase responses of type I units. The concentrations of LY341495 used were sufficient to block all known types of mGlu receptors. In contrast the concentrations of (RS)-4C3HPG used were only sufficient to block mGlu1 receptors (Kingston et al. 1995). The results suggest that Group I mGlu receptors modulate either neurotransmission or excitability at the Merkel-neurite junction.

Fagan BM & Cahusac PMB (2001). Neurorep **12**, 341-347.

Hitchcock IS et al. (2004). Neurosci Lett **362**, 196-199.

Kingston AE et al. (1995). Neuropharm **34**, 887-894.

Tachibana T et al. (2003). Histochem Cell Biol **120**, 13-21.

This work was supported by the Wellcome Trust, grant reference 061023.

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

C42

Innocuous warm transduction in chick primary sensory neurones depends on TRPV3

G. Reid

Faculty of Biology, University of Bucharest, Bucharest, Romania

Innocuous warming in mice is detected by the ion channel TRPV3, expressed in the skin but not in sensory neurones (Moqrich et al. 2005). This finding may be of limited relevance to human warm sensing: a substantial number of human dorsal root ganglion (DRG) neurones express TRPV3 (Smith et al. 2002), and lack of TRPV3 expression in mouse DRG may simply reflect the fact that warm receptors are very scarce in rodents.

Hatchling chicks may be a better model for studying cutaneous warm sensing: they thermoregulate only behaviourally, being unable to generate enough body heat to survive, and require high ambient temperatures (~37°C) for survival. My aim here was to investigate innocuous warm transduction in chick sensory neurones.

Chicks 3 - 6 days after hatching were humanely killed, and DRG cultures and measurements of $[Ca^{2+}]_i$ were made as described (Reid et al. 2002). Heat ramps (29 - 38°C or 29 - 53°C, 0.5°C/s) were applied with a Peltier-based system (Reid et al. 2001).

Of 1321 chick DRG neurones, 100 (7.6%) responded to warming to 38°C with an increase in $[Ca^{2+}]_i$ ($\Delta F/F_0$ 0.255 ± 0.136, mean ± SD, $n = 100$). Thresholds were normally distributed (33.7 ± 2.0°C, $n = 100$), and heating to 53°C revealed a bimodal distribution of thresholds with distinct groups of neurones responding to warmth and to noxious heat (Fig. 1). Warm-sensitive neurones were significantly smaller than noxious heat-sensitive neurones (20.7 ± 3.5 μm, $n = 92$, vs. 24.5 ± 4.0 μm, $n = 157$; $p < 0.001$).

Responses to warming depended on extracellular Ca^{2+} ($n = 7$) and were strongly inhibited by the TRPV antagonist ruthenium red (75 ± 18% inhibition at 10 μM, $n = 18$). Warm responses were unaffected by reducing pH to 6.5 ($n = 19$), suggesting TRPV1 is probably not the transducer. All warm-sensitive neurones were activated or sensitised by 50 μM 2-aminoethoxydiphenyl borane (2-APB), an agonist of TRPV1, 2 and 3 but not TRPV4 ($n = 16$), and by 2 mM camphor, a TRPV3 agonist which is without effect on other TRPV channels ($n = 16$; Fig. 2). RT-PCR showed TRPV3 mRNA to be highly expressed in chick DRG.

In conclusion, chicks have substantial numbers of warm-sensitive DRG neurones, and TRPV3 plays an important role in warm transduction in these neurones. This is the first report of innocu-

ous warm transduction in DRG neurones, and of the function of TRPV3 in the DRG. Chicks are likely to be a more useful model than rodents in understanding the roles of TRPV3 in human sensory neurones.

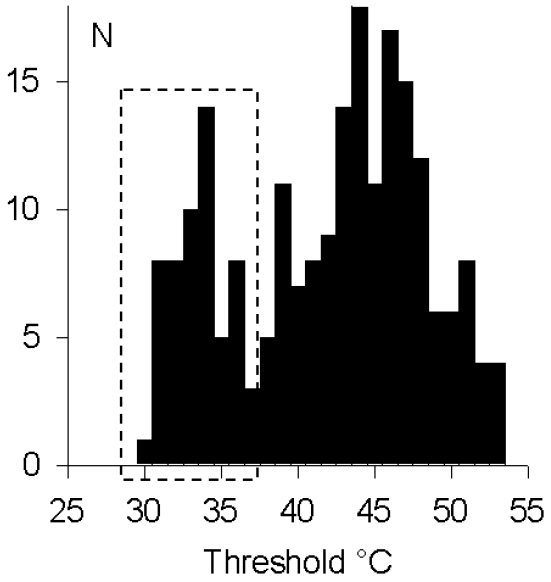

Figure 1. Thresholds of chick DRG neurones during 53°C ramps show distinct groups activated by warming (box) or by noxious heat.

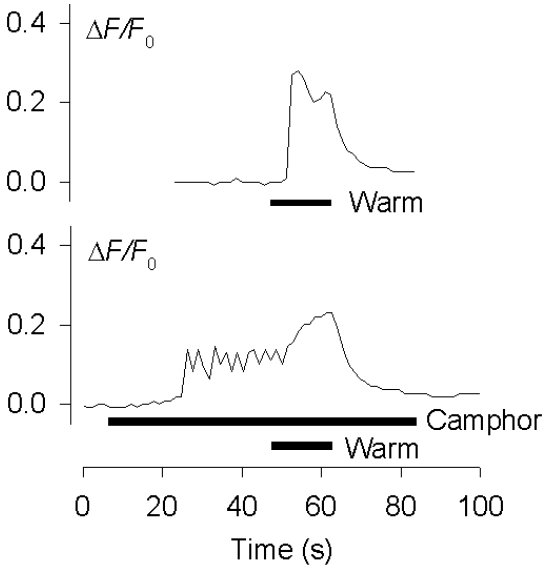

Figure 2. Activation of a chick DRG neurone by 38°C ramp (top) and by 2 mM camphor (bottom, same neurone).

Moqrich A et al. (2005). Science 307, 1468-1472.

Reid G et al. (2001). J Neurosci Methods 111, 1-8.

Reid G et al. (2002). J Physiol 545, 595-614.

Reid G et al. (2001). J Neurosci Methods 111, 1-8.

Funding was from the Physiological Society and the Volkswagen Foundation.

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

C43

A novel type of cold-sensitive neurone in rat dorsal root ganglia (DRG) with rapid adaptation to cooling

A. Babes, D. Zorzon and G. Reid

Dept. of Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, Bucharest, Romania

At least two types of neurone are involved in cold sensitivity of rodent DRG cultures (Thut et al. 2003; Babes et al. 2004). One group is menthol-sensitive and probably expresses TRPM8, the cold and menthol receptor; the other group, which is menthol-insensitive, has not been yet completely characterised, but it does not appear to express the other cold-activated ion channel, TRPA1. Within this latter group we have identified a new type of cold-sensitive neurone, which responds to fast cooling with a transient increase in $[Ca^{2+}]_i$. Wistar rats were humanely killed. Neurones from DRG levels L1 to S1 were kept in primary culture for up to 3 days (Reid et al. 2002). Temperature steps from 32 to 20°C with a 10 s duration were applied by fast (<100 ms) switching (the fast temperature controlled application system will be demonstrated at the meeting). Calcium Green-1 fluorimetry was used to monitor changes in $[Ca^{2+}]_i$ during cooling or application of chemicals. During fast cooling steps some neurones adapted rapidly (a in Fig. 1), while others showed almost no adaptation (b in Fig. 1). While cells showing a sustained response were all menthol-sensitive and thus probably express TRPM8, rapidly adapting cells appear to belong to a novel group of cold-sensitive neurones. Of 2205 imaged neurones, 82 (3.8%) adapted during the cooling step to less than 35% of the initial rise in calcium. The signal depended on extracellular calcium, demonstrating that it was due to Ca^{2+} entry. Slower cooling ramps (0.5°C/s) were less optimal stimuli for activating these rapidly adapting (RA) neurones: only 8 of 27 RA cells responded during such a ramp between 32 and 20°C. RA neurones were insensitive to agonists of known cold-activated ion channels. None of them was stimulated or sensitised by 100 μM menthol: instead, interestingly, they were slightly inhibited by it. They were also insensitive to 200 μM cinnamon aldehyde, a TRPA1 agonist, and to 50 μM icilin, an agonist for both TRPM8 and TRPA1. RA neurones were activated by pH 8 and ruthenium red (10 μM). Capsazepine, an antagonist of both TRPV1 and TRPM8, inhibited RA neurones at 50 μM. Our data provide evidence for the existence of a new type of cold receptor, distinct from the cloned TRPM8 and TRPA1.

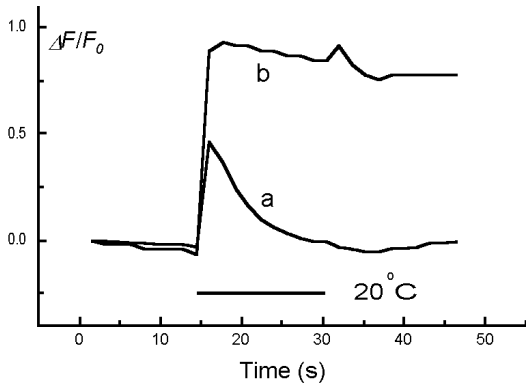

Fig. 1. A rapidly adapting (a) and a non-adapting response (b) to a fast cooling step from 32 to 20 C, with a duration of 10 s.

Babes et al. (2004). Eur J Neurosci 20, 2276-2282.

Reid et al. (2002). J Physiol 545, 595-614.

Thut et al. (2003). Neuroscience 119, 1071-1083.

Funding was from the Volkswagen Stiftung, the Physiological Society and the Romanian Research Council (CNCSIS).

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

C44

Distinct electrophysiological properties *in vivo* of C-fibre nociceptive DRG neurones that bind IB4 relate to the expression of Nav1.9 Na^+ channels in rat

X. Fang[1], L. Djouhri[1], S. McMullan[2], C. Berry[1], K. Okuse[3], S.G. Waxman[4] and S.N. Lawson[1]

[1]Department of Physiology, University of Bristol, Bristol, Avon, UK, [2]Hypertension & Stroke Research Laboratory, University of Sydney, Sydney, NSW, Australia, [3]Department of Biology, University College London, London, UK and [4]Department of Neurology, University of Yale, New Haven, CT, USA

Small-sized dorsal root ganglion (DRG) neurones have been divided into two subgroups: those that bind the isolectin B4 (IB4+) and those that do not (IB4-). The latter group tend to express trkA, the high affinity NGF receptor. We have previously shown that in rat strong IB4 binding is limited to a subgroup of C-fibre nociceptive-type (nociceptive and unresponsive) neurones. In mouse small DRG neurones isolated *in vitro*, IB4+ neurones had longer somatic action potential (AP) duration and higher densities of TTX-resistant Na^+ currents compared with IB4- neurones (Stucky & Lewin, 1999). We have examined whether this pattern holds for APs in C-fibre nociceptive-type DRG neurones *in vivo* in the rat and if so, whether it is related to expression of two TTX-resistant Na^+ channels Nav1.8 and Nav1.9.

We therefore made intracellular recordings *in vivo* in L4-L6 DRG neurones of adult female Wistar rats. The rats were deeply anaesthetized with sodium pentobarbitone (70-80mg/kg, i.p.) and neuromuscularly blocked with regular doses of pancuronium (0.6mg/kg, i.v.) always given with an additional dose (20mg/kg, i.v.) of anaesthetic. End-tidal CO_2 and blood pressure were monitored throughout. For each neurone, after recording the somatic AP shape, and recording sensory and electrophysiological properties, fluorescent dye was injected into the soma. The rats were killed with anaesthetic overdose and perfused through the heart with Zamboni's fixative. Immunostaining to show IB4 binding, Nav1.8 and Nav1.9 was carried out on different frozen sections (7μm) from the same dye-injected DRG neurones.

IB4+ C-fibre nociceptive-type neurones were compared with those that were IB4-. They had longer median somatic AP rise times (IB4+ 2.4ms, n=14; IB4- 1.4ms, n=4, $P<0.05$, Mann Whitney test) and lower median conduction velocities (0.38m/s, n=20; 0.59m/s, n=9, $P<0.05$). They also had a higher median relative staining intensity for Nav1.9 (67%, n=14; 19%, n=6, $P<0.05$), but not Nav1.8 (46%, n=13; 50%, n=6, $P>0.05$). This confirms that IB4+ C-fibre nociceptive-type neurones have distinct membrane properties from those that are IB4- and suggests that Nav1.9 may contribute to these distinct membrane properties.

Stucky CL & Lewin GR (1999). J.Neurosci 19, 6497-6505.

Supported by BBSRC and Wellcome grants to S.N.L. We thank L.F. Reichardt for the gift of anti-trkA antibody and C. Berry and B. Carruthers for technical assistance.

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

C45

Effect of opioid blockade on the human nociceptive flexion reflex and pain in patients with essential hypertension

L. Edwards[1], C. Ring[1], D. McIntyre[1], D. Carroll[1], C.R. France[2], M. al'Absi[4] and U. Martin[3]

[1]School of Sport and Exercise Sciences, University of Birmingham, Birmingham, UK, [2]Department of Psychology, Ohio University, Athens, OH, USA, [3]School of Medicine, University of Birmingham, Birmingham, UK and [4]Behavioral Sciences, University of Minnesota, Duluth, MN, USA

Hypertensive hypoalgesia may be explained by opioid insensitivity (McCubbin & Bruehl, 1994). Opioid antagonism may therefore be expected to have different effects on hypertensives and normotensives during pain assessment. Following ethics committee approval and in accord with the Declaration of Helsinki, this double-blind placebo-control study examined the effects of the opioid antagonist, naltrexone, on the nociceptive flexion reflex (NFR) threshold (Willer, 1977) and electrocutaneous pain tolerance, in 35 unmedicated hypertensive patients (14 female, 47 ± 14 years; mean ± s.d.) and 28 normotensive controls (16 female, 38 ± 13 years). They completed two 3-h sessions, 48 h apart, which began with ingestion of either 50 mg of naltrexone or placebo. The sural nerve was electrocutaneously stimulated to obtain an NFR threshold using an adaptive staircase procedure (Edwards et al. 2001). Pain tolerance was determined using an ascending method of limits. Sural nerve stimulation intensity was increased in 2 mA steps from 0 until participants rated the stimulation as maximum tolerable pain; they then completed a McGill pain questionnaire (Melzack, 1987). A series of 2 Group × 2 Sex × 2 Tablet ANCOVAs, with age as covariate, were performed. During placebo NFR thresholds were not significantly different between hypertensive (16.1 ± 10.5 mA) and normotensive (14.6 ± 7.6 mA) groups, whereas during naltrexone NFR thresholds were higher ($p<0.05$) in hypertensives (16.6 ± 10.5 mA) than normotensives (12.5 ± 5.6 mA). Opioid blockade significantly reduced NFR thresholds in normotensives but not hypertensives. During pain tolerance assessment, although stimulus intensity at maximal tolerable pain was higher, albeit not significantly, in hypertensives (29.4 ± 8.2 mA) than normotensives (27.1 ± 9.0 mA), the hypertensives rated this stimulus as less ($p<0.05$) painful (8.3 ± 4.7 arbitrary units (au)) than normotensives (12.0 ± 7.2 au). However, naltrexone had no significant effect on maximal pain tolerance in either group. The lower pain ratings in hypertensives during the maximal pain tolerance assessment agree with previous observations of hypertensive hypoalgesia. In conclusion, the decrease in NFR thresholds with naltrexone in normotensives but not hypertensives confirms that opioids play a role in dampening nociceptive responding in

healthy individuals and suggests that hypertensives are characterised by opioid insensitivity during noxious stimulation.

Edwards L et al. (2001). Psychophysiol 38, 712-718.

McCubbin JA & Bruehl S (1994). Pain 57, 63-67.

Melzack R (1987). Pain 30, 191-197.

Willer JC (1977). Pain 3, 69-80.

Research supported by grants from the NIH (HL64794) and BHF (FS/03/128).

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

C46

The effect of an A1 adenosine receptor agonist, GW684067, on capsaicin-induced secondary hypersensitivity in the dorsal horn of the anaesthetised rat: an electrophysiological study

J.C. Martindale[1], P.M. Headley[2] and I.P. Chessell[1]

[1]Pain, GSK, Harlow, Herts, UK and [2]Physiology, University of Bristol, Bristol, UK

The effects of the A1 agonist GW684067, (2R,3R,4S,5R)-5-ethynyl-2-[6-tetrahydro-2H-pyran-4-ylamino)-9H-purin-9-yl]tetrahydrofuran-3,4-diol) were assessed on the response of dorsal horn neurones sensitized by the intraplantar injection of capsaicin. Single unit recordings were made using multi-electrode arrays in the spinal cord of 12 Saffan-anaesthetized male CD rats 200-250g, from 59 neurones that responded to pinch of the hind paw receptive field (RF), recorded at depths of 444-1029 μm from the surface. Saffan was administered by intravenous perfusion (10 mg/kg/h). Each ml of Saffan contains 9 mg alfaxalone and 3 mg/kg alfadolone acetate. Baseline responses were evoked by mechanical stimulation (von Frey filaments, 1-164 g, 10s) to the RF before and after capsaicin injection (10 μg in 10μl) approximately 15 mm outside the RF. GW684067 or vehicle (saline) was administered either i.v. (0.1 mg/kg) or topically to the spinal cord (1.5 μg in 150 μl) immediately after capsaicin injection. Mechanical stimulation was continued every 15 min for 75 min. Counts of action potentials per stimulus were analysed by Student's paired t test.

Capsaicin caused a significant enhancement of responses to 164 g von Frey stimulation; following vehicle administration either topically or i.v., responses were increased by 414 ± 133% (n=16, 3 rats) and 307 ± 84% (n=14, 3 rats), respectively (mean ± s.e.m; $P<0.05$ vs pre-capsaicin control). In contrast, in the presence of GW684067 administered topically to the spinal cord, capsaicin inhibited any sensitisation compared to vehicle or baseline (29 ± 10% decrease compared to baseline, $P<0.02$, n=13, 3 rats). GW684067 i.v. also significantly reduced the sensitisation when compared to vehicle (16 ± 14% increase compared to baseline, $P<0.02$ vs vehicle control, n=16, 3 rats). GW684067 significantly inhibited the spontaneous firing rate of the neurones when administered either topically to the cord or i.v. (39 ± 4% and 38 ± 7% decrease; $P<0.05$ vs. pre-capsaicin baseline). Vehicle had no significant effect the spontaneous firing rate.

These data indicate that the adenosine A1 agonist GW684067, whether administered systemically or spinally, significantly reduced both the ongoing discharge of dorsal horn neurones and the secondary hypersensitivity induced by intraplantar capsaicin.

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

PC67

The chemokine fractalkine modulates nociceptive behaviour after injury to the peripheral nervous system

F.E. Holmes[1], N. Arnott[2], N. Isles[2], C.K. Thomas[1], C. Combadiere[3] and D. Wynick[1]

[1]LINE, University of Bristol, Bristol, UK, [2]Neurotargets, Bristol, UK and [3]INSERM, Paris, France

Fractalkine is the only member of the CX3C subgroup of chemokines. It exists as a trans-membrane protein that can be cleaved to release its chemokine domain. Fractalkine activates only one known receptor, CX3CR1, which has been shown to be present in the dorsal root ganglion (DRG). Using semi-quantitative RT-PCR analysis we have shown that CX3CR1 mRNA is present in the intact adult mouse DRG and that levels increase 5.8-fold at 1 week and 6.2-fold at 3 weeks after sciatic nerve axotomy. Immunohistochemistry of the DRG has shown that CX3CR1 and fractalkine proteins are present predominantly in glia and neurons respectively. These results suggested that fractalkine-CX3CR1 might be involved in the adaptive response of the PNS to injury, and thus may play a role in modulating nociception and/or neuronal regeneration after injury. To investigate the effect of fractalkine on pain-related behaviour, it was administered intra-sciatically into mice (1). Fractalkine had no effect on either thermal or mechano-sensory pain behaviours in intact mice. However, when administered at the time of spared nerve injury (SNI) surgery (a model of neuropathic pain) (2), it attenuated mechano-sensory allodynia. Intra-sciatic fractalkine also reduced nociceptive behaviour in response to intra-dermal formalin (3). Studies of CX3CR1 knockout and wildtype mice demonstrated that in naive animals the knockouts have lower withdrawal thresholds to both thermal and mechanical stimulation. Furthermore, following SNI (2), the knockouts developed more allodynia than wildtypes. These results imply that CX3CR1 activation by fractalkine has an anti-nociceptive role after nerve injury when expression of the receptor is markedly up-regulated. Before surgery, animals were anaesthetised with Hypnorm:Hypnovel:water at a ratio of 1:1:2 and a dose of 5 µl/g. Animals were killed humanely 2 weeks after surgery.

Thompson SW *et al.* (1997). *Eur J Neurosci* **9**, 1244-12251.

Holmes FE *et al.* (2003). *Proc Natl Acad Sci USA* **100**, 6180-6185.

Sufka KJ *et al.* (1998). *Eur J Pain* **2**, 351-358.

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

PC69

The effect of a P2X2/3 antagonist A-317491 on dorsal horn neuronal responses following chronic constriction injury of the sciatic nerve in the anaesthetised rat

C.J. Sharp, S.D. Collins, J.C. Martindale, R.S. Sargent, A.J. Reeve and I.P. Chessell

Neurology and GI CEDD, GlaxoSmithKline, Harlow, Essex, UK

The effects of the P2X2/3 antagonist A-317491 (Jarvis et al., 2002) were assessed on the response of dorsal horn neurones in chronic constriction injury (CCI) operated and control rats. Single unit recordings were made using multi-electrode arrays from second order dorsal horn neurones in the spinal cord of 26 saffan-anaesthetized male Random Hooded rats (150-270g), at depths of 1036.9 ± 40.5µm from the surface of the spinal cord. In CCI-operated animals, electrophysiology was carried out 15-29 days post-surgery. Baseline responses were evoked by electrical stimulation (20 pulses, 0.5Hz; 2ms, 6mA) and mechanical stimulation (9 von Frey Filaments, force 1-165g, 10 second application per filament) of the hind paw. A-317491, or vehicle (1% DMSO, 66% PEG200, 33% saline), was administered i.v. (1, 3 and 10mg/kg). Electrical and mechanical stimulation were applied every 15 min for 45 min following each dose. Counts of action potentials per stimulus were analysed by Student's unpaired t-test. Data is presented as mean ± sem. A P value of <0.05 was taken as significant.

A-317491 had no effect on the Aβ-fibre-evoked responses in both control and CCI operated rats (naive n=30, 6 rats; CCI n=30, 6 rats). The Aδ-fibre-evoked responses were significantly inhibited compared to vehicle control at 3 ($P<0.05$) and 10mg/kg ($P<0.01$) in both naive (n=30) and CCI (n=28) rats. In naive animals, the C-fibre-evoked response was inhibited by 24.2 ± 8.6% at 10mg/kg (n=28; $P<0.05$ vs. time-matched vehicle controls); in CCI-operated rats, the C-fibre evoked response to electrical stimulation following A-317491 was significantly inhibited at both 3 (51.3 ± 7.9%) and 10mg/kg (74.0 ± 4.6%; $P<0.001$ vs. vehicle control; n=18), and wind-up was significantly reduced by 39.2 ± 19.3% at 3mg/kg ($P<0.05$ vs. vehicle control, n=12). Spontaneous activity was significantly inhibited by 25.9 ± 10.3% and 35.6 ± 9.4% at 3 and 10mg/kg respectively in CCI rats ($P<0.05$ vs. vehicle control, n=30). There was no inhibitory effect on responses to mechanical stimulation in either control or neuropathic animals.

These data indicate an increased involvement of the P2X2/3 receptor following chronic constriction injury of the sciatic nerve, thus suggesting a role for P2X2/3 receptors in the modulation of neuropathic pain.

Jarvis MF et al. (2002). PNAS 99, 17179-17184.

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

PC70

Selective expression within dorsal root ganglion (DRG) neurones of the α3 Na^+/K^+ ATPase isoform by muscle spindle afferents

A.J. Campbell[1], A. Parekh[1], L. Djouhri[1], X. Fang[1], S. McMullan[2], C. Berry[1], B. Carruthers[1] and S.N. Lawson[1]

[1]Physiology, University of Bristol, Bristol, UK and [2]Hypertension & Stroke Research Laboratory, University of Sydney, Sydney, NSW, Australia

The α3 isoform of the Na^+/K^+ ATPase is selectively expressed in a subpopulation of large diameter primary afferent neurones and in fibres that project to muscle spindles (Dobretsov et al. 2003). In the present study we examined immunocytochemically the distribution of this isoform in dorsal root ganglion (DRG) neurones classified according to sensory receptor properties and conduction velocity in deeply anaesthetised rats as

previously described (Lawson et al. 1997). α3-like immunoreactivity was examined with an affinity purified polyclonal antibody, previously fully characterised with Western blotting and causing staining patterns similar to other anti-α3 antibodies.
Young adult female Wistar rats were deeply anaesthetized throughout the experiments with sodium pentobarbitone (70-80mg/kg, i.p.) and during recording, regular doses of pancuronium (0.6mg/kg, i.v.) were given always with additional (20mg/kg, i.v.) anaesthetic. End-tidal CO_2 and blood pressure were monitored throughout. Intracellular recordings were made in 81 L4-L6 DRG neurones with dye-filled glass microelectrodes. For each neurone, after identification of sensory and electrophysiological properties, the fluorescent dye was electrophoretically injected into the neuronal soma. Rats were killed with an anaesthetic overdose and perfused with Zamboni's fixative. DRGs were post-fixed and frozen sections (7 μm) cut. ABC immunostaining for α3 on dye-injected DRG neurones enabled measurement of relative staining intensity (percentage maximum staining in that section). In addition, double-label fluorescence immunocytochemistry was carried out on lumbar DRGs from 4 rats, with antibodies to neurofilament (RT97) and α3. Muscle spindle afferents (MSAs) did not have cutaneous receptive fields, usually showed ongoing firing resulting from stretch of the leg and presence of muscle relaxant, showed altered firing rates with gentle pressure on muscle or altered leg position and followed a rapid vibratory stimulus. Of the Aα/β-fibre neurones examined, all 17 MSAs stained positively for α3, with dense staining over the cell membrane - appearing as a stained 'ring' around the cell. Furthermore, in MSAs stained positively for α3, there were significant correlations ($P<0.05$) between relative ring staining intensity and a) membrane potential magnitude (positive correlation, $r^2=0.48$), and b) action potential fall time and duration at base (negative correlations, $r^2= 0.38$ and 0.52, respectively). In contrast, the ring staining was low (weakly stained or absent) in other Aα/β-fibre groups (nociceptive units and cutaneous low threshold slowly and rapidly adapting units). The absence of detectable ring staining seen in 14/15 slowly conducting afferents studied (i.e. those with Aδ- or C-fibres), was consistent with the lack of clear α3 membrane staining in most neurones with neurofilament-poor cytoplasm. Thus the α3 Na^+/K^+ ATPase isoform is highly expressed in MSAs and may contribute to their functional properties.

Dobretsov M, Hastings SL, Sims TJ, Stimers JR & Romanovsky D (2003). Neurosci 116, 1069-1080.

Lawson SN, Crepps BA & Perl ER (1997). J Physiol 505, 177-191.

Supported by the Welcome Trust and BBSRC grants to SNL.

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

PC71

Presynaptic depression by metabotropic glutamate receptor agonists in rat somatosensory cortex as assessed by intracellular recordings *in vivo*

P.M. Cahusac

Department of Psychology, University of Stirling, Stirling, UK

It was previously shown that the metabotropic glutamate (mGlu) receptor agonist 1S,3R-ACPD had a variety of actions in rat somatosensory cortex, prominent among them was a depression of synaptic responses (Cahusac, 1994). The present study used intracellular recordings to determine whether the depression was due to an action at pre- or postsynaptic receptors.
Male Wistar-derived rats (N = 5, 346 - 567g) were anaesthetized with urethane (2g/kg I.P.). At the end of the experiment they were humanely killed by anaesthetic overdose. Intracellular recordings were made from cortical neurones using pipettes filled with 1.6M K citrate. The recording pipette was glued to a 7-barrelled iontophoresis pipette such that the recording pipette protruded 60 μm ahead of the iontophoresis pipette tip. Different iontophoresis barrels contained one of the following: monosodium L-glutamate (0.5 M, pH 8.5), (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD, 0.1 M, pH 8), (RS)-4-Carboxy-3-hydroxyphenylglycine (4C3HPG, 0.1 M, pH 8.5), α-methyl-(2S,3S,4S)-2-(Carboxycyclopropyl)glycine (MCCG, 0.05 M, pH 8.8), GABA (0.5 M, pH 3.5), Pontamine Sky Blue dye (2% in 0.5 M Na acetate) and 1 M NaCl (for current balancing and current controls). Stimuli consisted of brief (3 - 10 ms) deflections of selected vibrissae by piezoelectric bimorphs.
A total of 7 cells were recorded from for sufficient time to evaluate the effects of the agonists. In 6 of 7 cells tested, synaptic transmission was depressed by mGlu agonists. This was associated with complete abolition of EPSPs, suggestive of a presynaptic action. Typically, there were no marked changes in membrane potential (mean before = -53.8±2.45 mV, mean during drug = -54.5±2.98 mV, mean change = 0.70±0.65 mV, $t(3) = 1.07$, $P = 0.365$). In contrast when tested with GABA, synaptic depression was associated with the continued presence of EPSPs, indicating a postsynaptic action. The depressant effect produced by 1S,3R-ACPD was reversibly antagonised by the Group II mGlu receptor antagonist MCCG.
These results suggest that the depression of synaptic transmission by mGlu receptor agonists is due to an action at Group II mGlu receptors situated on presynaptic terminals (Schoepp, 2001).

Cahusac PMB (1994). Eur J Neurosci **6**, 1505-1511.

Schoepp DD (2001). J Pharmacol Exp Ther **299**, 12-20.

Supported by grants from the BBSRC (GR/H05739) and the MRC (G9021772N).

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

PC73

TTX-blocked sensory C-fibre terminals regain responsiveness upon cooling in the isolated rat skin-nerve preparation

K. Zimmermann[1], J. Kobayashi[2], R.W. Carr[1] and P.W. Reeh[1]

[1]Dept. of Physiology and Pathophysiology, University Erlangen-Nuremberg, Erlangen, Germany and [2]Dept. of Fixed Prosthodontics, Kyushu University, Fukuoka, Japan

Studying TTX-resistance in cutaneous receptive fields we discovered the phenomenon described in the title first in mechano-cold sensititve C-fibres (CMC). We extended the study including mechano-heat sensitive (CMH) and mechanosensitive fibres (CM) (obtained from humanely killed animals) using controlled electrical stimulation to assess excitability. The most sensitive spot in the receptive field (threshold current <50μA at 1ms) was electrically stimulated with a high impedance (9-12 MΩ) needle electrode and a constant-current stimulus isolator (max.

10mA) varying stimulus intensity and duration. In all fibre classes the terminals became less excitable with cooling, thus higher currents were needed to evoke action potentials, occuring at longer latencies. These effects were readily explained by the established influence of temperature on the kinetics of TTX-sensitive sodium channels (1). There was no consistent change in rheobase current; however, with cooling from 30° to 10°C strength-duration (S-D) time constant (2) increased, most distinctively in cold-sensitive units (6.2-fold ±2.8 S.E.M., n=4; other units: 1.5-fold ±0.4 S.E.M., n=11).

To apply TTX (1μM) and restrict its diffusion, the receptive field was covered with a cylindrical superfusion chamber (outer diameter 15mm) sparing a small hole (1.5mm) in the bottom. All CMC-units tested were blocked at 30°C within ≤2min after TTX application; the maximal current was insufficient to evoke action potentials. Nonetheless, when cooled down to 10°C the fibres started firing below their previously established threshold temperature (15-23°C) and clearly responded to mechanical and to electrical stimulation. However, threshold currents were largely increased (2-20-fold) and latencies prolonged by 2-8% in comparison to pre-TTX-values at 10°C. The latter phenomena also occurred upon cooling in some of the CM- and CMH-fibres that had previously been blocked by TTX at 30°C. However, the majority of the CMH-fibres were blocked only after a 30-40min exposure time and increasing TTX concentration to 6-12.5μM.

We suggest that the terminals cooling manoeuvre uncovered an, at least latent, capacity of nociceptive C-fibre terminals to generate action potentials in the presence of TTX. The large increase in S-D time constant during cooling (in cold-sensitive fibres) probably reflects an increase in membrane ('input') resistance such that generator potentials created by cold (3)- and mechanically activated (4) inward currents can reach the high threshold of TTX-resistant sodium channels and trigger (slowly propagated) action potentials. If these action potentials prove to rely on TTX-resistant sodium channels, our findings extend the possibilities to examine the pharmacology of TTX resistance in sensory nerve endings under physiological conditions.

Hille B (2001). Ion Channels of Excitable Membranes, 3rd ed. Sinauer Associates, Sunderland.

Bostock H (1983). J Physiol 341, 59-74.

Reid G & Flonta ML (2001). Nature 413, 480.

Drew LJ, Wood JN & Cesare P (2002). J Neurosci 22, 1-5.

We are grateful for valuable discussions with Oleg Krishtal (Bogomoletz Institute, Kiew, Ukraine).

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

PC74

The increase in cold sensitivity in rat dorsal root ganglion (DRG) neurones induced by nerve growth factor (NGF) is mediated by the high affinity NGF receptor

R. Linte, A. Babes and G. Reid

Dept. of Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, Bucharest, Romania

The neurotrophin NGF increases cold sensitivity of cultured rat DRG neurones, primarily through an effect on cold- and menthol-sensitive cells (Reid et al. 2002; Babes et al. 2004). Two NGF receptors with different affinities for NGF have been characterised so far: the low affinity receptor (Kd = 1 nM) identified as the non-specific neurotrophin receptor p75, and the high affinity receptor (Kd = 10 pM), composed of both p75 and the tyrosine kinase NGF receptor trkA (Mahadeo et al. 1994). The aim of this work was to identify which of the two receptors is involved in sensitising DRG neurones to cold.

Wistar rats were humanely killed. Primary DRG cultures were split in four groups and 7S NGF was added to the four fractions as follows: no NGF (control), 1 ng/ml (7.7 pM), 10 ng/ml (77 pM) and 200 ng/ml (1.5 nM). Cells were stimulated by cooling from 32 to 17 °C with a Peltier based temperature controlled application system, and changes in $[Ca^{2+}]_i$ were monitored using Calcium Green-1 fluorimetry (Reid et al., 2002). The following parameters were measured: fraction of menthol-sensitive (MS) neurones, amplitude and temperature threshold of the cold response, and sensitivity to capsaicin (CAP) and cinnamon aldehyde (CA). Data are presented as mean ± SD. χ^2 test and Student's *t* test were used to determine statistical significance.

NGF increased the fraction of MS cells in days 2 and 3 after the culture (14.3% for 7.7 pM NGF compared to 4.5% for control, in day 2, $p < 0.001$). The effect was significant for all three NGF concentrations tested in day 2 and for the two higher concentrations in day 3. The amplitudes of the calcium signals induced by cooling were not different in the four groups, but the threshold temperatures were significantly different from control for all three groups with added NGF (28.4 ± 2.7 °C for 7.7 pM NGF, $n = 31$, compared to 25.3 ± 3.5 °C for control, $n = 19$, $p = 0.001$). CAP sensitivity of MS neurones was increased by 77 pM NGF from 57% to 80% while, interestingly, co-expression of menthol and CA sensitivity was significantly decreased by addition of 77 pM NGF, from 58% to 39% ($p = 0.01$).

We conclude that the sensitising effects of NGF on MS neurones in rat DRG cultures and the degree of co-expression with CAP and CA sensitivities are all mediated primarily by the high affinity NGF receptor.

Babes et al. (2004). Eur J Neurosci 20, 2276-2282.

Mahadeo et al. (1994). J Biol Chem 269, 6884-6891.

Reid et al. (2002). J Physiol 545, 595-614.

Funding was from the Volkswagen Stiftung, the Physiological Society and the Romanian Research Council (CNCSIS).

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

PC75

Cannabinoid-mediated attenuation of network activity within the substantia gelatinosa of the rat spinal cord *in vitro*

P. Cilia La Corte[1], A.U. Asghar[2] and A.E. King[1]

[1]School of Biomedical Sciences, University of Leeds, Leeds, UK and [2]Department of Biological Sciences and Hull York Medical School, University of Hull, Hull, UK

Network behaviour within dorsal horn *in vivo* is implicated in spinal nociceptive signalling (Sandkühler & Eblen-Zajjur, 1994). In substantia gelatinosa (SG) of spinal dorsal horn *in vitro*, rhythmicity can manifest in the 4–12 Hz frequency band and is

dependent upon chemical and electrical neurotransmission (Asghar *et al.* 2005). The aim of this study was to investigate modulation of network activity within the SG by drugs that selectively target cannabinoid (CB) receptors. Wistar rats (12–15 days) were terminally anaesthetised with urethane (2 g/kg i.p.). Transverse lumbar spinal slices (300 μm) were cut and field recordings were made from SG. Transient pressure ejection of potassium (KCH_3SO_4, 1.5 M) evoked oscillations with a peak frequency of 7.8 ± 0.1 Hz (mean ± S.E.M.) and a duration of 12.4 ± 2.0 s (n=24). Perfusion of the broad–spectrum cannabinoid agonist CP55940 (1 μM, n=6) significantly attenuated ($P < 0.05$, paired *t* test) the power area (by 52 ± 8%) and power amplitude (by 58 ± 8%) of the rhythm. This cannabinoid inhibitory effect was reversed after drug removal. The CB1 specific receptor agonist ACEA (20 nM, n=6) caused a significant decrease ($P < 0.05$) in the power area (by 44 ± 8%) and amplitude (by 60 ± 8%) of the network activity. AM251 (1 μM, n=4), a CB1 receptor antagonist, had no significant effect on the power area or amplitude when tested alone ($P > 0.05$). However, AM251 (n=7) abolished the ACEA–induced inhibition of the rhythm (power area by 14 ± 7% and power amplitude by −12 ± 10%, $P > 0.05$). The results of this study demonstrate an inhibition of dorsal horn rhythmicity upon cannabinoid agonist application. These pharmacological data suggest that this modulation may be attributable to CB1 receptors that are localized to the dorsal horn (Farquhar-Smith *et al.* 2000). Further studies are required to clarify whether CB1-induced modulation of rhythmic behaviour is linked to the established anti-nociceptive role of cannabinoids in the SG.

Asghar AUR *et al.* (2005). *J Physiol* **562**, 183–198.

Farquhar-Smith WP *et al.* (2000). *Mol Cell Neurosci* **15**, 510–521.

Sandkühler J & Eblen–Zajjur AA (1994). *Neuroscience* **61**, 991–1006.

Supported by The Wellcome Trust. PFCLC is in receipt of a BBSRC PhD studentship.

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

PC76

The role of spinal and supraspinal NMDA NR2B receptors on inhibiting wide dynamic range (WDR) responses in anaesthetised rats with complete Freund's adjuvant (CFA) inflammation

M.J. Sammons and A.K. Houghton

Pharmacology, Organon Laboratories, Motherwell, UK

NMDA NR2B-containing receptor subtype are antinociceptive in preclinical models and lack effects on motor function, suggesting these compounds will have a better side-effect profile in man, compared to ketamine, a non subunit selective antagonist. Pharmaceutical companies are therefore developing selective NMDA NR2B-containing receptor subtype compounds. The activity of these selective NR2B compounds has been reported to be mediated only in the brain (Chizh et al. 2001). If so, preclinical testing cannot rely solely on withdrawal reflex tests. The aim of this study was to identify the site of action of the selective NR2B compound Ro-25,6981 in modulating noxious heat responses of WDR neurones in rats with inflammation.

Male Wistar rats (180-225g) with an intraplantar injection of CFA into one hind paw (100μl/paw); under brief isoflurane (2-3% in O_2) anaesthesia), were anaesthetised with isoflurane (2-3% in O_2). The trachea, two jugular veins and a carotid artery were cannulated for administration of isoflurane, α-chloralose, compounds and measurement of blood pressure, respectively. L4-L6 spinal cord was exposed in all animals for electrophysiological recording; in some animals T12 spinal cord was exposed and the cord transected. Once surgery was completed animals were transferred to α-chloralose anaesthesia (300mg/kg bolus then 100mg/kg/h infusion). Extracellular recordings of isolated neurones were made using tungsten microelectrodes (1.8-2.2Ohms); a peltier device was used to apply noxious heat (50-55°C) to the peripheral receptive field every 4min and responses recorded. Increasing doses of Ro-25,6981 and ketamine were administered to a maximum dose of 100μmol/kg and 120μmol/kg, respectively. Experiments were terminated with an overdose of pentobarbitone. ED_{50} values were calculated using XL fit software.

Ro-25,6981 (3.125-50μmol/kg; i.v.) inhibited noxious heat responses in animals with an intact spinal cord, with an ED_{50} value of 17.9μmol/kg (n=6); responses were reduced from baseline values of 25.3±3.4 to 10.3±2.5 spikes/s, after administration of a mean dose of 17.7±7.1μmol/kg. In contrast, Ro-25,6981 (6.25-100μmol/kg; iv) had no effect (n=6) in animals with a transected spinal cord. Ketamine was active; the ED_{50} values were 68 and 60μmol/kg in the intact and transected spinal cord preparation, respectively.

The present results show that the spinal cord needs to be intact to observe an antinociceptive effect of Ro-25,6981 suggesting that the brain plays a key role in mediating the antinociception of NR2B-containing receptor antagonists in rats with inflammation. This is in contrast to the non-subunit selective antagonist ketamine, which inhibited WDR responses with similar potency in both preparations.

Chizh BA et al. (2001). Neuropharmacology 40, 212-20.

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

PC77

Use of adenoviral vectors to map the pontospinal noradrenergic projection to the lumbar dorsal horn of rat

P.W. Howorth[1], A.G. Teschemacher[2], T. Lonergan[1], J.F. Paton[1], S. Kasparov[1] and A.E. Pickering[3]

[1]Physiology, University of Bristol, Bristol, UK, [2]Pharmacology, University of Bristol, Bristol, UK and [3]Anaesthesia, University of Bristol, Bristol, UK

Noradrenergic (NA) neurons in the pons that project to the dorsal horn are thought to form an important antinociceptive control system (Milan, 2002). These NA neurones are distributed across three areas A5, A6 and A7, close to vital brainstem centres. Hence it has been difficult to study the role of this descending NA system in isolation. We have used an ade-

noviral vector (Ad-PRS) containing a synthetic promoter element (Hwang *et al.* 2001) that is specifically active in catecholaminergic neurones (Lonergan *et al.* 2005) to retrogradely target NA neurones that project to the lumbar dorsal horn. We used Ad-PRS transfection to express enhanced green fluorescent protein (eGFP) or monomeric red fluorescent protein (mRFP) and thus mapped the anatomical organisation of the NA system.

Wistar rats (130-200g) were anaesthetised (ketamine 5mg/100g and medetomidine 30μg/100g i.m.) and 4-6 injections of Ad-PRS (500nl of 10^8–10^{10} plaque forming units/ml) were made into the lumbar dorsal horn (L4-5, 400μm lateral from midline, 500μm deep to dorsal surface). Animals were killed 1 to 14 days later with an overdose of pentobarbital, perfused with 4% formaldehyde in 0.1M phosphate buffer and the brainstem and spinal cord were removed. Fluorescence immunocytochemistry was undertaken for adenovirus hexon protein and dopamine β hydroxylase (DBH). Neuronal counts from day 7 post injection are reported as mean ± S.E.M.

Adenoviral hexon staining showed that the viral transfection at the injection site was confined to the dorsal horn. Retrogradely labelled neurones (127 ± 23, n=8) were only seen in the pontine A5, A6 and A7 areas. DBH staining showed that 95% of all retrogradely labelled neurones were noradrenergic. The majority of labelled cells were found in A6 (80%) with the remainder in A5 (15%) and A7 (5%). This retrograde labelling was seen as early as 1 day after lumbar injection. Unilateral spinal injection showed a predominantly ipsi- but also contralateral projection from A6. Indeed 4% of labelled A6 neurones were shown to project bilaterally to the lumbar dorsal horn using combined injections of eGFP and mRFP expressing vectors. We have demonstrated the use of an adenovirus to selectively target noradrenergic neurones projecting to the dorsal horn using the retrograde transport of the viral vectors by axonal terminals. Such adenoviral transfection strategy provides a powerful tool to explore the function of the noradrenergic system in nociceptive processing by the selective expression of genetic constructs.

Millan MJ (2002). Prog Neurobiol 66, 355-474.

Hwang DY et al. (2001). Hum Gene Ther 12, 1731-40.

Lonergan T et al. (2005). Physiological Genomics 20, 165-72.

AEP is a Wellcome Trust Advanced Fellow. AGT is a British Heart Foundation Research Fellow.

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

PC78

Neurochemical properties of neurones activated by C- or Aδ-nociceptors in the superficial dorsal horn of the spinal cord

A. Reid[1], T.T. Liu[1], D.J. Maxwell[1], S. Koutsikou[2], D.M. Parry[2] and B.M. Lumb[2]

[1]Institute of Biomedical & Life Science, University of Glasgow, Glasgow, UK and [2]Physiology, University of Bristol, Bristol, UK

Nociceptors in the C or Aδ conduction velocity range are likely to mediate slow and fast components of pain respectively. The aim of the present study was to investigate the properties of spinal neurones that are selectively activated by these two classes of afferent. Four Wistar rats were anaesthetised (pentobarbitone 31 mg kg^{-1} h^{-1} i.v.) and slow (2.5°C s^{-1}; n=2) or fast (7.5°C s^{-1}; n=2) ramps (30-55 or 57°C, respectively) of contact heat applied (six times in each animal) to the dorsal surface of the left hind paw to preferentially activate C- or Aδ- nociceptors, respectively (McMullan et al. 2004). Two hours later, anaesthesia was deepened and the animals were perfusion fixed. Transverse sections were cut from midlumbar segments and reacted immunochemically to visualise Fos protein in cells activated by the heat stimuli. Both types of stimuli activated clusters of cells in laminae I-II of the ipsilateral mid-dorsal horn. Sections containing Fos were reacted immunochemically for neurochemical markers that are associated with either excitatory (the neurokinin-1 receptor (NK-1), protein kinase Cγ (PKCγ), calbindin (Cal), the μ opioid receptor 1 (MOR-1)) or inhibitory (nitric oxide synthase, parvalbumin and choline acetyltransferase) spinal cord neurons (Todd & Spike, 1993; Kemp et al. 1996). A quantitative confocal microscopic study was performed to determine which of the markers was associated with Fos. None of the markers for inhibitory cells contained Fos but it was present in cells labelled with all the markers for excitatory cells in both fast (F) and slow (S) ramp experiments (NK-1, F=31% (32/102 Fos cells), S=21% (83/398 Fos cells); PKCγ, F = 23% (24/102 Fos cells), S=15% (59/398 Fos cells); Cal, F=28% (107/382 Fos cells), S=24% (204/849 Fos cells); MOR-1, F=10% (28/292 Fos cells), S=7% (62/909 Fos cells)).

The results suggest that both C and Aδ fibres activate excitatory neurons in the dorsal horn which are likely to include interneurons and projection cells. Further studies are in progress to determine if the number of cells activated by the two rates of skin heating differ significantly.

Kemp T, Spike RC, Watt C & Todd AJ (1996). Neurosci 75, 1231-1238.

McMullan S, Simpson DAA & Lumb BM (2004). J. Neurosci Methods 138, 133-139.

Todd AJ & Spike RC (1993). Prog Neurobiol 41, 609-638.

Supported by The Wellcome Trust, and University of Glasgow funds.

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

PC79

Peripheral vs central efficacy of NMDA receptor/glycine$_B$ site receptor antagonists

M. Sladek[2], C.G. Parsons[2] and P.M. Headley[1]

[1]Physiology, University of Bristol, Bristol BS8 1TD, UK and [2]Pharmacology, Merz Pharmaceuticals GmbH, D60318 Frankfurt am Main, Germany

NMDA receptors are present not only in the CNS but also in the periphery, and there is growing evidence for their involvement in visceral nociceptive processing. For example, the glycine$_B$ selective antagonists MRZ 2/576 (brain permeant) and MRZ 2/596 (brain impermeant (Danysz & Parsons, 1998) were both anti-allodynic in a rat model of Irritable Bowel Syndrome (IBS); the delayed rectal allodynia (10 h, 0.4 ml distension) induced by

LPS (1 mg/kg i.p.) was abrogated by MRZ 2/576 and MRZ 2/596 at 0.1 mg/kg by 79.7 and 52.6%, respectively (Sladek et al. 2002). In the current work, any preparative surgery was done under sodium pentobarbitone anaesthesia (60 mg/kg i.p); at the end of the experiments animals were humanely killed, as were all donor animals.

Brain penetration was examined in an in vitro model of the blood-brain barrier (BBB) using bovine brain capillary endothelial cells co-cultured with rat cortical astrocytes (Danysz et al. 2005). MRZ 2/596 showed a permeability coefficient similar to that of the BBB-impermeant marker sucrose, 1.11 ± 0.05 (SD) x 10^{-5} cm/s and 0.77 ± 0.02 x 10^{-5} cm/s, respectively. On the other hand, MRZ 2/576 showed a medium BBB permeability in vitro (pe-coefficient 34.56 ± 4.42 x 10^{-5} cm/s).

These in vitro data are consistent with brain microdialysis studies in awake rats (5 days after probe implantation), where MRZ 2/596 and MRZ 2/576 at the very high dose of 30 mg/kg i.p. generated maximal concentrations in brain of 0.11 ± 0.05 μM (S.E.M.) (n=3) and 1.34 ± 0.43 μM (n=5), respectively.

Central and peripheral NMDA receptor properties have also been compared in patch-clamp experiments. The IC_{50} rank order of steady-state inward current responses of cultured rat hippocampal neurons (Danysz et al. 2005) to NMDA (200 μM with glycine 10 μM) were memantine (NMDA receptor channel blocker) > MRZ 2/576 > MRZ 2/596 (n = 4-8). In contrast, in cultures of dorsal root ganglia (DRG) from adult rats, NMDA activated currents were antagonized by memantine, MRZ 2/576 and MRZ 2/596 with similar IC_{50}s of 1 μM (n=7-11).

Visceral DRG neurons were identified by retrograde labelling with Fast DiI-oil (Molecular Probes) injected into the muscle wall of the small intestine 10-14 days before cell preparation. The NR1 and all NR2 subunits were expressed as shown by Western blotting, immunostaining, and RT-PCR of both DRG cultures and cryosections (cf. Marvizon et al. 2002). However, for all NR1/NR2 subunits examined, no difference in the expression pattern was detected that might explain the different glycine-related pharmacology in DRG cells in comparison to the CNS.

Danysz et al. (2005) Neuropharma 48, 360-371.

Danysz W & Parsons CG (1998). Pharmacol Rev 50, 597-664.

Marvizon JC et al. (2002). J Comp Neurol 446, 325-341.

Sladek et al. (2002). Soc Neurosci Abst 28, 451.3.

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