C30

Cinnamaldehyde sensitises and activates rat primary afferent neurons *in vivo* and *in vitro*

J. Dunham, H. Kennedy and L. Donaldson

Department of Physiology, University of Bristol, Bristol, UK

Cinnamaldehyde (cinn) is a selective agonist at the Transient Receptor Potential A1 Receptor (TRPA1) that evokes pain in humans (Namer et al. 2005) and nociceptive behaviour in rodents (Bandell et al. 2004). We have determined the effect of cinn on single mechanosensitive primary afferent units *in vivo*, and on spontaneous activity in dissociated rat dorsal root ganglion (DRG) neurones.

Recordings were made from single cutaneous mechanosensitive fibres from teased filaments of the saphenous nerve, in deeply anaesthetised male Wistar rats (250-350g, sodium pentobarbital ~20mg kg^{-1} h^{-1} i.v.). Thresholds for activation were determined using receptive field stimulation with graded von Frey hairs, and units were classified as either low or high threshold mechanoreceptors (LTM or HTM) on their baseline responses. Responses to von Frey stimulation were recorded after application of vehicle (DMSO) and cinn (10%) directly to the receptive field. In separate experiments, cinn was administered by close arterial injection (C.A.I.) through a femoral arterial cannula. For *in vitro* study, DRG were isolated and plated on collagen-coated glass coverslips and incubated in F12 medium until used in experiments. DRG were used on days 3–4 of culture. All data shown are median ± semi-interquartile range.

Topical cinn increased the evoked activity in 10 of 19 mechanically sensitive units, representing 9/15 HTMs and 1/4 LTMs. In these units cinn decreased mechanical thresholds compared to vehicle (120 ± 86g DMSO vs. 6 ± 13g cinn, $p<0.05$, Wilcoxon signed rank test). In the same units, cinn also increased the rate of spontaneous firing (0.03 ± 0.08 Hz veh. vs. 0.12 ± 0.2 Hz cinn, $p<0.01$, Wilcoxon) and the response evoked by suprathreshold noxious mechanical stimulation (0.1 ± 0.45 Hz veh. vs. 0.6 ± 1.7 Hz cinn, $p<0.01$, Wilcoxon).

C.A.I. cinn (0-80mM) resulted in a dose-related transient increase in action potentials (AP) in the 60 s after cinn administration, in a population of predominately HTMs (0 + 0.9 veh vs. 3.5 ± 6.8 AP cinn, $p<0.05$ Kruskal Wallis test + Dunns). In 4 cinn responsive units, the cinn-evoked response was unaffected by capsaizepine (20µg/100µl), suggesting that altered activity was not attributable to TRPV1 activation. In isolated DRG neurons, bath application of cinnamaldehyde (250µM) evoked trains of action potentials in a population of small DRG neurons.

Our data suggest that activation of TRPA1 receptors both directly activates, and sensitises primary afferent nociceptors to mechanical stimulation. TRPA1 receptors may play a role in mechanonociception and contribute to the development of peripheral hyperalgesia.

Bandell M et al. (2004). Neuron 41, 849-857.

Namer B et al. (2005). NeuroReport 16, 955-959.

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

C31

Antidromic activity in sensory nerves contralateral to an inflamed rat knee joint may contribute to the symmetrical spread of arthritis

S. Kelly, J.P. Dunham and L.F. Donaldson

Physiology, University of Bristol, Bristol, UK

Rheumatoid arthritis is a symmetrical disease where inflammation spreads from one joint to involve the mirror image joint, a phenomenon also seen in animal models of arthritis (Donaldson et al. 1993). Clinical evidence from patients (Veale et al. 1993), and from experimental studies (Donaldson et al. 1995; Kidd et al. 1995), suggests that a neural mechanism contributes to the spread of arthritis.

We investigated whether there are changes in antidromic neuronal activity in the homologous nerve contralateral to that innervating an inflamed joint, which could account for the spread of arthritis through a neurogenic mechanism. Complete Freund's Adjuvant (CFA; 250µg/100µl) was injected intra-articularly into the right knee joint of male Wistar rats (n=20) under halothane anaesthesia (3% in O_2). Intra-articular injection of CFA resulted in significant swelling of the injected joint ($p<0.001$, vs contralateral, paired t test). 3-5 days post-CFA, rats (n=16) were re-anaesthetised (60mg/kg pentobarbital i.p.) and the external jugular vein and trachea cannulated. Extracellular recordings were made from teased filaments of the left saphenous nerve, contralateral to the inflamed knee joint, sectioned distally. Data are means ± SEM.

The frequency of centrally generated spontaneous efferent activity recorded from the contralateral saphenous nerve was significantly higher in rats with CFA-induced inflammation (2.9 ± 1Hz, n=16) than that seen in control non-injected rats (0.1 ± 0.05Hz, n=5; $p<0.01$, Mann Whitney). Capsaicin (2%) applied directly onto the nerve proximal to the recording site in CFA rats (n=6) significantly reduced activity levels (0.2 ± 0.1Hz, 1-way ANOVA) indicating that the recorded activity was predominantly in VR1 expressing sensory neurons. In a separate set of experiments, CFA-inflamed rats (4 days) and control rats (n=4 each group) received an injection of Evan's Blue dye (50mg/kg i.v.) under pentobarbital anaesthesia. Following removal of knee joints and formamide extraction, Evan's Blue dye extravasation (measured spectrophotomically) was significantly higher in contralateral knee joints of CFA rats than in the knee joints of control rats ($p<0.01$, 1-way ANOVA). These data indicate that vascular changes had occurred in the uninvolved joint, contralateral to the arthritis.

Here we report that a unilateral arthritis results in the generation of contralateral spontaneous antidromic activity in capsaicin-sensitive sensory nerve fibres. In addition, vascular changes (dilatation/increased permeability) were found in knee joints contralateral to arthritis when no overt contralateral inflammation was present. In conclusion, increases in antidromic activity in sensory nerve fibres and vascular changes occur contralateral to CFA-induced arthritis.

Donaldson LF et al. (1993). J Neurosci Meth 49, 5-10.

Donaldson LF et al. (1995). J Physiol 486, 473-482.

Kidd BL et al. (1995). Brain Res 688, 72-76.

Veale D et al. (1993). Br J Rheumatol 32, 413-416.

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C32

Exogenous galanin has both excitatory and inhibitory effects in the peripheral nervous system *in vivo*

R.P. Hulse[2], D. Wynick[2] and L.F. Donaldson[1]

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

Galanin is known to have both excitatory and inhibitory effects in the CNS, effects that are known to be concentration dependent (Wiesenfeld-Hallin et al. 1989). Galanin (Gal) activates two G protein coupled receptors (GalR) in DRG neurones, GalR1 and GalR2 (Waters & Krause, 1999). Thus Gal may have differing functional effects on different DRG neuronal populations. Recently Gal was shown to have both inhibitory and facilitatory effects on knee joint afferent responses to noxious joint movement (Heppelmann et al. 2000), supporting this hypothesis. The aim of this study was to determine the effect of close arterial Gal administration on mechanically- and cold-evoked responses of cutaneous afferents. As Gal peptide is rapidly degraded *in vivo* in the systemic circulation, repeated measurements could be made in the same afferents.

Recordings were made from 16 single cutaneous high threshold mechanosensitive (HTM) fibres from teased filaments of the saphenous nerve, in deeply anaesthetised male Wistar rats (250-350g, sodium pentobarbital ~20mg kg^{-1} h^{-1} i.v.). Conduction velocities were determined by electrical stimulation of the receptive field (RF); all were $<5ms^{-1}$. Mechanical thresholds for activation were determined using graded von Frey (VF) hairs (0.2-180g). Responses to cold were activated by skin cooling by acetone. Data are medians ± semi-interquartile range unless otherwise noted.

Galanin (10^{-4}M) altered mechanically evoked activity in 13/16 HTMs, increasing firing frequency in 12 and decreasing activity in 1 of the units ($p<0.05$ Chi squared cf. saline vehicle). In the HTM population in which activity was increased, planned comparisons showed that Gal significantly increased responses of HTMs to 4g (0+0.1 cont., 0.23 ± 1Hz Gal, p=0.03, Wilcoxon signed rank) and 10g (0.2+0.6 cont., 0.7 ± 1.8Hz Gal, p=0.02, Wilcoxon signed rank). 6 HTM units also responded to cooling, increasing their firing rate in response to cutaneous acetone (2.4±1.1 cont, vs 3.2±1.1 Hz, acetone; $p<0.01$ ANOVA + Bonferroni; means ± s.e.m.). This was significantly attenuated by Gal (2.2±1.0 Hz acetone + Gal: $p<0.001$ ANOVA + Bonferroni; mean ± s.e.m.).

Thus, in cutaneous high threshold mechanonociceptors, 10^{-4}M galanin exerts excitatory effects by enhancing their responses to non-noxious stimuli (4-10g). In HTMs also responsive to cold, however, 10^{-4}M Gal inhibits activity evoked by skin cooling. We suggest that the differential effects of galanin on different sensory stimuli reflects differences in primary afferent GalR receptor expression. Future work will concentrate on the effects of GalR2 specific agonists on primary afferents.

Waters SM & Krause JE (1999). Neuroscience 95, 265-271.

Wiesenfeld-Hallin Z et al. (1989). Brain Res 486, 205-213.

Heppelmann B, Just S & Pawlak M (2000). Eur J Neurosci 12, 1567-1572.

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C33

Group II mGlu receptor agonist 2R,4R-APDC causes presynaptic depression of excitatory transmission in rat barrel cortex

P.M. Cahusac

Department of Psychology, University of Stirling, Stirling, UK

Group II metabotropic glutamate (mGlu) receptors are known to play an important role in regulating the release of excitatory transmitter in a number of brain areas (Schoepp, 2001). Previous experiments in rat barrel cortex suggested that synaptic responses were depressed by the agonist 1S,3R-ACPD (Cahusac, 1994). To further clarify the role of these receptors in cortical somatosensory processing, experiments were done using a more selective receptor agonist and antagonist.

Single cell recordings were made from 4 adult urethane anaesthetized (2g/kg I.P.) Wistar-derived rats. Micropipettes were used in which the central barrel contained a carbon fibre for recording action potentials. Surrounding barrels contained combinations of the following: monosodium L-glutamate (0.5 M, pH 8.5), (S)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA, 10 mM in 75 mM NaCl, pH 8), GABA (0.5 M, pH 3.5), (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD, 0.1 M, pH 8), (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate (2R,4R-APDC, 0.1 M, pH 8), (2S)-α-ethylglutamic acid (EGLU, 0.1 M, pH 10), 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 ms) deflections of the principal vibrissa by a piezoelectric bimorph.

The selective Group II receptor agonist 2R,4R-APDC (40 – 100 nA) depressed synaptic transmission in 9 of 20 neurones studied (mean effect was 61 ± 2.4% (S.E.M.) of control responses). In some experiments 2R,4R-APDC (40–100 nA) was studied alongside 1S,3R-ACPD (20–100 nA). No excitatory effects of 2R,4R-APDC were seen, and analyses only include cells which were depressed. The same depressant effects on synaptic responses were seen with both compounds in all 5 cells tested (57 ± 2.1% and 47 ± 9.1%) of control responses, respectively). The synaptic depressant effects observed were selectively antagonised relative to GABA by the selective Group II receptor antagonist EGLU (70–150 nA) in 7 out of 10 cells tested. The interaction between drug and time was statistically significant ($F(2,12) = 9.13$, $p = 0.004$), as were the relevant multiple comparisons, which showed that only Group II agonist-produced depressions were antagonised during EGLU. In 2 experiments where selective antagonism by EGLU was achieved, the same Group II agonist currents were used against postsynaptic excitations produced by iontophoretic AMPA. In each case the Group II receptor agonists had little or no effect on the excitations, while GABA had the expected depressant effect.

These results are consistent with work in cat visual cortex (Beaver et al. 1999), and provide evidence that Group II mGlu receptors mediate presynaptic depression of excitatory synaptic transmission. Such a role may be important in keeping excitatory activity within certain physiological limits, or may play more specific roles in the coding of different types of somatosensory information in primary sensory cortex.

Beaver CJ et al. (1999). J Neurophysiol 82, 86-93.

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

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

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