S33

## Spinal memory of peripheral inflammation

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The mechanisms underlying hyperalgesia induced by peripheral injury include both peripheral and central components. The central components of hyperalgesia (central sensitisation) is characterised by an increased excitability of spinal neurones manifested as hyperreflexia, increased receptive field sizes, decreased thresholds and prolonged afterdischarges. In this talk I will address two questions of particular relevance to the understanding of central sensitisation. Firstly I will examine whether the isolated spinal cord, in the absence of peripheral and descending inputs, can show hyperreflexia. Secondly I will review several mechanisms which may lead to increased spinal excitability during hyperalgesic states, and in particular I will consider the possible role of KCNQ potassium channels.

All the work to be discussed was performed on live Wistar rat pups (behavioural experiments) or on the *in vitro* hemisected spinal cord obtained from the same animals (electrophysiological experiments). Inflammations were induced in a group of animals by intraplantar injection of carrageenan (25 $\mu$l) in a hindpaw. The spinal cord was extracted under I.P. urethane anaesthesia, hemisected and maintained *in vitro* using standard procedures (Hedo *et al.* 1999). Dorsal root–ventral root reflexes (DR-VRRs) were obtained from the lumbar segments L4 or L5 using suction electrodes. Changes in these reflexes induced by an experimental inflammation performed 3, 6 or 20 h prior to the extraction of the spinal cord and by the superfusion of potassium ion channel modulators were analysed.

Induction of paw inflammation caused a behavioural hyperalgesia to mechanical stimuli which developed shortly after the injection of carrageenan and lasted for more than 20 h. The DR-VRRs obtained from animals that had suffered an inflammation 6 or 20 h (but not 3 h) prior to extraction of the spinal cord were significantly greater than those obtained from naive animals. This observation indicates that the consolidation of memory traces of injury in the spinal cord requires prolonged time periods which are consistent with the synthesis, trafficking and/or phosphorylation of relevant synaptic proteins. The nature of these relevant proteins and the processes involved in their regulation are not fully established at present.

KCNQ proteins are the molecular substrate for M-currents, which are known to be involved in the regulation of neuronal excitability. Ongoing work in our laboratory using isolated spinal cords from naive animals shows that superfusion of XE-991, an M-current blocker (Zaczek *et al.* 1998), enhances spinal reflexes whereas superfusion of retigabine, an M-current opener (Rundfelt, 1997), produces inhibition of reflexes. These experiments indicate the presence of functional M-currents in the spinal cord and bring into focus the possible role that these currents may play during altered algesic states.

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S34

## Cellular and molecular mechanisms of $GABA_B$ modulation in the spinal cord dorsal horn

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Among the intrinsic bioelectrical properties of deep dorsal horn neurons (DHNs), $Ca^{2+}$-dependent plateau potentials play a major role in processing of nociceptive information. In the first part of the talk, I will present the inhibitory $GABA_B$ modulation of plateau-generating DHNs and the ionic basis of this control.

Application of the $GABA_B$ agonist baclofen on rat spinal cord slices induced a hyperpolarization of the DHNs, associated with a decrease in input resistance. *V–I* relationships indicated the activation of potassium conductances. Baclofen also reduced the amplitude and duration of plateau potentials. Using whole cell voltage-clamp recordings, we further examined potassium currents activated by baclofen. Bath-application of the agonist induced outward current at resting potential that reversed near the $K^+$ equilibrium potential. The cord conductance increased for more negative potentials. The current was sensitive to the inward rectifier channels ($KIR_3$) blockers $Cs^+$ and $Ba^{2+}$ at low concentrations. Baclofen-induced current was blocked by the $GABA_B$ antagonist CGP55845. Baclofen also reduced a dihydropyridine-sensitive $Ca^{2+}$ current. Inhibition of DHN plateau properties via induction of a KIR current and inhibition of a $Ca^{2+}$ current could underlie the antinociceptive effect of $GABA_B$ agonists.

In the second part of this abstract, I will show that the $GABA_B$-receptor-mediated inhibitory modulation of deep DHNs is dynamically balanced by an excitatory modulation via mGlu receptors. This dual modulation has a profound impact on processing of sensory information in the dorsal horn. Deep DHNs present three possible types of firing: (i) tonic discharge, (ii) plateau potentials, and (iii) oscillations and rhythmic bursts. The proportion of each type of firing depends on sustained modulatory inputs. In basal condition, the tonic state is predominant (90.2 % of cells), and oscillations almost inexistent. When $GABA_B$ receptors are blocked (CGP55845) or group I mGluRs activated (ACPD/DHPG) a much larger proportion of neurons expressed plateau potentials (respectively 46.15 % and 45.9 %). Rhythmic bursts are elicited in 8 % of the cells only when mGluRs are activated. Finally, when ACPD and CGP55845 are applied together, the majority of the neurons (90 %) display regenerative properties, i.e. 60 % rhythmic bursts and 30 % plateau potentials. These effects partly relied on the modulation of an inwardly rectifying $K^+$ current (Kir3), activated by the $GABA_B$ agonist baclofen and depressed by DHPG. Immunohistochemical analysis showed that DHNs with plateau properties are contacted by both GABAergic and glutamatergic input.

Using the hybrid network technique, connecting a model of a nociceptive afferent fibre to a biological DHN, we investigated the incidence of the firing type on input-output transfer. The significant differences in the cross-correlation and contribution indexes between afferent and DHN spikes indicate that the three modes of firing impose three different functional states to nociceptive signal transmission. Each state could preferentially participate to different pathophysiological conditions: acute nociceptive transmission, pain sensitisation, and loss of coding related to chronic pain.

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S35

## Striking a balance between anti- and pro-nociceptive actions of adenosine within the spinal cord *in vitro*: a role for the nucleoside transporter ENT1?

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Adenosine is a ubiquitous nucleoside that, in addition to its established role within metabolism, has widespread neuromodulatory actions in the CNS through an interaction with heterogeneous P1 purinoreceptors (A1, A2A, A2B, A3). The concept of 'homeostatic neuromodulation' by adenosine has been introduced to account for the co-existence of a predominant synaptic inhibition by A1 and facilitation by A2 receptors (Cunha, 2001). A1 receptors localized to the substantia gelatinosa of the rat spinal cord exhibit antinociceptive actions that are attributed to presynaptic inhibition of glutamatergic synaptic transmission (Lao *et al.* 2001). Pro-nociceptive actions have been attributed to spinal A2 receptors (Burnstock & Wood, 1996). Given this complexity of action, up- or down-regulation of the adenosine concentration within the synaptic environ may impact on neurotransmission that is modulated by P1 receptors. In this respect, the equilibrative (ENT) and concentrative (CNT) nucleoside transporters that move adenosine into and out of cells could provide a means to achieve this. In this study, we have used immunohistochemical techniques to assess the cellular and regional distribution of the adenosine transporter ENT1 within the spinal dorsal horn. In addition, we have tested the functional consequences of reduced adenosine uptake via the ENT1 transporter on nociceptive synaptic transmission in rat substantia gelatinosa neurons *in vitro*. In the light of data showing a morphine-induced release of spinal adenosine via a putative action on nucleoside transport systems (Sweeney *et al.* 1993), we have evaluated whether $\mu$ opioid receptor-induced synaptic inhibition in substantia gelatinosa could be partly mediated by endogenous adenosine acting through presynaptic A1 receptors.

Immunoblotting of dorsal horn samples with ENT1 antisera revealed a band at ~50 kDa, consistent with the size that is predicted from the established sequence of rat ENT1. Immunohistochemical analysis of spinal cord sections using selective ENT1 antisera revealed dense staining for ENT1 within laminae I and II and moderate to low staining in deeper laminae. This distribution of ENT1 overlaps with expression of the A1 receptor, which is especially dense within substantia gelatinosa. Ultrastructural analysis of ENT1 expression within the substantia gelatinosa with electron microscopy and immuno-gold labelling revealed localization of ENT1 to presynaptic terminals and postsynaptic neuronal elements. Significant A1 receptor immunoreactivity was also found predominately within presynaptic terminals. In patch clamp recordings of rat substantia gelatinosa neurons *in vitro*, monosynaptic EPSCs were significantly attenuated by either adenosine (1 $\mu$M) or the selective A1 receptor agonist 2-chloro-N6-cyclopentyl adenosine (CCPA, 1 $\mu$M). This synaptic inhibition was mimicked by a reduction of adenosine uptake with the selective ENT1 inhibitor nitrobenzylthioinosine (NBMPR, 1–1000 nM). These data suggest inhibition of synaptic transmission as a consequence of an accumulation of endogenous extracellular adenosine and activation of A1 receptors. Further evidence for this mechanism of action is the finding that facilitated breakdown of endogenous adenosine by inclusion of adenosine deaminase (30 mg ml$^{-1}$) in the superfusate partially reversed NBMPR-induced attenuation of the EPSC amplitude. The selective A1 receptor antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) offset EPSC inhibition by both adenosine and NBMPR. Analysis of the paired-pulse ratio for evoked EPSCs and spontaneous miniature EPSC amplitude and frequency revealed a presynaptic locus of action and a reduced probability of transmitter release after NBMPR. An AMPA-evoked inward current in single cells was unaltered by NBMPR indicating that postsynaptic modulation cannot account for these effects. The $\mu$ opioid receptor agonist D-ala(2), *N*-Me-Phe(4), Gly-ol(5) enkephalin (DAMGO, 1 $\mu$M) significantly reduced the evoked EPSC amplitude and this action was partially offset by DPCPX. These data suggest a contributory role of adenosine and presynaptic A1 receptors to opioid-induced antinociception.

In interpreting these data, we propose that adenosine transporters, including the ENT1 subtype, may play a role in adenosine homeostasis within the vicinity of synapses expressing adenosine receptors. A corollary of this is that altered activity of these nucleoside transporters within substantia gelatinosa will indirectly modify nociceptive synaptic transmission as a secondary consequence to manipulation of extracellular adenosine concentrations. Furthermore, a closer evaluation of the proposal that opioid-induced antinociception may be enhanced by inhibition of adenosine reuptake (Keil & Delander, 1995) may be justified.

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S36

## Neuronal cyclooxygenase and pain

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Conventional thinking concerning the mode of action of non-steroidal antiinflammatory (NSAIDs) drugs suggests that they inhibit cyclooxygenase (cox) enzymes in damaged peripheral tissues thereby reducing inflammation and pain. Over the last 10 years, a number of studies have shown that cox enzymes in peripheral and central neurones appear to have an important role in regulating the nociceptive pathway that contributes to pain processing.

In peripheral non-neuronal tissues, the response to tissue damage involves the synthesis of a number of inflammatory mediators including prostaglandins from damaged cells or immune cells that invade the injury site. Prostaglandins induce nociceptor sensitisation by binding to prostaglandin receptors on nerve terminal which modulate the activity of ion channels in the cell membrane. Following tissue damage, the inducible

isoform of cox-2 is upregulated and prostaglandin synthesis is markedly increased.

Molecular, biochemical and immunocytochemical studies have shown that cox enzymes are expressed in peripheral and central neurones. Cox-1 is expressed in subsets of small diameter dorsal root ganglion neurones suggesting that it is preferentially expressed in primary afferent nociceptors. The role of cox enzymes in these neurones has not been fully determined but there is good evidence that they have an important role in relaying signals from Gq coupled GPCRs to ion channels that regulate the excitability and/or firing pattern of these neurones.

Almost 10 years ago it was demonstrated that NSAIDs have a pronounced spinal action which occurs in addition to the established peripheral effects. Since these original studies were performed, several groups have shown that both cox-1 and cox-2 isoforms are expressed in the spinal cord neurones and there is now good evidence that spinal cox-2 gene expression is upregulated in animal models of peripheral inflammatory pain. Recent evidence from neuroanatomical, immunocytochemical and electrophysiological studies have provided good evidence that prostaglandins have a role as neuromodulators in spinal nociceptive pathways.

In summary, non-neuronally derived, and neuronally derived prostaglandins have an important role in regulating the excitability of primary afferent neurones as well as synaptic transmission within the spinal cord. NSAIDs have actions at several sites which individually contribute to the overall analgesic actions of this important group of drugs.