Peripheral and central mechanisms of pain

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Our understanding of nociceptive processing has progressed significantly in recent years, largely because of an improved understanding of afferent fibre physiology and synaptic processing in the dorsal horn of the spinal cord. These developments are the result of the use of several experimental approaches, including behavioural studies, in vivo and in vitro electrophysiology, molecular biology and anatomical studies.

It has become apparent that the responses of the spinal cord to afferent input are altered after persistent activation, that is, sensitization/excitation, of primary afferent nociceptors. Experimental models of peripheral inflammation and studies in man have been useful in providing information on the response of spinal cord neurons to persistent activation of nociceptors. They indicate that, far from being a simple relay in the pathway to the brain, the spinal cord processes nociceptive input in very specific ways that are not static, but change with time. These changes manifest themselves as an increased sensitivity of spinal cord neurones to afferent input and an expansion of peripheral receptive fields, termed spinal hyperexcitability. The excitability of spinal cord neurones is dependent on the balance of inputs from primary afferent nociceptors, intrinsic spinal cord neurones and descending systems projecting from supraspinal sites. An indication of neurotransmitter systems that may have an important role under these conditions comes from experiments that have measured the release of neurotransmitters and the effect of neurotransmitter receptor antagonists, and from those that have examined the expression of genes involved in neurotransmitter systems. Many of these genes are up- or down-regulated in models of inflammatory pain (and neuropathic pain), suggesting that they may be particularly important in initiating or maintaining spinal components of pain.

The potential importance of this type of research is in identifying possible new targets for analgesic therapy and in understanding the timing of events that contribute to the development of these hyperexcitability phenomena in the spinal cord. It is conceivable that timing the administration of drugs targeted at individual neurotransmitter systems (receptors or enzymes involved in neurotransmitter synthesis) to pre-empt or coincide with known changes in neurotransmitter gene expression may provide a useful approach to the management of pain.

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Peripheral nociceptors are distinguished from other sensory nerve fibres on the basis of morphology, conduction velocity and responsiveness to mechanical stimuli. Nociceptors have poorly differentiated terminals, slow conduction velocities (C fibres, < 2.5 m s⁻¹; A-δ fibres, 2.5–20.0 m s⁻¹) and are normally activated by potentially damaging or damaging stimuli, that is, stimuli of strong to noxious intensity.

Primary afferent nociceptors terminate mainly in the superficial (C and A-δ fibres) but also in the deep (A-δ fibres) dorsal horn of the spinal cord, where they release several neurotransmitters. Glutamate, an excitatory amino acid (EAA) released from these central terminals of the nociceptors,₂⁵ ₄⁶ elicits fast synaptic responses in second-order neurones ⁴² that are mediated by at least two EAA receptor sub-types, the (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and the N-methyl-D-aspartate (NMDA) receptors, so called because they are activated by these selective ligands. A proportion of primary afferent nerve fibres also express and synthesise neuropeptides that are co-released with glutamate within the spinal cord. Substance P, neuropeptide A and calcitonin gene-related peptide (CGRP), which are found in 20%, ²⁹ ³⁰% (joint afferents ¹⁸) and 25–47%, ²⁵ ³⁰% of primary afferent nerve fibres respectively, but predominantly in nociceptors, are three such peptides. They are released in response to noxious thermal or mechanical stimuli or in response to electrical stimuli applied to sensory nerves at a stimulus intensity sufficient to excite C and A-δ afferent nerve fibres. ¹³ ₁⁴ ₁⁵

Many dorsal horn neurones project to the brain, mainly in the spinothalamic, spinoreticular and spinomesencephalic tracts. On termination in these regions the information may be processed to produce a perception of pain or to elicit output that is relayed to the spinal cord by way of descending fibre tracts mainly originating in the medulla or cortex.

Responses of afferent nerve fibres and spinal cord neurones to persistent afferent input

ELECTROPHYSIOLOGICAL CHANGES

During peripheral inflammation nociceptors within damaged tissues are readily excited by non-noxious stimuli (alldynia) or show an enhanced response to noxious stimuli (primary hyperalgesia). This sensitization of nociceptors is produced by physical changes in the damaged tissues (oedema, synovial

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effusion, etc.) and by inflammatory mediators, for example, prostaglandins, serotonin and bradykinin, which alter the sensitivity of nerve endings to mechanical and thermal stimuli. In addition to the reductions in mechanical threshold, some nociceptors are also directly activated by the inflammatory mediators present, such that continuous low-frequency firing (~1.0 Hz) can be detected in the afferent axons of these nociceptors.43

Following the development of peripheral inflammation the responses of spinal cord neurones to peripheral stimulation are also altered. This has been demonstrated most conclusively in neurones located in the deep dorsal horn and ventral horn of the spinal cord, which receive input from both nociceptors and low-threshold mechanoreceptors, that is, wide-dynamic-range (WDR) neurones. As with primary afferent nociceptors, peripheral inflammation elicits a reduction in the mechanical threshold for activation of these neurones as the duration and intensity of the inflammatory lesion develop. The second change is an enlargement of the peripheral receptive fields,17,32 which can be interpreted as increased sensitivity of spinal cord neurones to subliminal inputs from the periphery of the receptive field. Under normal circumstances WDR neurones have moderately sized receptive fields most readily activated by mechanical stimuli applied to the centre of the receptive field. If the stimulus is applied nearer the edge of the receptive field the mechanical threshold for activation of the spinal cord neurone generally increases, indicating that the size of this synaptic input to that neurone is smaller. Stimuli applied to the edge of the receptive field do not elicit cell firing but may elicit sub-threshold responses (excitatory post-synaptic potentials (EPSPs)).

When inflammation develops in the periphery, however, there is persistent firing in the afferent nociceptors, which continuously release a number of neurotransmitters into the synapses. It has been established that the amount of glutamate released into the synapse increases,46 which will elicit rapid (AMPA-receptor dependent) and slower (NMDA-receptor dependent) EPSPs. Other neurotransmitters, such as substance P,45 neurokinin A24 and CGRP,37 whose release is also continuous and is also increased under these circumstances, contribute to the prolonged depolarization of the post-synaptic neurone. This action is enhanced by the resistance of some neuropeptides, for example neurokinin A, to enzymatic degradation, which may mean that they persist in the synapse and that they may diffuse and act in a paracrine manner.

The net effect of the release of all of these neurotransmitters is to excite the neurone and move the membrane potential closer to threshold for the firing of action potentials. When in this condition the neurones are more sensitive to smaller inputs from low-threshold (tactile) and high-threshold (nociceptors) mechanoreceptors,27,49 such as those in the subliminal regions of the receptive field. While the EPSPs evoked by stimulation of these regions fail to elicit cell firing under normal conditions, they now elicit action potentials because the depolarization required to move the resting membrane potential to threshold is reduced.

The result of this is that firing is elicited by stimulation of the former subliminal zone and the receptive field apparently expands. This is a form of spinal hyperexcitability (sometimes called central sensitization) and is the mechanism thought to underlie the phenomenon of secondary hyperalgesia. This phenomenon has been observed in a wide variety of experimental paradigms including acute32 and more chronic models of joint inflammation17 and in man.149 Indeed, this expansion can be very marked, such that weak synaptic inputs from the contralateral hindlimb begin to excite neurones receiving input predominately from the ipsilateral leg.45

Experimental paradigms have been developed that enable one to examine spinal hyperexcitability and investigate the underlying pharmacology. If, in the normal animal, that is, in the non-inflamed state, trains of electrical stimuli are applied to sensory nerves at an intensity sufficient to excite C fibres or Aδ fibres or both, and the responses of spinal cord neurones are monitored by measuring neuronal activity directly with intracellular or extracellular electrodes or by recording motor outflow from the ventral roots or muscle (EMG activity), the phenomenon of wind-up can be observed.10,48,52 This is an enhanced response of spinal cord neurones to successive stimuli in the train and occurs because of the summation of EPSPs (a single shock elicits an EPSP lasting several seconds), which results in a progressive depolarization and enhanced cell firing following each stimulus.46 This is clearly a frequency-dependent effect and can be observed at stimulus frequencies as low as 0.5 Hz, a frequency that is not dissimilar to the background firing rate of sensitized nociceptive afferents. It is possible therefore that the wind-up paradigm mimics, in some respects, the situation observed in animal models of inflammation where spinal cord neurones are rendered hyperexcitable by persistent low-frequency input from sensitized afferent nociceptive fibres. Wind-up and central sensitization are not, however, identical processes although they share many features.51

PHARMACOLOGY OF SPINAL NOCICEPTIVE PROCESSING

Wind-up can be blocked by administration of NMDA receptor antagonists, for example, ketamine or AP-5, through a reduction in the magnitude and duration of these long-duration EPSPs elicited by single nerve shocks, thereby preventing summation and reducing cell firing.46 Neurotransmitter receptor antagonists that selectively block the action of other primary afferent neuropeptides, for example substance P (NK2),57,59 also reduce wind-up when applied in the vicinity of spinal cord neurones, indicating that the pharmacology of wind-up is complex. Opioid peptides are also effective in reducing wind-up but only when applied pre-emptively,8 presumably through activation of opioid receptors that are known to be present on the terminals of primary afferent nerve fibres (μ and δ subtypes) and on spinal cord neurones (μ, δ and κ subtypes). Opioid peptides probably exert their effects at one or both of these sites by blocking voltage-gated calcium channels that will prevent neurotransmitter release from primary afferent nerve fibres,24 or by opening potassium channels30 that will result in neuronal hyperpolarization, or both.

In man, electrical stimulation at an intensity sufficient to elicit pain also produces temporal summa-
tion.\textsuperscript{2,40} In these studies electrical stimuli were applied to the skin and either EMG or M-VAS scores were recorded. The summation of these nociceptive responses can be blocked by NMDA receptor antagonists,\textsuperscript{1,40} indicating that some similarities exist between the pharmacology of wind-up in man and in experimental paradigms.

Drugs acting at the same receptors are effective in preventing the development of neuronal hyperexcitability or in reversing it once it is established in experimental models of inflammatory pain, for example NMDA receptor antagonists,\textsuperscript{3,14} NK\textsubscript{1} receptor antagonists,\textsuperscript{35} NK\textsubscript{2} receptor antagonists,\textsuperscript{38} and CGRP receptor antagonists.\textsuperscript{37} NMDA receptor antagonists administered pre-emptively, alone or in combination with other analgesics, in a variety of clinical paradigms, seem to produce signs of improved post-operative pain control.\textsuperscript{4,9,15,26,40} Similarly, pre-emptive administration of morphine also improves post-operative pain control in man.\textsuperscript{41}

Both human and experimental data suggest that several neurotransmitter systems are involved in the initiation of spinal hyperexcitability, or are able to modulate it once established, or both.

### NEUROCHEMICAL CHANGES IN THE SPINAL CORD

While electrophysiological measures of the changes in neuronal responsiveness and measurements of the release and actions of some neurotransmitters provide valuable information about the processing of nociceptive input, new information has become available from studies of gene expression in primary afferent nerve fibres and spinal cord neurones in models of inflammatory disease. These studies have shown that the genes encoding the sequence of some primary afferent neuropeptides are upregulated when a peripheral inflammation is induced. Two examples of these are the preprotachykinin gene, which encodes substance \textit{P}, the endogenous NK\textsubscript{1} receptor ligand and neurokinin \textit{A}, the endogenous NK\textsubscript{2} receptor ligand, and the gene encoding the sequence for CGRP. In Freund's adjuvant-induced monoarthritis in the rat, both genes are up-regulated\textsuperscript{12} with a time course (2–3 weeks) that mimics closely the physical changes (swelling) in the joint. This also results in an increase in the amount of these peptides in the dorsal root ganglia\textsuperscript{19} and presumably to increased release within the spinal cord, although this has not been measured in this model of arthritis. One could argue that this increase in gene expression occurs so as to synthesize more peptide as part of the response to increased activity in the afferent nerve fibres. This is not true, however, for two other neurotransmitter systems, the endogenous opioid and eicosanoid systems in intrinsic neurones of the spinal cord.

In the same model of inflammation, preprodynorphin (and to a lesser extent preproenkephalin) gene expression is up-regulated in the spinal cord\textsuperscript{12,25} but with a quite different time course to that observed for the primary afferent neuropeptide genes. In this case there was rapid (3–4 h) induction of the preprodynorphin gene as measured by mRNA synthesis\textsuperscript{11} but the increases in dynorphin (1–8) synthesis did not occur for 2–5 days,\textsuperscript{25} which is significantly delayed compared with the time course of substance \textit{P} synthesis.\textsuperscript{19} This indicates that an endogenous pain control mechanism is switched on at a defined time after the induction of arthritis to regulate substance \textit{P} activity.\textsuperscript{47} Cyclo-oxygenase (cox)–2, a gene that encodes the protein that converts arachidonic acid to the prostaglandin precursor molecule PGH\textsubscript{2}, is up-regulated but only very acutely, that is in the 24–48 h after the induction of arthritis.\textsuperscript{6,16,22} Prostaglandins, which are important in spinal nociceptive processing,\textsuperscript{28,34,36,52} seem, therefore, to have a role as neurotransmitters or neuromodulators only during a very defined time period.

It is clear that we still have much to learn about these changes in gene expression in the spinal cord and how they are induced, the role of immediate early genes such as \textit{c-fos} and \textit{c-jun} and why they occur at different times. Only by understanding the timing of induction and the precise roles of both excitatory and inhibitory elements in the nociceptive system shall we be able to gain a better insight into the complexities of pain processing. One hope is that a better understanding of the roles of these different neurotransmitter systems at different stages of inflammatory disease may help with the identification of novel therapeutic targets and with the timing of administration of analgesic drugs.

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