Inflammatory mediators of pain

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Normally when fine afferent C- and A-δ fibres are activated by brief, high intensity stimuli, which induce little or no tissue damage, transient pain is induced and serves as a physiological warning. However, during the inflammation produced by mild tissue damage or infection (e.g. postoperative pain, toothache, cystitis), afferent fibres are activated by lower intensity stimuli and the pain produced differs in quality and may be more persistent. A number of operational changes in afferent neurone function and in central processing of nociceptive signals may account for this [43, 53, 57]. These events can also be considered as a physiological protective response; the nociceptive system reverting back to a normal state once the underlying injury has healed. In chronic pain conditions, however, there may be spontaneous pain as well as intermittent or persistent pain which also involve complex changes in peripheral and central signal processing. In these circumstances the physiological relevance of the nociceptive signal is less clear and the protective function of afferent activity is obscure. Chronic pain can be associated with chronic inflammation in which lesioning or tissue damage is obvious but also there are conditions in which no obvious pathology or degenerative process can be found. Chronic pain is symptomatic of numerous conditions including rheumatoid arthritis, osteoarthritis, fibromyalgia, low back pain, pelvic and abdominal pain, cancer and neuropathic pain and migraine. The mechanisms of chronic pain are still poorly understood and the pain is difficult to ameliorate [20]. It is likely, however, that in the vast majority of pain conditions, whether inflammatory or neuropathic, there is an associated phase of inflammation in which a variety of chemical mediators are able to alter the functions of peripheral afferent fibres. This review will address the actions of these substances on sensory neurones and the way in which they are involved in pain signalling and in the functional remodelling of peripheral afferent fibres.

Key words

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PERIPHERAL AFFERENTS; CHEMICAL SIGNALLING

Although the majority of nociceptors, the polymodal nociceptors, respond to thermal and mechanical stimulation, chemical signalling is likely to be the most common and diverse form of signal generation in all types of fine afferent fibres. A small proportion of afferent fibres, the “silent” or “sleeping” nociceptors, which have been identified in the skin as well as in visceral organs, may be unresponsive, even to intense stimuli. They may represent the extreme range of afferent fibre sensitivity. However, when influenced by inflammatory mediators, or after the administration of irritants, they exhibit spontaneous activity or become sensitized and responsive to sensory stimuli [47, 48]. Many afferent fibres (somatic and visceral) can be affected by chemical stimuli without this inducing specific sensations as those occurring after the activation of nociceptors. This would depend on the generation and propagation of nerve impulses to the spinal dorsal horn, and synaptic interactions in spinal cord. Some chemicals however may induce little obvious excitability change but rather have a trophic influence on afferent fibre function [17, 43].

Several important features are associated with inflammation besides the stimulation of peripheral nerve fibres to induce pain there are changes in local blood flow and vascular permeability, activation and migration of immune cells and changes in the release of growth and trophic factors from surrounding tissues. These events represent an orchestrated series of responses in which afferents play an important role [17, 43]. In theory the afferent nerve terminal may be exposed to a great many substances during tissue injury and inflammation but there is little information about the composition or concentrations of various components. However, these can produce manifold and complex changes in afferent fibres ranging from overt activation, or sensitization to other stimuli, to alterations in the phenotype and structure of sensory nerves [17, 29, 43, 57]. Enormous potential thus exists for interactions between different substances and between neural (sensory and sympathetic nerves) and non-neural systems (sensory nerves and immune cells).

Although some mediators (e.g. protons, 5-hydroxytryptamine (5-HT)) can act directly on membrane ion channel proteins to change permeability and cell excitability, for the most part,
chemical interactions occur via the activation of membrane receptors which are usually, but not exclusively, coupled with a number of regulatory intermediates such as G-proteins and second messengers. Activation of the latter, in turn, activates specific kinases that phosphorylate cellular proteins, thereby inducing changes in membrane ion channels or cellular enzymes. For example, receptor (adenosine, bradykinin, 5-HT, prostanoids) coupled with activation or inhibition of cAMP is a common mechanism by which membrane excitability is regulated by altered potassium ion permeability.

Because of the technical difficulties of studying individual fine afferent fibres, much of our understanding of their signalling properties has been obtained from studies of their cell bodies in sensory ganglia. For this, cells are often maintained in vitro in defined media whose chemical composition is likely to differ from that occurring pathophysiologically. This environment influences cellular properties in addition to those imposed by the stresses of preparing cells for maintenance in culture. Caution is thus required in extrapolating findings in cultured cells to the operation of sensory neurones in vivo.

As in other excitable cells sensory neurones express a diversity of ion channels [40]. However, there may be differences in different sensory fibres. For example, most nerve fibres possess voltage gated Na channels which are important for nerve conduction and which can be blocked with the puffer-fish toxin, tetrodotoxin. A large number of fine afferent fibres can be distinguished by having tetrodotoxin-resistant Na channels. These may be differentially regulated by inflammatory mediators. Indeed, nerve growth factor (NGF) has been shown to promote the expression of a gene for the PN1 type of Na channel which is found only in peripheral afferent nerves and sympathetic fibres. The significance of this is not yet understood though it may be related to the increased afferent excitability and hyperalgesia caused by NGF (see later).

Sensory neurones also possess calcium-activated potassium channels which several pro-inflammatory mediators block to increase excitability and thereby induce hyperalgesia. As in other neurones, membrane voltage-dependent T, N and L-calcium channels contribute to the excitability of sensory neurones but N-channels are particularly important as they control the release of neurochemicals from peripheral and central terminals of sensory neurones. N and L-channels can be blocked by a number of drugs (dihydropyridines) and chemical transmitters (opioids, GABA, neuropeptide Y (NPY)) to prevent nociceptive signalling in the spinal cord and to modify excitability at peripheral nerve terminals which indirectly alters the neurogenic effects produced by sensory neuropeptides. Recently abnormal calcium channel activity has been implicated in the ectopic discharges of sensory ganglion cells after peripheral nerve transection. These events may be related to neuropathic injury pain. Spontaneous discharges could be blocked by verapamil and a number of N-channel, ω-conotoxin derivatives [59, 60].

Chemical factors produced during tissue damage

A variety of substances are released upon damage to cells while others are synthesized during the events that follow tissue injury. These have a profound qualitative and quantitative impact on the production of inflammation and on afferent fibre activity.

Reactive oxygen species

Reactive oxygen species have received relatively little attention with respect to afferent fibre activation during tissue injury and inflammation. Reactive oxygen species (ROS) include hydrogen peroxide, superoxide and hydroxyl species and are normal products of cellular electron transfer reactions which are important for the regulation of a number of gene transcription activities. Normally the production of ROS is finely controlled by the anti-oxidative activity of superoxide dismutase and catalase. During the ischaemia which follows the rapid vasoconstriction response upon tissue injury ROS concentrations decrease to unphysiologically low levels and thereby switch off antioxidant activity. Then tissue reperfusion creates an oxidative stress in which oxygen and nitrogen species are produced in abundance leading to the induction of a number of factors such as NF-kB and the fos/jun dimer, AP-1. These stimulate the production of a second wave of gene products encoding enzymes with free radical scavenging (catalase), tissue repair activity (collagenase, stromelysin) as well as the production of cytokines, cell surface receptors, adhesion molecules and growth factors.

Though the production of ROS is important in inflammation evidence for nociceptor activation is sparse. However, hydrogen peroxide has been shown to enhance the effects of other inflammatory mediators including bradykinin and prostaglandin E2 (PGE2) while nitric oxide (NO), another reactive molecule, induces a delayed burning pain upon intradermal injection [25] and NO donors have been postulated to activate cerebral sensory fibres directly, causing release of the vasodilator calcitonin gene-related peptide (CGRP) [55]. Indeed, NO has been suggested to contribute to migraine and other types of head pain [41].

The major route of nitric oxide formation is from l-arginine. This occurs in many cell types but particularly in small and medium sized sensory neurones [54], following the activation of nitric oxide synthase (cNOS), by calcium and other co-factors. NO alters cellular processes mainly via the activation of guanylate cyclase. In keeping with this, increased production of cGMP occurs in satellite cells in the dorsal root ganglia (DRG) upon administration of NO donors. During inflammation or nerve injury, however, an inducible and calcium-independent form of NOS occurs [54] which allows a greater capacity for NO synthesis. It is unclear what function this serves but there may be other indirect consequences as i-NOS has an important role in the regulation of an inducible form of cyclo-oxygenase
activity [46] and hence the production of pro-inflammatory prostanoids. In addition, NO may alter the responsiveness of sensory neurones to inflammatory chemicals such as bradykinin [17, 45]. This may involve a cGMP-dependent regulation of bradykinin receptor-effector coupling mechanisms, but the details of this interaction are not known. Presently there is little evidence for direct activation of sensory neurones by NO but recently it has been suggested that NO formation contributes to the ectopic discharges induced by peripheral nerve lesions as the increased sensory neuronal excitability found in this condition is reduced by NOS inhibition [54].

Protons

Proton production is increased in inflammation and is likely to be involved in inflammatory hyperalgesia and in the sensation of muscle aching and discomfort due to the hypoxia/anoxia of muscle exercise. Indeed, direct activation of nociceptors accounts for the sharp stinging pain produced by intradermal injections of acidic solutions and low extracellular pH enhancing the effects of other inflammatory mediators [2, 17].

Exogenously administered acidic solutions produced a rapid but transient increase in membrane cation permeability as well as a more prolonged permeability increase in sensory neurones. This can give rise to sustained nerve activation as well as an enhanced mechanosensitivity [49]. The mechanism of proton-induced activation of sensory neurones has not been fully elucidated but seems to be remarkably similar to that of capsaicin [2, 4], the pungent principle obtained from Capsicum peppers, with highly specific actions on polymodal nociceptors [16]. Thus, protons and capsaicin activate sensory membranes through identical ionic conductance mechanisms and capsaicin and proton sensitivity are both regulated by the presence of NGF. Capsaicin has been shown to activate nociceptors via a specific membrane receptor which is blocked by the competitive antagonist, capsazepine [3, 16]. Although protons might appear as a likely candidate for a capsaicin receptor, studies with capsazepine have been inconclusive. Thus, capsazepine was ineffective against proton-induced activation of sensory neurones [2] but blocked proton-induced membrane changes and proton-induced neuropeptide release (heart, trachea) in other studies [2]. It is possible that protons induce the release of capsaicin-like molecules or that visceral and somatic fibres differ in their mechanism of activation by protons. In support of these suggestions capsazepine was shown to block the release of CGRP induced by proton activation of PGI₂ production [2]. In addition, proton-induced activation of tracheal afferent fibres was also antagonized by capsazepine [21].

Kinins

Kinins exert a number of proinflammatory effects including the release of prostanoids, cytokines and free radicals from a variety of cells. They also stimulate postganglionic sympathetic neurones [23] to affect blood vessel calibre. Kinins degranulate mast cells to release histamine as well as other inflammatory mediators and also cause plasma extravasation by contraction of vascular endothelial cells. Kinins are potent allogenic substances and induce pain by directly stimulating nociceptors in skin, joint and muscle, as well as by sensitizing them to heat and mechanical stimuli [1, 43]. There is also a strong synergism between the actions of bradykinin and other allogenic substances, for example prostaglandins, 5-hydroxytryptamine. Sympathetic neurones, which are activated by B2 kinin receptors, may also be involved in bradykinin-mediated mechanical hyperalgesia [23, 29] though in the skin the mechanism for this is unclear as sympathectomy did not alter heat hyperalgesia induced by bradykinin [17, 43].

Kinin production occurs from separate precursors within the blood and other tissues. In the blood, bradykinin is formed as part of the clotting cascade by enzymatic processing of high molecular weight kininogen precursor. In other tissues, however, processing of low molecular weight kininogen forms kallidin (lysyl-bradykinin) [18, 22]. Both bradykinin and kallidin are rapidly degraded by kininas to generate the active metabolites desArg²bradykinin or desArg⁹kallidin, respectively as well as a number of inactive metabolic products.

The effects of kinins are mediated via two distinct receptors, B1 and B2 [18, 22, 37]. The endogenous agonists for the B2 receptor are bradykinin and kallidin. Studies with a number of B2 receptor-selective antagonists have confirmed that bradykinin is an important mediator of pain, since these antagonists significantly attenuated the pain and hyperalgesia associated with a number of inflammatory conditions [42, 51]. These data also indicate that B2 antagonists are likely to be useful in future analgesic therapy. The B1 receptor has not been as extensively characterized but this receptor is preferentially activated by the kinin metabolites desArg²bradykinin or desArg⁹kallidin while desArg⁹Leu³bradykinin has been used as the prototypic antagonist at this site. B1 receptors are encountered infrequently under normal conditions but their expression is increased rapidly during inflammation or infection. This is the result of the influence of immune cell products including cytokines such as IL-1β [34]. The significance of this is not entirely clear but B1 receptors make an important exogenous contribution to hyperalgesia as selective B1 receptor antagonists produce analgesia [13, 18]. So far there is little evidence for a direct activation of sensory neurones through B1 receptors and it is likely that B1 receptor-induced hyperalgesia is mediated indirectly via release of other mediators (e.g. prostaglandins) from macrophages and leucocytes [13, 18, 34].

B2 receptors are present on sensory neurones where they are coupled with a G protein to induce phospholipase C activation. This generates the second messengers, 1,4,5-inositol-trisphosphate (IP3) and diacylglycerol (DAG) following cleavage
of membrane phospholipids. IP3 stimulates the release of calcium from intracellular stores while DAG activates protein kinase C (PKC) to phosphorylate cellular proteins including membrane receptors and ion channels. PKC plays a key role in the excitation of afferent fibres by bradykinin which is associated with an increase in membrane ion permeability, mainly to sodium ions [43]. However, bradykinin-evoked depolarization also induces a calcium influx [52] causing both the release of neuropeptides such as substance P and the stimulation of arachidonic acid production via the activation of phospholipase C [43]. In visceral sensory neurones (nodose ganglion cells) increased excitability can also occur through the inhibition of a long-lasting spike after-hyperpolarization (slow-AHP). This is regulated by a cAMP-dependent potassium conductance mechanism. The slow-AHP reduces cellular excitability and limits the number of action potentials that can be evoked upon stimulation [56]. Prostaglandins and bradykinin (through prostanoid formation), inhibit the slow-AHP by stimulating c-AMP formation, allowing the cell to fire repetitively. A number of substances which cause hyperalgesia may work by a similar mechanism.

**Prostaglandins**

Prostaglandins (prostaglandins, leukotrienes, hydroxy-acids) are among the most important mediators of inflammatory hyperalgesia and are generated from arachidonic acid by cyclo-oxygenase and lipoxygenase enzyme activity. Prostaglandins act via a number of receptors coupled with second messengers [9] but the EP receptor for PGE; and the IP receptor for PGI; (prostacyclin) are probably the most important for their effects on sensory neurones. Indeed a receptor subtype, the EP3, has recently been identified in the majority of small sensory neurones. Prostaglandins do not usually evoke pain when injected intradermally into human skin [11] although PGE; and prostacyclin (PGI;2) have been reported to increase the activity of nociceptors directly [6, 47] and PGE; stimulated the release of substance P from sensory neurones in culture. These depolarizing effects may have been due to an increase membrane Na+ conductance. More usually prostaglandins are normally generated by the constitutive form of cyclo-oxygenase (COX-1) and serve a number of physiological functions [9, 38], during inflammation prostaglandin formation is enhanced by the induction of another form of the enzyme COX-2 [24]. Non-steroidal anti-inflammatory drugs (NSAID) owe their analgesic and anti-inflammatory properties to a block of COX enzymes but compounds which select for COX-2 produce analgesia with fewer side effects [38].

Intradermal injection of leukotriene B4 (LTB4, a product of the 5-lipoxygenase pathway) or 8R,15S-diHETE (a product of the 15-lipoxygenase pathway) also decrease the nociceptive thresholds [29, 43]. LTB4 appears to act via the release of 8R,15S-diHETE from polymorphonuclear leukocytes [29] while 8R,15S-diHETE produces hyperalgesia directly, by decreasing the mechanical and thermal thresholds of C-fibres. The hyperalgesic effect of 8R,15S-diHETE and LTB4 can be inhibited by the isomer 8S,15S-diHETE [29] indicating that there may be complex inhibitory as well as facilitatory interactions of prostanoids with nociceptors.

**Adenosine triphosphate**

Adenosine triphosphate activates sensory neurones and increases their permeability to cations. This may account for the sharp, transient pain that ATP produces when administered intradermally. Adenosine, formed by the breakdown of ATP also provokes pain and hyperalgesia when administered i.d., i.v. or onto a blister base [7, 29]. This is likely to be due to the activation of adenosine A2 receptors which are coupled with adenylate cyclase [29]. The production of cAMP and a reduction of potassium ion permeability accounts for the hyperexcitability of afferent fibres. On the other hand adenosine may also activate A1 receptors which are negatively coupled to cAMP activation to cause a reduced afferent excitability by blocking Ca2+ conductance or increasing K+ permeability. This may cause antino-ciception, as demonstrated with adenosine ligands [29, 43].

**Serotonin**

Serotonin can cause direct excitation of sensory neurones by increasing sodium permeability via 5-HT3-receptor activation. This can be blocked ICS 205,930 and accounts for mild, and transient pain produced when serotonin is applied to a blister base [44]. A similar effect may occur when serotonin is released from platelets and mast cells, during, injury or inflammation. Since the 5-HT3 receptor binding site is part of a cation (Na+) selective ion channel it is possible that ICS 205,930 may produce its effects via Na+ channel block. 5-HT also activates sensory neurones via G-protein-coupled 5-HT_{1} and 5-HT_{2} receptors [29]. This induces a decrease in potassium ion permeability and a membrane depolarization which may sensitize nociceptors and lower their threshold to heat and pressure stimuli but may also induce repetitive neuronal firing [1, 17]. Activation of cAMP-dependent processes appears to be required for 5-HT-induced sensitization, since hyperalgesia could be blocked by an inhibitor of cAMP and augmented by inhibition of phosphodiesterase [29]. The cAMP-mediated reduction of K+ permeability has also been proposed to be the mechanism that attenuates the slow-post spike AHP which increases sensory neural excitability and provokes repetitive firing [43].

5-HT_{1D}-like receptors have also been postulated to be present on fine afferent fibres innervating the dura mater of the brain. Their activation may reduce afferent excitability, plasma extravasation and the vasodilatation brought about by sensory neuro-
Histamine

Histamine can be released following mast cell degranulation by a number of inflammatory mediators including substance P, interleukin-1 (IL-1) and NGF. It can then act on sensory neurones to produce itching at low concentrations and pain at higher concentrations [50]. Indeed, sensory neurones express histamine H2 receptors and H1 receptor activation increases membrane calcium permeability in a variety of sensory neurones [43]. This is likely to evoke the release of sensory neuropeptides as well as the release of prostaglandins and monohydroxyeicosatetraenoic acids (HETEs) from endothelial cells leading to hyperalgesia and other pro-inflammatory effects [43].

NEUROGENIC SUBSTANCES AND AFFERENT FIBRES

Normally neuropeptides (neurokinins, CGRP) released from sensory nerve endings exert efferent and trophic effects on target tissues [17,43]. During inflammation however, the neurokinins substance P and neurokinin A (NKA) contribute directly and indirectly to neurogenic inflammation and hyperalgesia in the periphery and to the excitability changes in the spinal dorsal horn associated with the transmission of pain signals. Studies with a number of antagonists indicate that the effects of neurokinins are mediated through the activation-specific neurokinin receptors [31]. To date, NK1 and NK2 rather than NK3 receptors have been most prominently involved in the pro-inflammatory and hyperalgesic effects of neurokinins. During inflammation, however, the neuropeptide content of sensory nerves is increased by the actions of neurotrophins such as NGF [15] so that effects of substance P and NKA are more pronounced causing vasodilatation, plasma extravasation and mast cell degranulation to allow the release of other inflammatory mediators. CGRP, however, does not produce plasma extravasation but is a powerful arteriolar vasodilator and by increasing blood flow into venules acts synergistically with substance P to enhance plasma extravasation. Other sensory peptides such as galanin and somatostatin may reduce neurogenic inflammation since they decrease afferent excitability and thereby reduce neuropeptide release from sensory fibres. Plasma extravasation induced by NK1 receptors seems to be of special significance in dural blood vessels and may be important in vascular headache. Indeed, NK1 receptor antagonists potently attenuate the plasma extravasation induced by sensory nerve stimulation [39] and are predicted to have clinical anti-migraine activity.

Neurokinins may also directly depolarize sensory neurones by reducing potassium permeability [19] and separate studies have postulated that NK1 receptors are present on primary afferent nerve terminals [32]. Thus neurokinins may be important for direct regulation of afferent nerve excitability though presently it is not clear whether this is an autoregulatory mechanism via neuropeptide release from afferent fibres themselves or from other tissues such as sympathetic fibres.

A role for neurokinins in inflammatory hyperalgesia is further supported, however, by the fact that NK1 antagonists produce behavioural analgesia. Thus, the non-peptide antagonist RP67580 was shown to reduce mechanical hyperalgesia in rats with streptozotocin-induced diabetic neuropathy but not in normal animals and a similar antagonist CP 96,345 abolished carrageenan and formalin-induced inflammatory hyperalgesia [5]. These antagonists may induce their effects both within the spinal cord as well as at peripheral sites.

Interactions of sympathetic neurones and nociceptive afferents have been postulated during inflammation. However, direct interactions of sympathetic nerves or sympathetic transmitters with afferent fibres have been demonstrated only after peripheral nerve damage or inflammation [28,36,53]. Thus, during inflammation afferent fibres can be sensitized by the release of prostanoids, or possibly substance P, from activated sympathetic fibres [27,29]. In addition, sympathetic nerve stimulation or the direct administration of noradrenaline was able to excite fine afferent fibres after injury to a sensory nerve trunk [14,29]. This could be attenuated by the alpha-adrenergic receptor blocker, phentolamine, suggesting that alpha-adrenergic receptors were expressed on fine afferent fibres [29] as well as on large A-fibre afferents [14].

Although NPY receptors (Y1 and Y2) have been found on sensory neurones it is unclear what function they serve though they are likely to be involved in regulating nociception. NPY can be released from sympathetic fibres in which it is co-localized with sympathetic transmitter, or from large afferent fibres in which it is expressed after nerve injury [33,35]. Y1 receptor activation increases DRG excitability and peptide release through an increased calcium permeability but Y2 receptor activation inhibits calcium conductance and transmitter release [10, thereby reducing neurogenic inflammation [29].

Inflammatory mediators from immune cells

A number of cytokines (IL-1β, IL-6, IL-8, TNFα) are released from a variety of immune cells and can induce powerful hyperalgesia. This is mediated indirectly via several mechanisms including prostaglandin release, increasing the expression of NGF or bradykinin receptors, or by affecting sympathetic fibres [12,13,27]. So far there is no evidence that cytokines directly affect the excitability of sensory fibres.

Neurotrophins, especially NGF are normally produced by the peripheral target tissues of afferent fibres and by supporting cells including fibroblasts, Schwann cells and keratinocytes. NGF is essential for the survival and development of sensory neurones and for maintaining their phenotype [30] acting via a specific tyrosine kinase receptor (trkA) to regulate specific gene transcription processes. During inflammation, however, NGF production is stimulated...
by other inflammatory mediators such as cytokines (IL-1β and TNFα). NGF increases the synthesis of several neuropeptides including neurokinins and CGRP and regulates a number of other proteins such as the capsaicin receptor, membrane Na+ channels and proton-activated ion channels [2, 43]. Accompanying this is an increased sensitivity to exogenous stimuli producing hyperalgesia. In keeping with this, anti-NGF antibodies reduce the hyperalgesia and neurochemical changes induced by NGF and inflammation [30, 58].

**Summary**

While sensory fibres normally respond to a range of physical and chemical stimuli their activity and metabolism are profoundly altered by a variety of mediators generated by tissue injury and inflammation. These include substances produced by damaged tissue, substances of vascular origin as well as substances released by afferent fibres themselves, sympathetic fibres and various immune cells. The effects of inflammatory mediators, to activate or sensitize afferent fibres, are produced by changing membrane ion channels which are coupled directly via receptors or more commonly are regulated through receptor-coupled second messenger cascades. These latter processes also have the potential to alter gene transcription and thereby induce long-term alterations in the biochemical of sensory neurones. This can have far-reaching consequences as the expression of novel proteins for ion channels (Na channels) and receptors (capsaicin, NPY) as well as the induction of novel enzymes (i-NOS) can profoundly affect the properties of nociceptors and their ability to transmit pain signals. However, such changes may be targeted successfully for the development of new analgesic and anti-inflammatory agents.

**References**


