Novel pharmacological strategies for analgesia

Martin Perkins, Andy Dray

Acute transient pain, associated with negligible tissue damage, serves as a physiological warning of potential tissue damage. Somewhat more persistent pain, associated with hyperalgesia and tenderness, is often associated with inflammation. This is also a normal protective response to mild injury and resolves rapidly once the injury has healed. However there are various persistent pain conditions in which the stimulus and pain are unrelated. Thus the pain sensation outlasts its biological usefulness. In these conditions pain can no longer be regarded as a purely physiologically protective symptom. This may arise as a result of chronic pathological lesions or degenerative processes, but in many cases there may be no discernible pathology. These types of chronic pain state—which occur with migraine, rheumatoid arthritis, osteoarthritis, low back pain, cancer pain, and neuropathic pain—are poorly understood and for the most part difficult to treat. Thus chronic pain sufferers of one kind or another account for approximately 10–20% of the adult population, while about 5–8% experience pain which is inadequately treated.¹

Present treatment for most pain states has relied heavily on the use of non-steroidal anti-inflammatory drugs (NSAID) and opioid analgesics. Although variants of these classes of drug have been developed there has been little conceptual innovation in analgesia for many years. Furthermore both classes of drug produce side effects, such as gastrointestinal disturbances, gastric ulceration, renal damage, and hypersensitivity reactions with NSAID, and nausea, constipation, confusion, respiratory depression, and possibly dependence with opioids. Despite considerable effort these problems have not yet been overcome. An important reason for this has been the relatively slow advance, until recently, in understanding the pathogenesis of chronic pain. Some of these advances have been reviewed^{2,3} and are leading a conceptual shift in pain pathophysiology and management. In the light of this we shall discuss some recent initiatives for the discovery and development of new analgesic drugs based on knowledge of the pathophysiological and neurochemical changes that occur in the nociceptive pathway after injury.

Kinin receptor inhibition

Kinins are a family of small peptides which are produced locally in response to tissue injury or trauma and particularly during inflammation.

The two most studied of these are bradykinin and kallidin, a nonapeptide and a decapeptide respectively. Both are cleaved from precursor molecules following the activation of a biochemical cascade. In the plasma, bradykinin (Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg) is produced from high molecular weight kininogen (HMWK) whereas in tissue kallidin (lysyl-bradykinin) is liberated from its precursor, low molecular weight kininogen (LMWK)⁴ by the action of proteolytic enzymes (fig 1).

Both bradykinin and kallidin are rapidly degraded by peptidases known as kininases giving rise to inactive metabolites with the exception of des-Arg⁹bradykinin or des-Arg¹⁰kallidin which lack the carboxy-terminal arginine. These are the only metabolites of bradykinin or kallidin with significant activity.⁴

Bradykinin and kallidin mediate their effects through two main classes of receptor, B₁ and B₂.⁵ The genes for both of these receptors have recently been cloned and have been shown to belong to the superfamily of receptors of G protein coupled, 7-transmembrane spanning domains receptors.^{6,7} Less is known about the molecular characteristics of the B₁ receptor but it has little homogeneity with the B₂ receptor⁸ and in keeping with current pharmacological studies it seems likely that antagonists for this receptor will be different from those developed at B₂ sites.

Bradykinin and kallidin acts preferentially on the B₂ receptor, and this accounts for the majority of the acute pharmacological effects of bradykinin. The preferred agonists for the B₁ receptor, however, are the active metabolites, des-Arg⁹bradykinin and des-Arg¹⁰kallidin.

Bradykinin exerts a variety of effects on many body tissues but it is its actions on nociceptive neurones that are of most relevance to pain and analgesia. Bradykinin has two main effects on these neurones—activation and sensitisation—and both of these actions have been shown to be mediated primarily by the B₂ receptor.

Bradykinin depolarises sensory neurones by activation of phospholipase C and stimulation of protein kinase C, with an increase in membrane sodium ion conductance.^{9,10} This probably underlies the overt pain response to bradykinin. With respect to sensory neurone activity, bradykinin has been shown to increase nociceptor C fibre discharge in somatic and visceral sensory neurones both *in vitro*^{10,11} and *in vivo*.¹²

Sandoz Institute for Medical Research,
Gower Place, London
WC1E 6BN, United Kingdom
M Perkins
A Dray

Correspondence to:
Dr M N Perkins.

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Following administration to somatic and visceral tissue sites bradykinin elicits powerful nocifensor behaviours.¹³⁻¹⁵ Bradykinin is also algogenic in man following topical application or intracutaneous injection.^{16, 17} Bradykinin also has a powerful sensitising action on nociceptors, enhancing their responsiveness to mechanical and thermal stimulation.¹⁸⁻²⁰ This property of bradykinin is possibly of more relevance in man, increasing both the perception and duration of pain.

Although bradykinin is one of the first inflammatory mediators to be produced in response to trauma or inflammation, prostaglandins also play a major role in inflammation and enhance bradykinin induced responses. Not only is the neuronal activation by bradykinin potentiated by prostaglandins but they may also enhance bradykinin evoked release of the neurogenic inflammatory mediators substance P and calcitonin gene related peptide (CGRP).²¹

There are therefore strong experimental grounds for targeting the B₂ receptor in the treatment of inflammatory pain and consequently selective B₂ receptor antagonists based on the peptide structure of bradykinin have been developed (fig 2), the most potent being HOE 140.²² The use of this and other peptidergic antagonists has shown that the acute activation and sensitisation of sensory neurones by bradykinin are mediated by the B₂ receptor.^{5, 23} Furthermore, in several models of nociception, selective B₂ receptor antagonists^{24, 25} are antinociceptive.

Recently several studies have showed that during inflammation there is an increasing contribution of B₁ receptors to the accompanying hyperalgesia. Thus B₁ receptor antagonists such as Leu8,des-Arg⁹bradykinin are consistently antinociceptive.^{23, 26, 27-28}

The B₁ receptor appears to be expressed or synthesised de novo in tissues following trauma or when inflammatory mediators such as cytokines are present.^{23, 29} Indeed a close interaction has been shown between kinins other inflammatory mediators, particularly cytokines. In particular, interleukin-1 β upregulates B₁ and B₂ receptors as well as enhances responses to bradykinin and desArg⁹ bradykinin.^{23, 27, 30-32} The potential exists, therefore, for a powerful reinforcement between kinins and other inflammatory mediators leading to a sustained hyperalgesic state.

Cyclo-oxygenase inhibition

One of the major treatments for inflammatory pain has been the reduction of prostaglandin levels by inhibition of cyclo-oxygenase (COX) activity with NSAID. However, the development of tolerance and unacceptable side effects, such as gastric haemorrhage and nephrotoxicity, have severely limited the usefulness of this approach with respect to chronic or persistent inflammatory pain. It had been thought that there was a single cyclo-oxygenase enzyme responsible for prostaglandin production; however, recently two isoforms of cyclo-oxygenase have been demonstrated—a constitutive and an inducible enzyme (fig 3).³³ The

Figure 1 The formation of kinins following trauma and/or an inflammatory insult. Both bradykinin and kallidin act on the B₂ receptor. In addition, kallidin can be converted to bradykinin by the action of aminopeptidases. The preferred agonists for the B₁ receptor are desArg⁹bradykinin (desArg⁹Bk) and desArg¹⁰kallidin (desArg¹⁰Kd) which are formed by the action of kininase I-peptidase which removes the carboxy-terminal arginine residue of bradykinin and kallidin, respectively. Bradykinin and kallidin act directly on nociceptive neurones to cause overt excitation, leading to pain, and sensitisation of the terminals resulting in a reduction in threshold for activation. In addition, both these kinins act on immunocompetent cells (for example, macrophages) to release other inflammatory mediators such as prostaglandins and cytokines. The B₁ receptor is expressed on smooth muscle and other tissues such as endothelial cells and is upregulated following inflammation.

Bradykinin receptor ligands	
Agonists	Antagonists
B₁	Des-Arg9 Leu8 Bradykinin H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-OH
B₂	HOE 140 (Icatibant) H-(D)Arg-Arg-Pro-Hyp-Gly-Thi-Ser-(D)Tic-Oic-Arg-OH
Bradykinin H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH	NPC567 H-(D)Arg-Arg-Pro-Hyp-Gly-Phe-Ser-(D)Phe-Phe-Arg-OH
Kallidin H-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH	NPC16731 H-(D)Arg-Arg-Pro-Hyp-Gly-Thi-Ser-(D)Tic-Tic-Arg-OH

Figure 2 Structures of agonists and antagonists for the kinin B₁ and B₂ receptors.

constitutive enzyme (COX-1) is present in virtually all tissues and is important for the maintenance of normal cell function, particularly in the gastrointestinal tract and kidney. It is inhibition of this isoform that is probably responsible for the gastric and renal toxicity associated with sustained NSAID treatment.³⁴ COX-2 is virtually absent in normal tissues but is induced following an inflammatory insult such as carrageenin or endotoxin administration.^{35–38} The underlying trigger for the induction of COX-2 is still not entirely clear but interleukin-1 has been shown to induce the expression of mRNA for COX-2 as well as increasing its activity in many cell types, including primary human cell cultures or cell lines.^{35, 39, 40}

It has been proposed that the inhibition of COX-2 produces an anti-inflammatory and analgesic effect whereas the inhibition of COX-1 induces the toxic side effects attributed to NSAID. All the NSAID in current use inhibit both enzyme isoforms. However, most of them are either more potent inhibitors of COX-1 than COX-2, for example, indomethacin, ibuprofen and aspirin, or approximately equipotent, for example, diclofenac and acetaminophen,⁴² and all of these are associated with toxic side effects. Ibuprofen is, however, less ulcerogenic than aspirin and this may be related to observation that it is more potent at inhibiting COX-2 than aspirin.⁴² Dexamethasone also preferentially inhibits inflammation induced COX-2 activity.³⁷ Recently, much more selective COX-2 inhibitors have been developed, such as NS398, BF-389, and SC-58125, which have been shown to possess anti-inflammatory and anti-nociceptive properties in animal models, with no evidence of gastric toxicity.^{36, 37, 42} Selective inhibition of COX-2 may therefore provide a means of alleviating inflammatory pain without the damaging side effects associated with present treatments.

In addition to the development of NSAID with COX 2 selectivity there is the possibility that some of the NSAID may have an analgesic action distinct from inhibition of prostanoid synthesis. A recent study has suggested that diclofenac maybe have an anti-hyperalgesic action through the downregulation of

sensitised nociceptors, possibly through the nitric oxide-cGMP system.⁴¹ Further work is needed to establish whether it would be possible to develop NSAID which effectively reverse nociceptor sensitisation without inhibition of prostaglandin production.

Nitric oxide synthase inhibition

In the last few years there has been great interest in the physiology and pharmacology of nitric oxide (NO). This small gaseous molecule is generated from L-arginine by nitric oxide synthase (NOS) activity.⁴³ Once generated, NO acts as an intercellular messenger, diffusing to its target tissue, and stimulates soluble guanylate cyclase, thus increasing cGMP levels. The increased intracellular concentration of cGMP then regulates a variety of intracellular enzymes including protein kinases and phosphodiesterases.

Nitric oxide synthase, like cyclo-oxygenase, has also been shown to exist primarily in two forms, a calcium-calmodulin dependent enzyme which is expressed constitutively in neuronal and other tissues (cNOS) and an inducible form of the enzyme (iNOS), the activity of which is calcium independent. In addition, however, there are two isoforms of cNOS, so far located in endothelial cells and brain.⁴⁴ In the CNS, iNOS has been primarily located to glial cells⁴⁵ but recently there has been a report showing iNOS in cerebellar neurones.⁴⁶ The relative roles of constitutive and inducible NOS in neurones, remain to be clarified.

Nitric oxide synthase has been localised in both somatic and visceral sensory ganglia, particularly in small capsaicin sensitive cells with a dense concentration of the enzyme in the superficial laminae of the spinal cord where nociceptive afferents terminate, supporting a central as well as a peripheral role for NO in nociception.^{47, 48} In addition, following peripheral nerve lesion there is an increase in immunoreactive NOS and mRNA for NOS in dorsal root ganglia suggesting that it may play an important role in the peripheral neuropathy.⁴⁹

Studies to elucidate the functional role of NO have either used inhibitors of nitric oxide synthase based on guanidino-monosubstituted derivatives of L-arginine, such as L-N^G-nitro arginine methyl ester (L-NAME) and

Figure 3 The two isoforms of cyclo-oxygenase subserve different functions within the body. COX-1 is the constitutive enzyme and is involved in normal, physiological regulatory processes such as signal transduction. COX-2 is induced in immunocompetent cells following trauma and/or damage and has a role in the generation and maintenance of inflammation. Existing NSAID (eg, aspirin, ibuprofen) inhibit both isoforms of the enzyme but it is their action on COX-1 that is thought to result in renal and gastric toxicity. The induction or activity of COX-2 can be blocked by dexamethasone and, more recently, by specific inhibitors of this enzyme. Such compounds have been developed with the expectation that they will be anti-inflammatory but devoid of the toxic side effects seen with existing cyclooxygenase inhibitors.

L-N^G-monomethyl arginine (LN^GMMA), or compounds which increase NO levels such as L-arginine and sodium nitroprusside.⁴³ Neither L-NAME nor L-N^GMMA, however, discriminate between neuronal and endothelial nitric oxide synthase. Recently, a novel inhibitor of nitric oxide synthase, 7-nitro-indazole, has been described which shows selectivity for the neuronal form of the enzyme.⁵⁰ This agent has been shown to be anti-nociceptive in mice at doses that have no effect on blood pressure or vascular smooth muscle in vitro.⁵⁰ This raises the possibility that a nitric oxide synthase inhibitor could be developed that is anti-nociceptive but devoid of cardiovascular side effects.

There are many studies supporting a role for NO in nociception but the precise involvement is not yet clear. NO is pro-nociceptive in animal models of hyperalgesia, and inhibitors of nitric oxide synthase are antinociceptive against thermal and chemical stimuli. Thus L-NAME, but not its inactive isomer D-NAME, reversed formalin induced foot licking and acetic acid induced writhing in mice and carrageenin induced thermal hyperalgesia in rats. These effects were reversed by the NO precursor L-arginine.⁵¹⁻⁵⁵ NO does not, however, appear to be involved in the mechanical hyperalgesia as L-NAME has no effect in carrageenin induced hyperalgesia.⁵⁵ However NO has also been shown to be involved in thermal hyperalgesia in neuropathic pain in the rat.⁵⁶ In addition, L-NAME reduced the ongoing discharge seen in dorsal

root ganglia in rats following a peripheral nerve injury. This suggests a role for NO in the maintenance of spontaneous pain following neuropathic lesions.⁵⁷ Although there are few studies relating to NO and pain in man, a recent report showed that NO was algescic when injected intracutaneously.⁵⁸

Although the bulk of the evidence supports a pro-nociceptive role for NO there are also reports suggesting an anti-nociceptive action for this mediator. Most of this evidence relates to the administration of the precursor for NO, L-arginine. Thus, L-arginine has been shown to be anti-nociceptive when given centrally or peripherally, possibly through an endogenous opioid action as its actions were reversed by opioid receptor antagonists.⁵⁴⁻⁵⁹⁻⁶⁰ In addition, L-arginine itself can potentiate opioid induced analgesia.⁶¹ There is also evidence that NO mediates some of the nociceptive actions of NMDA within the spinal cord.⁵⁶

Part of the confusion may lie with the different roles that NO may subserve in nociceptive perception and transmission. NO may well be involved as a second messenger mediating some of the effects of neuronal transmitters both centrally and peripherally, but it may also contribute directly to the generation and maintenance of hyperalgesia. In the former case, therefore, inhibition of nitric oxide synthase could lead to hyperalgesic or analgesic responses, depending on whether the neuronal circuits that use NO as a second messenger raise or lower nociceptive thresholds. Where NO is one of the primary hyperalgesic agents

such inhibition would be expected to be antinociceptive, and indeed this is largely the case.

An additional complication lies in the specificity of some of the pharmacological tools used to manipulate NO levels. In particular, L-arginine, which is used to raise NO concentrations in many studies, may also be effective as a precursor of kyotorphin which is a substance reported to be an endogenous Met-enkephalin releaser.⁶² Increasing NO levels within the CNS by the use of L-arginine may therefore also increase the levels of this endogenous opioid. Such a mechanism may contribute to the reported anti-nociceptive actions of L-arginine, particularly where there is evidence for an opioid involvement in this phenomenon.^{59 61}

In summary, the evidence relating NO to nociception suggests a complex interrelationship between it and other mediators involved in the generation and maintenance of hyperalgesia. The development of inhibitors which are more selective for the neural form of nitric oxide synthase, including ones which are restricted in their action to the periphery, will aid in clarifying the role of NO in nociception.

Capsaicin analogues

Capsaicin is the major pungent extract from hot peppers of the capsicum family. It has the unique property of selectively activating polymodal nociceptors following local or systemic administration. It has long been established that large systemic doses of capsaicin in the neonatal and adult animal are neurotoxic to small unmyelinated C-fibres⁶³ and these high dose treatments lead to a very long lasting or even permanent increase in nociceptive thresholds.^{63 64} However, lower doses of capsaicin result in an acute reversible antinociceptive effect, distinct from the toxic effects seen with much higher doses.^{65 66}

The pharmacological and physiological basis for the acute antinociceptive activity remains unclear but may well be related to the action of capsaicin on neuronal ion channels. In particular, activation of cation specific ion channel causes initial depolarisation followed by an inactivation of voltage gated calcium channels and a subsequent reduction of calcium coupled neurochemical release from either the peripheral or central terminals of the sensory neurones.⁶⁷ Such a mechanism may underlie the short lasting (two to four hour) antinociceptive and anti-inflammatory actions of capsaicin observed with acute systemic applications. With higher or more prolonged administration of capsaicin other mechanisms may be responsible for the loss of sensitivity to both capsaicin and other stimuli, probably involving receptor desensitisation, an impairment of signal transduction, or conduction block in the sensory nerve fibre, or a combination of these.^{64 68} It is likely that capsaicin acts on a specific receptor, localised to sensory neurones, and studies with the first competitive capsaicin antagonist, capsazepine, have supported this hypothesis.⁶⁹

The anti-nociceptive properties of capsaicin, together with its selectivity for polymodal nociceptors, have made it of particular interest to those studying pain mechanisms and as a possible target for future drug development.⁶⁴ Locally applied capsaicin has been used clinically to alleviate painful conditions such as postmastectomy pain, reflex sympathetic dystrophy pain, diabetic neuropathy, and post-herpetic neuralgia.⁷⁰⁻⁷³ However, the usefulness of capsaicin itself in these conditions is severely limited by the initial burning sensation experienced with this compound, which can be severe enough to lead to patients discontinuing the treatment. It has therefore been challenging to separate the excitatory and pungent actions from the antinociceptive properties, and indeed analogues of capsaicin such as NE19550 and NE21610 have been shown to have less agonistic activity^{74 75} and yet retain analgesic as well as anti-inflammatory activity. It therefore seems likely that capsaicin analogues with desirable features can be developed into analgesics of the future (fig 4).

Tachykinin receptor antagonism

Among the most extensively studied of the potential nociceptive mediators within the CNS are tachykinins, a class of neuropeptides including substance P, neurokinin A, and neurokinin B. Of these, substance P has been most extensively investigated with respect to nociceptive mechanisms. There are three receptor types, NK-1, NK-2, and NK-3; substance P acts preferentially on the NK-1 receptor.⁷⁶ Substance P is located in sensory neurones and is released from their central terminals in the superficial laminae in the dorsal horn of the spinal cord.⁷⁷ It both directly depolarises dorsal horn neurones and enhances the responsiveness of cells in the dorsal horn to an excitatory amino acid, NMDA, known to be involved in the transmission of nociceptive information. In particular, the phenomenon known as "wind up", characterised by an increase in the excitability of dorsal horn neurones following repetitive C fibre stimulation,

Figure 4 The structures of capsaicin and two analogues olvanil (NE 19550) and nuvanil (NE 21610) which retain the anti-nociceptive properties of capsaicin but show much reduced agonist activity. Capsazepine is the first example of a competitive antagonist of capsaicin.

is reduced by NK-1 receptor antagonists, suggesting that substance P is involved in the underlying mechanism.⁷⁸

The pharmacology of tachykinins with respect to nociception appears to differ between acute and persistent nociceptive conditions. In acute nociception, NK-2 receptors appear to be the predominant receptor involved, with the endogenous mediator being NKA rather than substance P. Thus NK-1 antagonists are less effective in acute nociceptive models than NK-2 receptor antagonists such as MEN10376 and L659874.^{78,79} In persistent inflammatory hyperalgesia, however, it is substance P acting on the NK-1 receptor that is primarily involved, with a corresponding upregulation of mRNA for this receptor subtype.

An important advance in this field has been the development of non-peptide antagonists for tachykinin receptors, particularly the NK-1 subtype. In functional tests these antagonists, such as CP96345 and RP67580, are anti-nociceptive in models of persistent hyperalgesia.^{80,81} The emergence of these non-peptide tachykinin antagonists gives rise to the prospect of a novel class of centrally acting, orally available analgesics. Several of these compounds are undergoing clinical trials at present.

Excitatory amino acid receptor antagonism

Excitatory amino acids (EAA), and in particular glutamate, play a pivotal role in the transmission of nociceptive signals within the dorsal horn of the spinal cord. Glutamate is one of the major neurotransmitters of sensory fibres, being released from C fibre afferents in response to noxious stimuli, and it therefore mediates the first transmission step in the neural nociceptive pathway. Consequently, antagonism of this class of neurotransmitters has been an obvious and attractive target for novel analgesic drugs.

EAA are hyperalgesic when applied intrathecally and induce behavioural responses such as biting and scratching suggesting an algescic reaction; these responses are inhibited by antagonists at EAA receptors.^{82,83} Of particular interest in recent years has been the role of the NMDA receptor (upon which glutamate acts) in the increase in spinal hyperexcitability produced by persistent noxious input into the spinal cord and associated with chronic pain. This phenomenon of wind up has already been referred to and it is clear that NMDA receptors play a key role in the generation and maintenance of this state.^{78,84} NMDA receptor antagonists such as D-AP5, CPP, MK801, and more recently memantine, inhibit wind up and have been shown to be anti-nociceptive in animal models of persistent hyperalgesia and neuropathic pain.⁸⁵⁻⁸⁹ However, a major drawback in the development of such compounds as analgesics is the presence of unacceptable side effects at, or close to, the analgesic dose. Both motor disturbances such as ataxia and psychomimetic-like behavioural responses have been reported in animal models when

NMDA antagonists are given.⁹⁰ Similar symptoms have also been seen in man when an NMDA antagonist was given intrathecally to relieve neuropathic pain.⁹¹

Although the best evidence for EAA in nociception relates to central mechanisms, there have also been studies suggesting an involvement of peripheral EAA receptors in nociceptor activation. Thus functional non-NMDA receptors (kainate/AMPA) have been demonstrated on C fibre nociceptors in vitro, and the depolarisation of spinal neurones following peripheral kainic acid application was blocked by spinally applied morphine.^{92,93} This raises the possibility that peripherally released glutamate or a related amino acid may contribute to the activation or sensitisation of nociceptor terminals, in which case a peripherally acting receptor antagonist may be analgesic.

It may prove impossible to separate the undesirable side effects of centrally active NMDA antagonists from their anti-nociceptive properties, but if this can be achieved there is the prospect of a novel class of analgesics with efficacy in both acute and chronic pain. On the other hand further work is needed to characterise the peripheral EAA receptors and to develop novel antagonists without the associated side effects seen with centrally acting agents.

In conclusion, great progress has been made in understanding the changes occurring in nociceptive pathways during chronic pain conditions. This has caused a fundamental shift in the way that pain therapy and the development of analgesic drugs are being directed. Mechanistic studies have brought to light a number of new targets both in the periphery (B₁ receptors, COX-2, nitric oxide synthase) and in the CNS (nitric oxide synthase, NMDA, tachykinin receptors). This has stimulated the development of antagonists for specific kinin receptors, selective COX-2 inhibitors, and capsaicin analogues which block the activity in fine afferent fibres. In the CNS, there are various opportunities to develop NK1 receptor antagonists and to overcome the difficulties associated with the present generation of NMDA antagonists. Such developments will inevitably supplement or replace the traditional use of NSAID and opioids in the treatment of pain

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