Anti-inflammatory effects of κ-opioids: relevance to rheumatoid arthritis

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Therapy with opioids is an exciting new development for arthritis, especially since there is the potential for fewer side effects from molecules that act outside the central nervous system. We have found κ-opioid drugs to be powerfully anti-inflammatory, reducing disease severity by as much as 80% and attenuating arthritis in a dose-dependent, stereoselective, antagonist-reversible manner. In contrast, opioids acting at other receptors were therapeutic only at near toxic doses. Currently, however, no pure κ-opioids are available for clinical use. The hypothalamic-pituitary-adrenal axis was found to be only partially involved; thus we investigated other neural and immune mechanisms. The results showed that the κ-opioid anti-inflammatory actions were exerted via: (1) reduced adhesion molecule expression; (2) inhibition of cell trafficking; (3) reduced tumour necrosis factor release; and (4) alterations in mRNA expression and substance P (SP) and calcitonin gene-related peptide protein in joint tissue. The ability of κ-opioids to act at multiple sites in the inflammatory cascade, as suggested by the presence of opioid receptors at various locations throughout the cascade, may explain their powerful actions. κ-Opioids are, however, most therapeutic during disease onset; thus it is likely they exert their anti-inflammatory effects predominantly via changes in cellular activation and cytokine expression rather than via the nervous system. The involvement of SP and the efficacy of neurokinin 1 (NK1) antagonists predicts that combined opioid–NK1 regimens have therapeutic promise. Peripherally acting opioids may prove to be a potent new treatment for rheumatoid arthritis sufferers in the future.

Introduction

Opioid drugs are not currently used in the treatment of rheumatoid arthritis, partly because of their range of side effects and because their anti-inflammatory (as opposed to analgesic) actions have been largely unrecognized. The synthesis of peripherally selective κ-opioid agonists has allowed the analgesic and anti-inflammatory effects of opioids in arthritis to be studied, while mitigating the problems of tolerance and central side effects. They are powerfully anti-inflammatory in a dose-dependent, time-dependent, stereoselective and antagonist reversible manner.1 This brief report examines the anti-inflammatory effects of κ-opioids, both centrally active and peripherally selective κ-opioid agonists, with particular relevance to rheumatoid arthritis, and reports data on the mechanisms responsible for the anti-arthritic effects of κ-opioids in adjuvant arthritis.

Opioids exert their diverse physiological effects through three distinct membrane-bound receptor subtypes mu (μ), delta (δ) and kappa (κ)
in the central nervous system (CNS) and periphery. The different receptors have diverse behavioural characteristics; for example, euphoria, physical dependence and respiratory depression are mainly associated with μ- and δ-receptors. In contrast, opioids acting at κ-receptors produce dysphoric rather than euphoric effects, which limit their physical dependence liability. In this regard, κ-opioid agonists possess some advantages over μ-agonists: they are devoid of such side effects as dependence liability, constipation and respiratory depression.

Classically, opioids have been used in the treatment of pain rather than inflammation, partly due to their side effects and because their anti-inflammatory actions have been largely unrecognized. A great deal is known about the analgesic effects of opioids and the actions of opioids on the hyperalgesic aspects of inflammation have been comprehensively reviewed. Apart from our own work there have been relatively few studies of their peripheral anti-inflammatory effects, so a brief overview of these is presented here.

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There are conflicting reports concerning whether μ-opioids have anti- or proinflammatory properties. For example, morphine inhibits carrageenan-induced paw swelling and near toxic doses of morphine were able to attenuate adjuvant arthritis in rats. In contrast, low doses of morphine were proinflammatory in adjuvant arthritis. High doses would preclude the clinical use of morphine in arthritis, so specific attention was given to κ-opioids, particularly because they have a more favourable side effect profile.

Historically, opioids have been thought to produce their antinociceptive effects via actions in the CNS, but it is now well appreciated that opioid receptors are synthesized in the dorsal root ganglia and transported towards both central and peripheral nerve terminals. Furthermore, the peripheral axonal transport is upregulated during inflammation. Experimental and clinical studies have shown potent analgesic effects after peripheral administration of opioids. For example, the pioneering work of Robert Schmidt’s group in Germany has shown the local action of opioids in the knee joint of the cat. Morphine (μ-agonist) and PNU50488H (κ-agonist) reduce the action potential frequency in group III (Aδ) fibres of an articular nerves; reversal of this action by naloxone indicates an opioid receptor mediated action. This local action has clinical significance because intra-articular morphine produced pain relief after knee arthroscopy and in patients undergoing dental surgery after submucous injection, without overt systemic effects. A large body of work has also demonstrated that the local administration of low doses of opioid receptor agonists elicit potent analgesic effects in inflamed but not non-inflamed tissue. Clearly, there are functional opioid receptors on the peripheral terminals of afferent nerves that could well be exploited clinically.

κ-Agonists belong to four chemical classes, namely: the peptides (related to the endogenous ligand dynorphin), the benzomorphans (prototype ethylketocyclazocine), the aryacetamides (prototype PNU50488H), and the benzodiazepine derivative trifluadom. They include the centrally acting and prototype PNU50488H, which has served as the structural starting point for the synthesis of a multitude of compounds such as the centrally acting compounds: PNU62066E, PD117302 and GR89696A.

To minimize the problems of tolerance and central side effects, various chemical approaches have been utilized to make opioids less accessible to the brain without reducing κ-opioid activity. The structure of PNU50488H has been the basis for the development of many of these compounds. Asimadoline (EMD 61753; Merck KGaA), an amphiphilic compound that is orally active, is undergoing Phase II clinical trials in musculoskeletal pain. Most recently, other aryacetamide-derived peripherally selective agents, ADL 10-0101 and ADL-10-0116 (Adolor Corporation, USA), have been undergoing Phase I safety and efficacy studies.

In our laboratory we induce chronic polyarthritis in the rat by the administration of complete Freund’s adjuvant. This model of chronic arthritis has proved to be the most common; it is reproducible and shows many similarities to the human disease. We measure disease severity by using three quantitative indicators: paw swelling (oedema), radiological damage, and histological characteristics. We have tested the anti-inflammatory effects of κ-opioids in this model. κ-Opioids (e.g., PNU50488H and asimadoline) attenuated the progression of
experimental adjuvant arthritis via specific opioid receptors in the periphery using rigorous criteria such as reversibility by opioid antagonists, dose-dependency and stereospecificity.\textsuperscript{1,9,12,24,25} The anti-arthritic actions of \(\kappa\)-opioids were blocked by the opioid antagonist naloxone methiodide, which does not penetrate the CNS, and by specific \(\kappa\)-opioid antagonists; thus the anti-inflammatory effects are most likely exerted in the periphery via \(\kappa\)-opioid receptors.

This effect was seen with a number of \(\kappa\)-opioids, including low doses of centrally acting compounds (PNU50488H administered peripherally into a joint,\textsuperscript{24} PD117302\textsuperscript{26} and peripherally selective asimadoline,\textsuperscript{1} so our work has clinical potential because these drugs could be used with the minimal likelihood of central side effects such as addiction and tolerance. In contrast, morphine was anti-inflammatory only at very high doses (median effective dose \(\sim 58\) mg/kg).

The temporal details of the treatment regimens were found to be important. Opioid action is most significant in the first few days of treatment (i.e., at disease onset) and is cumulative over 21 days of treatment. These data support current opinion that aggressive drug therapy needs to be started as soon as possible after disease onset to prevent progressive joint destruction.\textsuperscript{1,24,27}

The finding that \(\kappa\)-opioids reduce inflammation in adjuvant arthritis raises interesting questions about the mechanisms and inflammatory mediators involved. The actions of \(\kappa\)-opioids are mediated via \(\kappa\)-receptors, which are found on immune system cells and on neural cells (for review see Figure 1 in Sharp et al., 1998\textsuperscript{28}). The close spatial and functional association between nerves and immune cells suggests that modulation of immune or neural proinflammatory pathways could contribute to the disease suppressing activity of these drugs.\textsuperscript{9}

A major point of neuro-immune convergence is in what is known as the hypothalamic–pituitary–adrenal (HPA) axis (Figure 1). Is it possible therefore that the anti-arthritic action of the \(\kappa\)-opioids is mediated via the HPA axis? Using the prototype PNU50488H in adrenalectomized rats, arthritis developed sooner and was more severe. However, PNU-50488H substantially reduced the pooled severity index (combined quantitative oedema, histological and radiological assessments) at day 18 in both control and adrenalectomized rats to an equal extent. Thus, the HPA axis is only partially involved in the anti-inflammatory actions of opioids.\textsuperscript{29} We therefore continued our investigations into other neural and immune mechanisms.

### Role of the nervous system

Neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP) are strongly implicated in the pathogenesis and/or spread of inflammatory arthritis.\textsuperscript{30} By using two differently acting drugs (the \(\kappa\)-agonist asimadoline, and the neurokinin 1 (NK1) antagonist GR205171) the roles that SP may play in the pathogenesis and maintenance of experimental arthritis were investigated. Depending upon the timing of their administration, both agents significantly attenuated adjuvant arthritis, putatively via a mechanism that involves SP. We hypothesize that asimadoline acts on peripheral terminals to modulate SP release, while GR205171 antagonizes the action of SP, either peripherally or centrally. Time-dependent multiphasic effects were found with asimadoline on SP tissue levels (i.e., protein) in the joints from rats with adjuvant arthritis. Treatment with asimadoline decreased SP levels in the joint during early arthritis (day 3) and increased levels were found during established disease (day 21\textsuperscript{25}).

Drug-induced changes in SP content could result from a change in either the release or synthesis from neural cells. We therefore further investigated neuropeptide production in cell bodies innervating the joints as a function of time. Asimadoline reduced the expression of both SP and CGRP mRNA in the dorsal root ganglion on either day 13 or day 21 postadjuvant.\textsuperscript{31} This reduction in peptide mRNA is likely to be a consequence of a negative feedback mechanism resulting from an increase in SP in the nerve terminals (and therefore enhanced axonal transport) rather than a direct action on peptide synthesis by the \(\kappa\)-agonist.\textsuperscript{32} Our studies confirm literature findings on the inhibitory actions of opioids on SP release from the peripheral endings of primary afferent fibres.\textsuperscript{32}
**Figure 1:** Schematic illustration of neuro-immune interactions in the control of inflammation. Opioid receptors are identified by the purple blocks and suggest possible sites of action. IFN-γ, interferon-γ; ICAM-1, intercellular adhesion molecule-1; PGE₂, prostaglandin E₂ (source: Wilson, 1998,²⁶ reproduced with permission from the author).
Role of the immune system

Opioids have been found to regulate lymphocyte proliferation, antibody production and natural killer cell activity as well as inhibiting the function of neutrophils, monocytes and macrophages. Thus the hypothesis that opioids may exert their anti-inflammatory actions via opioid receptors that exist on immune cells, as shown by Sharp’s group (see Figure 1 in Sharp et al., 1998), together with our own observations that opioids decrease the numbers of both mast cells and macrophages infiltrating the joint, is an attractive one.

The pretreatment of rats with a selectin blocker, fucoidin, abolishes peripheral opioid analgesia. The question arises of whether blockade adhesion molecules such as Intercellular adhesion molecule-1 (ICAM-1) abolishes inflammation in arthritis. The effect of the κ-opioid PD117302 on ICAM-1 expression was thus compared with the effects of a prototypic non-steroidal anti-inflammatory drug, naproxen, in the same model. In animals treated with PD 117302 and naproxen there was a significant attenuation of arthritis, however, only PD117302 was able significantly to inhibit the upregulation of ICAM-1 (a marker of inflammation) expression in arthritic joints.

Decreased cytokine release may explain the abrogation of the upregulation of ICAM-1 expression, reduced leucocyte recruitment, and less synovial inflammation observed in the adjuvant arthritic rats. In support of this, κ-opioids also directly inhibit the release of both interleukin-1 and tumour necrosis factor (TNF) from macrophages. We further investigated this using cultured macrophages in vitro and examined the effects of a range of κ-opioids on TNF release from these cells.

The production of TNF was significantly inhibited in the presence of asimadoline, PD117302 and PNU50488H in a dose-dependent, antagonist reversible, manner over the dose range of 10⁻¹¹ to 10⁻³ M with 100% suppression at a concentration of 10⁻³ M. Thus, κ-opioids may act directly to inhibit cytokine release from immune cells. Taken together with our earlier findings of decreased cell recruitment and reduced expression of adhesion molecules, these data help to explain the powerful anti-inflammatory effects observed clinically in the adjuvant arthritis model.

Summary

In summary, therapy with opioids is an exciting new development for arthritis, especially since there is the potential for fewer side effects from molecules that act outside the CNS. We found κ-opioid drugs to be powerfully anti-inflammatory, reducing disease severity by as much as 80%, attenuating arthritis in a dose-dependent, stereoselective, antagonist reversible manner. In contrast, opioids acting at other receptors were therapeutic only at near toxic doses. Currently, however no pure κ-opioids are available for clinical use. The HPA axis was found to be only partially involved; thus we investigated other neural and immune mechanisms. The results showed that the κ-opioid anti-inflammatory actions were exerted via: (1) reduced adhesion molecule expression; (2) inhibition of cell trafficking; (3) reduced TNF release; and (4) alterations in mRNA expression and protein levels of SP and CGRP in joint tissue. The mechanisms involved are summarized in Figure 1. The ability of κ-opioids to act at multiple sites in the inflammatory cascade, as suggested by the presence of opioid receptors at various locations throughout the cascade, may explain their powerful actions. κ-Opioids are, however, most therapeutic during disease onset; thus it is likely that they exert their anti-inflammatory effects predominantly via changes in cellular activation and cytokine expression, rather than via the nervous system. The involvement of SP and the efficacy of NK1 antagonists predict that combined opioid-NK1 regimens have therapeutic promise.

Thus our work supports the findings of Stein’s group in humans, that opioids do indeed have powerful actions in the periphery via specific receptors. This study examined intra-articular morphine in chronic arthritis. Synovial leucocyte counts were lower after morphine than after saline. Intra-articular morphine produced analgesia of a similar magnitude to dexamethasone and it may therefore have anti-inflammatory actions in human arthritis. Furthermore, unpublished data from our laboratory demonstrates
that the clinically available, oxycodone, which has κ-opioid activity, is also anti-arthritic in the Freund’s adjuvant model of arthritis.

Peripherally acting opioids may prove to be a potent new treatment for rheumatoid arthritis sufferers in the future.

Acknowledgements
I am very grateful to the dedicated students, post-doctoral fellows and entire research staff of my laboratory over the past decade. In particular, I would like to thank the following persons for their contributions to this research: Professor Richard Day, Associate Professor John Carmody, Dr Bruce Kirkham, Drs Jodie Wilson, Dr Waltraud Binder, Dr Caroline Scott, and Katherine Bush.

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