**Peripheral opioid analgesia**

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Major recent findings in peripheral opioid analgesia include the relative lack of tolerance under inflammatory conditions, tetrapeptides as novel peripherally restricted compounds, the potent anti-inflammatory activity of μ and κ agonists and the identification of selectins as important molecules governing the homing of opioid cells to injured tissue. Clinical studies have now moved into the field of chronic arthritic pain, a problem of major relevance and prevalence.

**Introduction**

The mediation of opioid analgesic effects has long been thought to occur exclusively within the central nervous system (CNS). Around a decade ago, reports on the existence of opioid receptors outside the CNS and the generation of analgesia by these peripheral receptors began to accumulate. Such analgesic effects are particularly prominent in painful inflammatory conditions and have been demonstrated both in animals and in humans (reviewed in [1]). Opioid receptors are present on peripheral sensory nerves and are upregulated during the development of inflammation. Their endogenous ligands, opioid peptides, are expressed in resident immune cells within peripheral inflamed tissue. Environmental stimuli (stress) and releasing agents (corticotropin-releasing factor, cytokines) can liberate these opioid peptides to elicit local analgesia, whereas suppression of the immune system abolishes these effects. These findings have led to the concept that endogenous opioid peptides can be secreted from immunocytes, occupy opioid receptors on sensory nerves and produce analgesia by inhibiting the excitability of these nerves and/or the release of proinflammatory neuropeptides (reviewed in [2]). This article will focus on publications of the last two years that have extended this concept. In particular, recent studies have examined the development of tolerance, anti-inflammatory effects, novel peripherally selective agonists, inhibition of visceral pain, adhesion molecules governing the homing of opioid-containing immune cells to injured tissue, and novel therapeutic applications.

**Peripheral opioid receptors**

Anatomical, molecular and electrophysiological studies have shown that all three opioid receptors (μ, δ and κ) are expressed within sensory neurons (reviewed in [3•]). They have been found on cell bodies in the dorsal root ganglia (DRG) and on peripheral terminals of primary afferent neurons in animals and in humans [3•]. A recent study used an antibody against the cloned δ-opioid receptor (DOR) and showed this receptor on unmyelinated sensory fibers, but not on postganglionic sympathetic neurons, in skin, lip and cornea [4•]. Together with previous functional studies using neurotoxins against primary afferent versus sympathetic neurons, these findings have corroborated the idea that peripheral opioid receptors mediating analgesia are localized exclusively on primary afferent neurons [3•].

The activation of these receptors by agonists can trigger several pathways, resulting in an attenuated excitability of peripheral nociceptive terminals and a decreased propagation of action potentials in sensory neurons. Such mechanisms include increased potassium [6] and decreased calcium currents [7–9] through interactions with G proteins (G<sub>i</sub>, G<sub>o</sub>), and the inhibition of tetrodotoxin-resistant sodium currents (for references see [3•]). Furthermore, opioids inhibit the calcium-dependent release of pronociceptive, proinflammatory compounds (e.g. substance P) from peripheral sensory nerve endings, which may account not only for antinociceptive but also for anti-inflammatory effects (see below) [2].

**Peripheral opioid receptors and inflammation**

Peripheral opioid analgesic effects have been observed mainly under pathological conditions like inflammation, neuropathy, colonic distension or bone damage. Inflammation was induced using Freund's adjuvant, formalin, carrageenan or prostaglandin in subcutaneous tissue, viscera or joints (reviewed in [1,10•]). During inflammation an increased axonal transport of receptors and receptor upregulation on peripheral nerve terminals is observed (for references, see [3•]). In addition, the specific milieu of inflamed tissue may increase opioid agonist efficacy by altering the interaction of receptors with G proteins (<i>G<sub>i</sub>, G<sub>o</sub></i>) and by increased neuronal cAMP levels [1,2]. Inflammation also increases the number of sensory nerve terminals and disrupts the perineurium, a diffusion barrier for high molecular weight or hydrophilic substances (such as exogenous and endogenous opioids) [3•]. Opioid receptors have also been demonstrated on immune cells but the functional significance of those receptors for the generation of analgesia has not been investigated so far [1,3•].

**Tolerance development**

Central side effects typically associated with opioids (e.g. tolerance, dependence, respiratory depression) might be avoided by the peripherally restricted application of agonists. Tolerance, however, has also been observed in peripherally
mediated opioid analgesia [11]; importantly though, the latter studies were performed in a model that does not entail inflammation. Opioid agonists were applied topically onto the uninjured skin of mouse tail and the reaction to heat stimuli (tail flick) was observed [11]. Another group used a model of hyperalgesia induced by local injections of bradykinin into the mouse paw [12]; again, signs of inflammation were not described. In this model peripherally (but not centrally) mediated morphine analgesia was reported to be resistant to tolerance development in two studies [12,13]. In a subsequent paper, the same group reported acute tolerance development of peripheral morphine- but not U-69593- (κ agonist) induced antinociception in the identical model [14]. However, this contradiction was not discussed by the authors. A lack of tolerance development was shown after repeated local administration of loperamide (a μ agonist, see below) in a thermal inflammation model [15*]. In the same study systemically applied morphine produced only partial cross-tolerance with loperamide [15*]. Clinical studies have also suggested a lack of cross-tolerance between local morphine- and endogenous opioid-induced analgesia [16]. These and earlier studies [1] have raised, but not conclusively answered, the important question whether tolerance does or does not develop in peripheral opioid analgesia. The weight of the evidence tends to argue for a relative lack of tolerance development in peripheral opioid analgesia under inflammatory conditions. Clearly more basic and clinical investigations are needed to resolve this important issue and the underlying mechanisms. Given the prominent role and the clinical relevance of models of inflammation, it seems that such models should be studied preferentially.

**Novel peripheral opioid agonists**

Many conventional opioid agonists produce potent opioid receptor-mediated analgesia when administered locally at small, systemically inactive doses into injured tissue of rodents and humans (reviewed in [1]). Recent studies have confirmed these findings in nonhuman primates [17,18]. Strategies to restrict the access of opioid agonists to the CNS include the incorporation of highly polar hydrophilic substituents [3*]. Loperamide, an old drug originally developed as an antidiarrheal agent, was recently presented as a peripherally active antihyperalgesic μ agonist in a model of thermal injury [15*]. Exclusion from the CNS is apparently due to the active removal of the drug by the multidrug resistance transporter, its high affinity to lipid membranes and its ability to decrease surface tension. This results in its accumulation in membranes and subsequent lack of systemic absorption [15*,19*]. Loperamide exhibits high affinity and selectivity for the cloned human μ-opioid receptor and was also effective after local administration in epidermal abraision, formalin-, Freund’s-adjuvant-induced inflammation and knee joint inflammation in the rat [19*]. After intravenous or oral application, loperamide apparently gets trapped in liver, kidney and lung, or stomach and intestines, respectively [19*].

Another approach based on the inclusion of both hydrophilic and hydrophobic portions in molecules was developed and preserved both peripheral selectivity and high antinociceptive potency [3*]. An example is asimadoline (EMD 61753). It has been described as a potent and selective κ agonist with restricted ability to cross the blood–brain barrier after systemic administration. In healthy human volunteers, asimadoline was rapidly absorbed into the blood and well tolerated after oral application. Unfortunately the oral application of this drug was ineffective in patients with postoperative pain [20]. In rats with Freund’s adjuvant-induced paw inflammation, it produced early κ opioid receptor-mediated antinociception but late non-opioid, non-NMDA-mediated hyperalgesia and proinflammatory effects [20]. In visceral pain models, asimadoline clearly inhibited the excitation of pelvic nociceptive afferents after colorectal distension and heat [21], as well as visceral nociceptive behaviors [22]. However, consistent with the above studies in paw inflammation, experiments using antisense oligonucleotides against the cloned rat κ opioid receptor suggested that asimadoline acted at an as yet unidentified non-κ opioid receptor in the colon [23*].

Recently, novel peptide κ ligands were identified by positional scanning of a tetrapeptide combinatorial library screened in opioid receptor radioligand binding assays [24]. These peptides have an all D-amino acid sequence and exhibit high selectivity for the κ receptor. Further preliminary studies showed that these ligands are potent κ agonists in vitro, potent antinociceptive agents in vivo and peripherally selective, as shown by the lack of sedative activity in the mouse rotarod test after systemic administration. Further chemical optimisation led to a second generation of tetrapeptide κ agonists with high peripheral selectivity and long duration of action [25]. In rats with Freund’s adjuvant-induced paw inflammation, two of these compounds (FE 200665 and FE 200666) were shown to be peripherally selective κ agonists with potent analgesic effects. Moreover, both peptides also exhibited significant anti-inflammatory properties as measured by paw volume and histological signs [26*].

These studies show that both novel and conventional opioid agonists are available for the local administration into injured tissue and are ready for clinical use. According to preliminary data the latter group of κ agonists may even be suitable for intravenous administration [25]. Agents for the oral route are not on the horizon yet.

**Anti-inflammatory effects**

An extremely intriguing feature of peripheral opioid agonists is their potential for disease-modifying anti-inflammatory activity. Evidence for such activity is accumulating in animal and human studies and the mechanisms underlying this have been examined extensively (reviewed in [3*]). Opioids inhibit neurogenic inflammation by decreasing the release of substance P from peripheral terminals of primary afferent neurons and opioid receptors on immune cells can mediate suppression of lymphocyte function as well as inhibition of the synthesis or release of cytokines. Recently, the locally administered μ agonist endomorphin-1 has been shown to reduce carrageenan-induced edema [27], the vascular...
response to electrical stimulation of the sciatic nerve and substance P-induced vasodilatation and plasma extravasation in the rat paw [28]. Asimadoline was shown to inhibit potently ankle edema as well as radiographically and histologically measurable ankle damage in Freund’s adjuvant-induced polyarthritis in rats [28]. Two novel κ agonists also exhibited a reduction of volume and histological signs of paw inflammation [26•]. A clinical study showed a reduced number of inflammatory cells in synovial fluid after intra-articular morphine in patients with chronic arthritis [30•]. On the other hand, two studies found non-opoid proinflammatory effects of asimadoline [20] and morphine [31]. However, in context with earlier findings [3•], the weight of the evidence clearly indicates a potential for anti-inflammatory activity of peripheral μ and (highly selective) κ agonists.

Peripheral endogenous opioid analgesia

Potent peripheral analgesia can be accomplished by the interaction of peripheral opioid receptors with opioid peptides released from immune cells [2,3•]. In rat paw inflammation, mRNAs encoding pro-opiomelanocortin (POMC) and proenkephalin (PENK) and the respective opioid peptide products β-endorphin (END) and enkephalin are found in lymphocytes, monocytes and macrophages. Small amounts of dynorphin are also detectable [1,2]. Recent studies indicate that END-producing immune cells home to inflamed tissue where they secrete the opioid to inhibit pain. Afterwards they travel to the regional lymph nodes, depleted of the peptide [32]. The mechanisms underlying the migration of opioid-containing cells to inflamed tissue are currently under investigation. Extravasation of immune cells involves the sequential activation of adhesion molecules. Initially, circulating leukocytes slow down and roll on the vascular endothelium, a process mediated by selectins on leukocytes (L-selectin) and endothelial cells (P- and E-selectin). Chemokines then lead to upregulation of integrins, which mediate the firm adhesion of leukocytes to endothelial cells. Finally, the cells transmigrate through the endothelial wall and are directed to the sites of inflammation. Interruption of this leukocyte–endothelial cell cascade (e.g. by antibodies against adhesion molecules or by other blocking agents) blocks immune cell extravasation [3•] and can also influence endogenous pain control. Immune cells containing opioids coexpress L-selectin [33••] and pretreatment of rats with a selectin blocker (fucoidin) abolishes peripheral opioid analgesia [34••]. This results from a blockade of the infiltration of END-containing cells and the consequent decrease of END content in the inflamed tissue [34••]. These findings indicate that the immune system uses mechanisms of cell migration not only to fight pathogens but also to control pain within injured tissue. Thus, pain may be exacerbated by measures inhibiting the immigration of opioid-producing cells or, conversely, analgesia may be conveyed by adhesive interactions that recruit those cells to injured tissue.

Clinical studies

Peripheral opioid actions are undoubtedly of clinical relevance. Opioid receptors on peripheral nerve terminals as well as opioid peptides have been demonstrated in human synovia [1]. A sizeable body of clinical literature has recently been reviewed and has demonstrated the analgesic efficacy of locally applied opioids in various clinical settings [35•]. The most extensively studied clinical situation (over 50 papers in peer-reviewed journals) is the intra-articular application of opioid agonists for pain control after knee surgery (reviewed in [1,35•]). Recent important additions to this field have demonstrated that intra-articular morphine analgesia is dose-dependent [36] and that intra-articular morphine is as effective as a standard steroid in reducing pain, as well as synovial inflammation in chronic arthritis [30•]. Not surprisingly, some reports have been unable to show analgesic effects [1]. Many such studies, however, were hampered by methodological shortcomings, such as lack of assay sensitivity owing to weak pain stimuli, lack of tissue inflammation and/or the superimposition of general or local anesthetic effects (e.g. [37]). Novel routes of administration include perineural [38], intra-abdominal [39], and orbital [40] and topical wound infiltration [41,42] with conventional opioids.

Conclusions

In summary, we have discussed recent developments in the field of peripheral opioid analgesia, including localization, signaling pathways and tolerance development of peripheral opioid receptors, novel peripherally restricted agonists, anti-inflammatory effects, the migration of opioid-containing immune cells and clinical applications. Major recent findings are the localization of opioid receptors to primary afferent (but not sympathetic) neurons, the relative lack of tolerance under inflammatory conditions, loperamide and all D-amino acid tetrapeptides as peripherally restricted compounds, potent experimental and clinical anti-inflammatory activity of μ and κ agonists and the identification of selectins as important adhesion molecules governing the homing of opioid cells to injured tissue. Clinical studies have now moved into the field of chronic arthritic pain, obviously a problem of major importance and prevalence. Although the studies on topical opioid application are very promising, a major long term goal remains to develop opioids with restricted access to the CNS. For widespread use in patients, such drugs should be active after oral, intravenous or subcutaneous administration. Other interesting avenues to be explored are the site-directed trafficking of opioid-containing immune cells to injured tissue and the transgenic enhancement of opioid production and release by such cells.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest

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Stein et al.


A detailed review with emphasis on peripheral opioid receptors, including electrophysiology, opioid producing immune cells and anti-inflammatory effects.


An excellent analysis of peripheral opioid receptors on sensory versus sympathetic neurons.


An interesting novel application of an old compound with peculiar antihyperalgesic action of a novel peripheral mu-opioid receptor agonist—loperamide.


