Endogenous opioid analgesia in peripheral tissues and the clinical implications for pain control

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Abstract: Opioid receptors are widely expressed in the central and peripheral nervous system as well as in numerous nonneuronal tissues. Both animal models and human clinical data support the involvement of peripheral opioid receptors in analgesia, particularly in inflammation where both opioid receptor expression and efficacy are increased. Immune cells have been shown to contain numerous opioid peptides such as β-endorphin (END), met-enkephalin (ENK), and dynorphin-A (DYN), although the predominant opioid peptide involved in immune-cell mediated antinociception is thought to be END. These opioid-containing immune cells migrate to inflamed tissues during a complex process of recruitment by chemokines, adhesion, and extravasation. In these tissues, opioid peptide is released from the immune cells upon stimulation with corticotrophin-releasing factor (CRF), noradrenaline, and interleukin 1β (IL-1β), and the immune cells return to the local lymph node depleted of peptide. Consistent with this model, systemic immunosuppression may lead to impaired endogenous analgesia as competent immune cells are essential to achieve release of endogenous opioid peptides within inflamed tissue. A further level of complexity is added by the observation that exogenous opioids may impair immune cell function, although there is some evidence to suggest that endogenous opioid peptides do not share this immunosuppressive effect. Improving our understanding of endogenous opioid mechanisms will provide valuable insight towards the development of novel treatments for pain with improved side effect profiles.

Keywords: Opioid, analgesia, inflammation, immunosuppression, immune cell, pain

Introduction

Pain arising from inflammatory conditions is often treated with agents that inhibit the immune response, such as corticosteroids, or modulators of the inflammatory cascade, such as nonsteroidal antiinflammatory drugs (NSAIDs) (Dequeker 1999). These drugs are associated with substantial adverse effects and risks with long-term use, ranging from gastrointestinal (GI) insult to opportunistic infections, and osteoporosis (Hirschowitz 1994; Dequeker 1999). While opioid receptor agonists have limited or, more likely, poorly understood antiinflammatory action, they remain useful effective analgesic agents in these conditions. However, respiratory depression, sedation, and constipation remain as dose-limiting side effects after systemic administration of this drug class. Tolerance, while not a common occurrence, can develop during long-term use (Sessle and Henry 1985; Mansour et al 1988; Marinangeli et al 2004; Jensen et al 2005).

The immune system is traditionally thought of as a contributor to inflammatory pain. However, a new analgesic role has been described for the immune system in the release of endogenous opioid peptides from immune cells within inflamed tissue (Stein et al 1997). The benefit of peripheral immune-mediated opioid analgesia is

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targeted analgesia at the site of inflammation, and thus avoidance of side effects mediated by activation of opioid receptors in the central nervous system (CNS), or on peripheral organs such as the GI tract. Although immune cell-mediated endogenous opioid analgesia appears to challenge our current understanding of the role of the immune system in inflammation, it is likely that the two approaches to inflammatory pain treatment are not in contrast, but complementary. The role of the immune system in peripheral analgesia is likely to be delicately balanced, depending on whether the action of infiltrating cells is proinflammatory, proalgesic, or both; either predicted by the immune cell, or by the injury, or disease status. In situations where endogenous antinociception exceeds hyperalgesia mediated by immune cells, immunosuppression or modulation of immune cell trafficking may increase pain (Hermanussen et al 2004).

A better understanding of the endogenous pathways and mechanisms involved in peripheral analgesia may not only enable us to target these pathways for novel antinociceptive approaches and avoid systemic side effects of opioid treatment, but it will also allow us to identify patients in whom immunosuppression may have detrimental effects on pain control and minimise the possible algesic effects of immunosuppression in these patients.

Opioid receptor distribution and mechanisms that mediate antinociception

Since their first characterization in 1973, 3 main opioid receptors, referred to as μ (MOR), δ (DOR), and κ (KOR), have been defined (Pert and Snyder 1973; Simon et al 1973; Terenius 1973; Law et al 2000). These opioid receptors can exist as several splice variants, which are differentially expressed in the central and peripheral nervous systems as well as varying nonneuronal tissues, presumably due to different messenger ribonucleic acid (mRNA) processing as they are derived from the same gene (Abbadie et al 2000; Law et al 2000).

Opioid receptor structure and function

Opioid receptors are prototypical G-protein coupled receptors belonging to the subfamily of rhodopsin receptors and consist of approximately 400 amino acids (Law et al 2000; Pil and Tytgat 2003). On a molecular basis, they possess 7 α-helical transmembrane domains and an extracellular N-terminus with multiple glycosylation sites (Law et al 2000). MOR, DOR, and KOR are highly homologous to each other at the sequence and post-translational levels, with particularly high conservation in the regions spanning the transmembrane domains and intracellular loops (Knapp et al 1995; Akil et al 1996; Law et al 2000; Pil and Tytgat 2003). Divergence can be found at the N- and C-termini as well as the extracellular loops, accounting for the unique pharmacological properties of these receptors (Knapp et al 1995; Akil et al 1996; Law et al 2000; Pil and Tytgat 2003). Opioid receptors can couple to both pertussis toxin-sensitive and insensitive G-proteins, with coupling characteristics differing slightly between the receptor types. G-protein coupling of opioid receptors to their effectors can be direct or involve intermediate or other indirect effector pathways (Connor and Christie 1999). Activation of inwardly rectifying K^+ channels ($GIRK^+$), inhibition of voltage-dependent Ca^{2+} channels as well as inhibition of adenylyl cyclase are direct G-protein-coupled effects of opioid receptor activation (Connor and Christie 1999; Law et al 2000). Conversely, opioid receptors can activate phospholipase C (PLC), the mitogen-activated protein kinase (MAPK) cascade, and large conductance Ca^{2+}-activated K^+ channels by utilising other intermediary messenger systems (Connor and Christie 1999; Law et al 2000). Modification of Ca^{2+} and K^+ conductance by opioid receptors can lead to a decrease in neuronal excitability, a decrease in neuronal firing rate, and inhibition of neurotransmitter release (Connor and Christie 1999; Law et al 2000). Functionally, opioid receptors have been implicated in regulation of pain, reinforcement, reward, neuroendocrine modulation, and alteration in neurotransmitter release (Mansour et al 1995). The consequence of opioid receptor activation appears to be correlated closely with the anatomical location as well as expression levels of the opioid receptor subtypes (Mansour et al 1995).

Opioid receptor expression and function in the central nervous system

Opioid receptors are widely and differentially distributed in the CNS. MOR, in particular, is widely distributed throughout the forebrain, midbrain, and hindbrain with greatest expression apparent in the neocortex, caudate-putamen, nucleus accumbens, thalamus, hippocampus, amygdala, and nucleus tractus solitarius (Mansour et al 1988, 1995). This distribution of the MOR is consistent with its suggested role in pain perception as well as sensorimotor integration. Conversely, KOR has been found to be expressed in moderate amounts in many brain areas, with

greatest expression in the caudate putamen, nucleus accumbens, hypothalamus, amygdala, and neural lobe of the pituitary (Mansour et al 1988, 1995). KOR has been implicated to be involved in feeding, pain perception, and neuroendocrine function. DOR is particularly highly expressed in olfactory related neural areas, the neocortex, caudate-putamen, nucleus accumbens, and amygdala while exhibiting limited binding in the thalamus, hypothalamus, and brainstem (Mansour et al 1988, 1995). This distribution corresponds with the suggested involvement of DOR in motor as well as olfactory and cognitive functioning.

The functional consequences arising from these expression patterns of opioid receptors may affect many other physiological systems, which can have important implications for the clinical use of opioids, particularly in the unwanted side effects of systemically administered opioid receptor agonists. Many side effects arising from systemic administration of opioids are due to activation of central opioid receptors. Administration of MOR agonists has been linked to centrally mediated cardiovascular and renal effects (Mansour et al 1988, 1995; Gutkowska et al 2004; Mao and Wang 2005). Of particular relevance to the use of opioids as analgesics are respiratory depression mediated though activation of MOR and DOR in the brainstem, as well as KOR-mediated sedation; both of which can be dose-limiting side effects of systemic treatment with opioids (Sessle and Henry 1985; Mansour et al 1988).

Opioid receptor expression and function in the spinal cord and peripheral nervous system

Opioid-mediated analgesia is mediated by modulation of ascending and descending pain pathways (Mansour et al 1988; Vaughan et al 1997; Pan et al 2004). Accordingly, MOR, DOR, and KOR expression has been confirmed in dorsal root ganglia, the spinal cord, and trigeminal nucleus of the ascending pain pathway as well as the central Gray area, pontine, gigantocellulare, and intermediate reticular nuclei of the descending pain pathway, with predominantly MOR and KOR expression in the median raphe and raphe magnus (Mansour et al 1988). In the periaqueductal gray region and the nucleus locus coeruleus, opioid receptor agonists produce analgesia by disinhibiting descending fibres through inhibition of Gamma-Aminobutyric acid (GABA)-ergic neuronal inputs, which modulates other descending pathways in turn, such as noradrenergic neurons, to produce analgesia (Vaughan et al 1997; Pan et al 2004).

In addition to spinal and centrally mediated opioid analgesia, opioid receptors expressed on peripheral neurons can also contribute to antinociception. Numerous animal models and human clinical trials in support of this peripheral opioid analgesia have been described and will be discussed in greater detail in the next section. While targeting these peripheral mechanisms may provide analgesic strategies that can avoid some or all of the central side effects of systemic opioid agonists, some peripherally mediated side effects may remain problematic. This is a result of opioid receptor expression on peripheral nervous system neurons innervating peripheral organs such as the skin and GI tract (Bagnol et al 1997; Fickel et al 1997; Bigliardi-Qi et al 2004; Holzer 2004). MOR and KOR have been found to be expressed in the stomach, duodenum, jejunum, ileum as well as proximal and distal colon, where their function is thought to include control of visceral pain, regulation of transit time of luminal contents, and mucosal transport of fluids and electrolytes (Bagnol et al 1997; Fickel et al 1997; Eastwood and Grundy 2000; Holzer 2004; Gray et al 2005). Accordingly, the adverse effect of systemic administration of opioid agonists on GI function is thought to arise primarily from modulation of the enteric pathways governing peristalsis by opioid receptor agonists (Bagnol et al 1997; Fickel et al 1997; Holzer 2004). The effect of opioid receptor agonists on gut motility and secretion has been utilised clinically in the symptomatic management of diarrhea (Sun et al 1997). Furthermore, opioid receptors may be involved in the modification of GI inflammation, possibly through the protein kinase C-mediated desensitization of chemokine receptors (Philippe et al 2003; Zhang et al 2003).

Opioid receptor expression and function in nonneuronal tissues

Opioid receptor protein and mRNA have also been described in several nonneuronal tissues such as vascular and cardiac epithelia as well as keratinocytes, although the significance of opioid receptor expression in these nonnociceptive tissues is less clear (Bidlack 2000; Cadet et al 2000; Mousa et al 2001; Bigliardi-Qi et al 2004). MOR, DOR, and KOR are also expressed on various immune cells including lymphocytes and macrophages. Activation of the receptors have been reported to modulate immune function including the macrophage oxidative burst and cytokine production (Bidlack 2000). Evidence for the expression of all 3 types of opioid receptors on immune cells stems from functional evidence suggesting that MOR, DOR, and KOR agonists

can modulate immune cell function, and includes immunohistochemical and binding studies which verify the expression of MOR, DOR, and KOR on numerous immune cells (Bidlack 2000). In addition, mRNA encoding for opioid receptors has been obtained from immune cells and supports the identification of opioid receptors expressed on immune cells as identical to neuronal receptors (Bidlack 2000).

In summary, opioid receptor protein and mRNA have been described in both central and peripheral nervous systems as well as in several nonneuronal tissues. Activation of these differentially distributed receptors by systemic treatment with opioid receptor agonists not only causes analgesia, but can be directly correlated to clinically observed adverse effects such as respiratory depression, sedation, and constipation (Sessle and Henry 1985; Mansour et al 1988, 1995; Fickel et al 1997; Vaughan et al 1997; Gutkowska et al 2004; Holzer 2004; Pan et al 2004; Mao and Wang 2005).

Peripheral opioid receptors and their contribution to analgesia

Including centrally mediated effects, the potential that opioid receptors of the peripheral nervous system have for mediating effective analgesia is well documented. Common strategies to isolate the activation of peripheral opioid receptors include the administration of local, systemically inactive doses of opioids, and the use of hydrophilic opioids that do not readily penetrate the blood-brain barrier.

Animal models

In animal models of inflammatory pain, peripheral MOR, DOR, and KOR participate in the suppression of pain. In the inflammatory phase of the formalin test in rats, novel peripherally restricted μ-opioid agonists, administered subcutaneously, produced dose-dependent antinociception (Furst et al 2005). This effect was reversed by the peripherally restricted antagonist naloxone methiodide, confirming the activation of peripheral MOR (Furst et al 2005). The DOR agonist SNC80 produced dose-dependent antinociception in PGE_2-induced hyperalgesia of the rat paw following subcutaneous administration (Pacheco and Duarte 2005). This effect was not observed after equivalent administration at a remote site, indicating that antinociception was delivered by peripheral DOR (Pacheco and Duarte 2005). KOR-selective opioid agonists, when administered subcutaneously to the paw, evoked potent dose-dependent increases in pain thresholds after Freund's complete adjuvant (FCA)-induced chronic inflammation, an effect antagonized by naloxone methiodide (Binder et al 2001). In an animal model of inflammatory bowel disease, peripherally restricted κ-opioid agonists produced dose-dependent, peripherally antagonized reductions in visceromotor responses and afferent nerve activity, providing further evidence that peripheral KOR effects antinociception (Sengupta et al 1999). In a single study using FCA-induced inflammation of the rat paw, locally acting MOR-, DOR-, and KOR-selective agonists delivered antinociception that was dose-dependent, stereospecific, and reversible by receptor specific antagonists (Stein et al 1989). In addition to inflammatory pain, peripheral opioid receptors reportedly attenuate nociceptive responses in rats following thermal injury (Nozaki-Taguchi and Yaksh 1999), and in animal models of uterine cervical distension (Sandner-Kiesling et al 2002), neuropathic pain (Pertovaara and Wei 2001; Truong et al 2003; Obara et al 2004), and nociceptive pain (Furst et al 2005). Moreover, a technique involving viral-driven transfer and overproduction of proenkephalin A, a precursor for the endogenous opioid peptide enkephalin in trigeminal ganglion neurons, resulted in long lasting attenuation of allodynia in a rat model of neuropathic pain (Meunier et al 2005). Immunohistochemistry indicated that transgene-derived opioids were transported predominantly to the peripheral ends of primary sensory neurons. The abolition of transgene-derived antinociception by naloxone methiodide confirms that peripheral opioid receptors were responsible for the attenuation of allodynia in this model (Meunier et al 2005).

Human clinical studies

Peripheral opioid receptor-mediated analgesia has also been demonstrated in controlled clinical studies. In experimental human pain models, administration of the peripherally restricted opioid agonists morphine-6-glucoronide (M6G) reduced hyperalgesia induced by freeze lesions and excessive muscle contraction, which was verified to be via peripheral action due to the absence of characteristic CNS effects (Tegeder et al 2003). Patients with chronic pancreatitis reported reductions in abdominal pain following intravenous infusion with a peripherally restricted κ-opioid agonist (Eisenach et al 2003). Similarly, the administration of locally acting opioids during knee and dental surgery significantly reduces postoperative pain and recovery time (Dionne et al 2001; Likar et al 2001; Kalso et al 2002). Topical application of opioids for the treatment of painful skin ulcers and oral mucositis has also achieved effective relief of pain (Twillman et al 1999; Cerchietti et al 2002).

Separate bioavailability studies indicate that therapeutic doses of opioids applied topically to skin ulcers are not readily absorbed into the blood and are therefore likely to act peripherally (Ribeiro et al 2004). Some clinical studies assessing the efficacy of locally acting opioids failed to show an improvement in pain intensity scores, but reported more subtle modifications such as increases in time to first analgesic request and a reduction in total analgesic requirement (Likar et al 2004; Zajaczkowska et al 2004).

Despite overwhelming evidence that peripheral opioid receptors mediate effective analgesia, there is limited direct evidence describing opioid mechanisms at peripheral nerve endings. This is because elucidation of opioid receptor mechanisms has relied heavily upon techniques that utilize cultured neurons and other cellular expression systems. However, experiments in animal models using ion channel blockers indicate that opioid receptors on peripheral sensory nerve endings mediate analgesia partly through local activation of adenosine triphosphate (ATP)-sensitive K^+ channels (Pacheco and Duarte 2005), and inhibition of L-type Ca^{2+} channels (Figure 1) (Shimizu et al 2004).

Inflammation promotes opioid receptor mechanisms in the periphery

An important determination from both animal and human studies is that peripherally acting exogenous opioids produce enhanced analgesia in the presence of inflammation. For example, in the rat paw model, locally acting opioid agonists significantly increase nociceptive thresholds to mechanical stimuli in inflamed tissue, but not in noninflamed tissue (Stein et al 1989; Binder et al 2001). In human studies, reports from dental surgery patients show that chronically inflamed tissue exhibits improved analgesic responses compared with acutely inflamed (Dionne et al 2001) and noninflamed tissue (Likar et al 2001). These findings indicate that inflammation promotes opioid receptor mechanisms in the periphery.

Opioid receptor expression

In primary afferent neurons, opioid receptors are manufactured in the dorsal root ganglion (DRG) and are transported both centrally and peripherally by axonal transport (Hassan et al 1993). In the rat paw model,

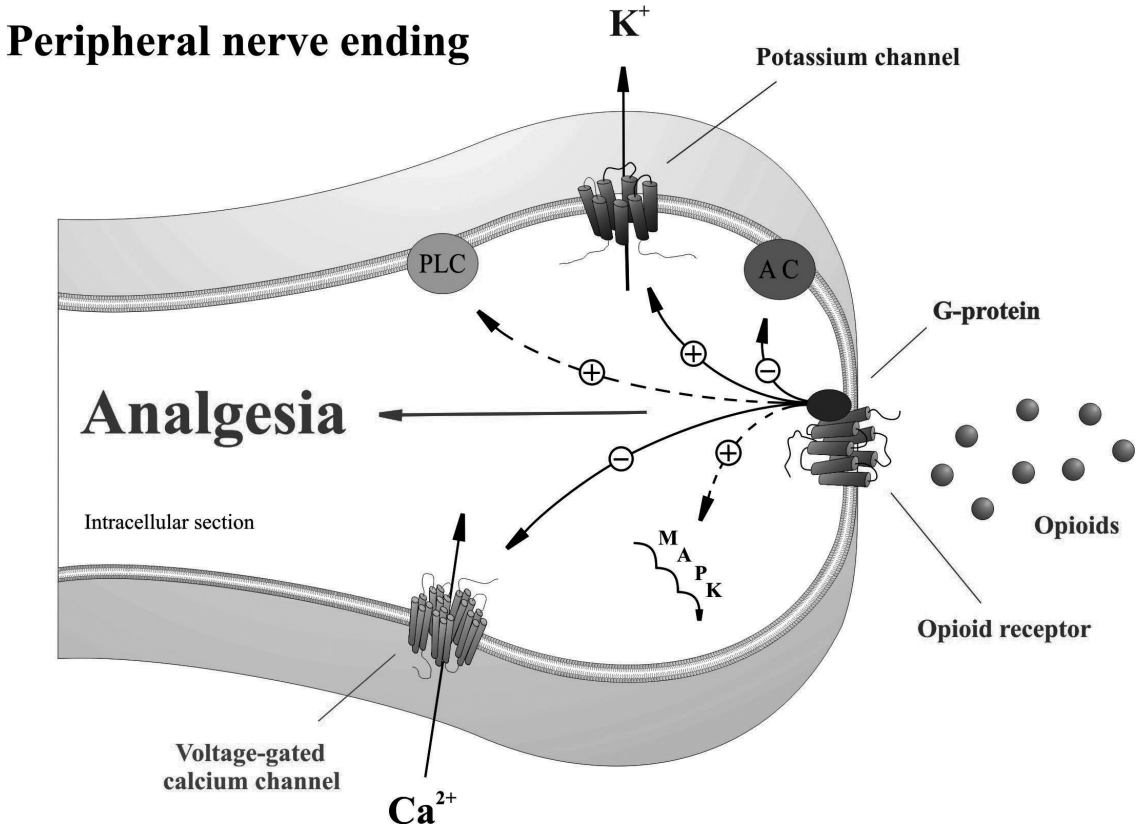


Figure 1 Proposed model for opioid receptor-mediated analgesia at peripheral terminals of primary sensory neurons. Activation of opioid receptors by endogenous or exogenous opioid agonists promotes G-protein coupling. Opioid receptor-coupled G-proteins directly activate inwardly rectifying K^+ channels ($GIRK^+$), inhibit voltage-dependent Ca^{2+} channels, and inhibit adenylyl cyclase (AC). Opioid receptors indirectly activate phospholipase C (PLC), the mitogen-activated protein kinase (MAPK) cascade, and large conductance Ca^{2+}-activated K^+ channels by utilizing other intermediary messenger systems.

inflammation induces a significant increase in MOR in the DRG (Ji et al 1995; Zhang et al 1998; Ballet et al 2003; Zollner et al 2003; Shaqura et al 2004) and in peripheral nerves innervating the inflamed tissue (Hassan et al 1993; Mousa et al 2001, 2002). In the spinal cord, there are reports of either no change (Ballet et al 2003; Shaqura et al 2004), or increase in MOR expression (Ji et al 1995; Mousa et al 2002); a discrepancy likely due to differences in disease state and the sensitivity of analytical techniques. Consistent with increased receptor expression, MOR mRNA levels are increased in both the DRG (Puehler et al 2004) and in the spinal cord (Maekawa et al 1996), which supports the thesis that inflammation increases MOR synthesis. With respect to DOR and KOR, the effect of inflammation on receptor expression is less clear in the rat paw model. In separate studies with similar disease states, DOR expression is reported to decrease in DRG (Ji et al 1995; Zhang et al 1998), increase in peripheral tissues (Hassan et al 1993), and both increase and decrease in the spinal cord (Ji et al 1995; Cahill, Morinville, et al 2003). DOR mRNA levels reportedly remain unchanged in the DRG (Puehler et al 2004) and in the spinal cord (Maekawa et al 1996). In independent studies, KOR expression has been found to both decrease and remain static in DRG (Ji et al 1995; Zhang et al 1998), and remain constant in the spinal cord (Ji et al 1995). A significant increase in KOR mRNA has been observed in the spinal cord of animals with peripheral inflammation (Maekawa et al 1996). Adding further complexity; changes in KOR and DOR expression have also been reported in nonneuronal cells. More specifically, the expression of KOR and DOR have recently been shown to be decreased in the fibroblast-like synoviocytes of patients with rheumatoid arthritis and osteoarthritis, particularly where inflammation was present (Shen et al 2005). Upregulation of the KOR occurred through exposure to the specific KOR agonist U69593 (Shen et al 2005), and while these changes occurred in cells isolated from patients and established in vitro, these observations could suggest that intra-articular or peripheral opioids may be useful in the management of these inflammatory conditions and could have an impact on pain states in these patients. Despite these apparently conflicting outcomes, DOR- and KOR-selective opioid agonists deliver potent antinociception in the inflamed rat paw model, suggesting the involvement of both DOR and KOR receptors in pain control during inflammation (Stein et al 1989; Binder et al 2001; Cahill, Morinville, et al 2003). In a separate inflammatory model, the inflamed mice intestine, MOR mRNA, and protein is upregulated in nerves innervating the gut (Pol et al 2001). Furthermore, intestinal inflammation significantly increases DOR mRNA in peripheral nerves and in the spinal cord, and increases DOR and KOR receptor protein in peripheral nerves (Pol et al 2003).

The mechanisms that affect opioid receptor upregulation during inflammation are not yet well understood, however, a number of recent studies provide insight into these mechanisms. The inflammatory mediator interleukin-6 (IL-6) strongly induces MOR, but not DOR transcription and translation in the human neuroblastoma cell line SH-SY5Y (Borner et al 2004). IL-6 evoked increases in MOR expression are mediated by the transcription factors signal transducers and activators of transcription 1 (STAT1) and transcription 3 (STAT3) (Borner et al 2004). In vitro results with IL-6 are supported by findings that IL-6 knock out mice have reduced MOR levels in the grey matter of the midbrain compared with wild type individuals and exhibit reduced analgesic responses to morphine (Bianchi et al 1999). Interleukin-4 stimulates MOR transcription in cultured immune cells and cultured neurons via transcription 6 (STAT6) activation of the MOR gene promoter (Kraus et al 2001). Furthermore, the proinflammatory cytokine tumor necrosis factor (TNF) induces MOR gene transcription, via the transcription factor nuclear factor κB (NFκB) in immune cells (Kraus et al 2003) and neurons (Borner et al 2002). Nerve growth factor (NGF) is produced by peripheral tissues, particularly during inflammation (McMahon 1996), and is implicated in opioid receptor regulation. Transgenic mice that overexpress NGF show an increase in MOR and KOR expression and a decrease in DOR expression in DRG compared with wild type mice, and exhibit increased nociceptive thresholds (Zwick et al 2003). Further evidence that NGF influences opioid receptor function is provided by findings that intrathecal NGF restored opioid effectiveness in a rat model of neuropathic pain (Cahill, Dray, et al 2003). In addition to the action of inflammatory cytokines and growth factors, the increased conduction of nociceptive signals that occurs during inflammation promotes opioid receptor transcription in sensory neurons, particularly during the early stages of inflammation (Puehler et al 2004). This was evidenced by blocking neuronal conduction with local anesthetic, which prevented the upregulation of MOR mRNA in the first 2 hours following induction of inflammation in the rat hind paw (Puehler et al 2004).

Opioid receptor efficacy

The ability of opioid agonists to stimulate radiolabelled Guanosine 5′-Triphosphate (GTP) ([^{35}S]GTP-γ-S) binding, provides a measure of G_i/G_o-protein coupling efficiency and

the functional status of the opioid receptor (Traynor and Nahorski 1995). In DRG, but not spinal cord tissue, (D-Ala, N-Me-Phe, Gly-ol)-Enkephalin (DAMGO) stimulated (^{35}S)GTP-γ-S binding is increased in animals with inflammation (Ballet et al 2003; Zollner et al 2003; Shaqura et al 2004). These results may simply reflect the increase in opioid receptor density that occurs in DRG during inflammation, but nevertheless represent an improvement in the efficacy of the μ-opioid agonist DAMGO to stimulate opioid receptor G-protein coupling and may explain the increased antinociceptive effects of exogenous opioids in inflammatory conditions (Zollner et al 2003). Moreover, in cultured sensory neurons, the expression of MOR mRNA correlates directly with opioid-evoked inhibition of voltage-gated Ca^{2+} channels (Silbert et al 2003), which also suggests that the increase in opioid receptor expression evident during inflammation translates into a functional enhancement of opioid efficacy. Inflammation also alters the subcellular distribution of opioid receptors, mobilizing receptors towards the cell membrane (Cahill, Morinville, et al 2003), and disrupts the perineurial barrier surrounding sensory nerve fibres, providing opioid peptides better access to cell membrane receptors (Antonijevic et al 1995). Some inflammatory chemokines have been reported to desensitize opioid receptors on peripheral sensory neurons in vitro and to suppress opioid antinociception to the noninflamed periaqueductal gray region of the rat brain when administered before opioid treatment (Szabo et al 2002; Zhang et al 2004). These findings suggest that elements of the inflammatory process may, in isolation, impair opioid efficacy. However, opioids can exert a reciprocal desensitization of chemokine receptors (Zhang et al 2003), which is evidence of the complexity of the interaction between chemokine and opioid receptors; a relationship that is not fully understood. Furthermore, in the inflamed rat paw, inflammatory chemokines recruit opioid-containing polymorphonuclear cells. The blockade of these chemokines reduces peripheral opioid-mediated antinociception, which is evidence that chemokines promote opioid mechanisms (Brack, Rittner, et al 2004b). Tissue acidosis is another characteristic of inflammation that may affect opioid efficacy. Interstitial pH as low as 5.6 has been observed in various inflamed tissues (Edlow and Sheldon 1971; Jacobus et al 1977; Ward and Steigbigel 1978; Simmen et al 1994; Andersson et al 1999). In NG108-15 cell membranes, acidic pretreatment increases opioid receptor-mediated inhibition of adenylyl cyclase by impairing GTPase activity (Selley et al 1993). Further evidence that acidic inflammatory pH improves opioid efficacy is provided by studies using DRG neurons, which show that morphine inhibition of Ca^{2+} transients is enhanced at low extracellular pH (Kapitzke et al 2005).

Taken together, the changes that occur during inflammation contribute to enhanced opioid receptor expression and efficacy, particularly for MOR.

Immune cells contain opioid peptides

Endogenous opioid peptides

In mammals, pro-opiomelanocortin (POMC), pro-enkephalin (PENK) and pro-dynorphin (PDYN) are the precursor proteins from which the main endogenous opioid peptides β-endorphin (END), met-enkephalin (ENK), and dynorphin-A (DYN), respectively, are derived (Nakanishi et al 1979; Kakidani et al 1982; Noda et al 1982). Dynorphin A and B, which are derived from PDYN, have relatively high affinity for KOR, but also some affinity for MOR and DOR. ENK and leu-enkephalin are endogenous ligands derived from PENK that display preferential binding to DOR, lower affinity for MOR, and negligible binding to KOR (Przewlocki and Przewlocka 2001). END, along with several nonopioid peptides, is derived from the precursor protein POMC and exhibits equipotent MOR and DOR binding while having lower affinity for KOR (Akil et al 1981; Przewlocki and Przewlocka 2001). Endomorphin-1 and endomorphin-2 are MOR-selective endogenous peptides of so far unknown origin that have been found in distinct regions of rat brain as well as primary neurons and the spinal cord (Martin-Schild et al 1997; Zadina et al 1997; Schulz et al 1998)

Opioid peptides in nonneuronal cells

The role of END, ENK, and DYN has been described in detail for both central and peripheral antinociceptive pathways (Przewlocki and Przewlocka 2001). Interestingly, nonneuronal tissues have also been described to contain opioid peptides that may contribute to peripheral antinociception.(Cabot et al 1997, 2001; Schafer et al 1994; Finegold et al 1999; Lin et al 2002; Mousa et al 2004). Opioid peptides have been shown to be expressed in keratinocytes (Khodorova et al 2003), and it has been suggested that these cells may be an important source of endogenous opioid receptor agonists. In particular, activation of both the endothelin-B and cannabinoid CB2 receptors

have been shown to elicit analgesia in vivo through release of END from keratinocytes (Khodorova et al 2003; Ibrahim et al 2005) This occurred in models where pain was induced either through injection of endothelin-1, or a noxious thermal stimulus, and may highlight that local release of opioid peptides can achieve anatomically specific analgesia (Khodorova et al 2003; Ibrahim et al 2005) .

Immune cells are of particular interest with respect to nonneuronal cell types involved in endogenous analgesic mechanisms. Expression of opioid peptides by immune cells have been extensively studied and have been shown to express both full-length and truncated mRNA encoding for the precursor proteins POMC, PENK, and PDYN. Moreover, the respective opioid peptides END, ENK, and DYN have been described in many immune cells of rodent and human origin (Lolait et al 1986; Smith et al 1986; Zurawski et al 1986; Oates et al 1988; Buzzetti et al 1989; Rosen et al 1989; Stein, Hassan, et al 1990; Hassan et al 1992; Przewlocki et al 1992; van Woudenberg et al 1992; Schafer et al 1994; Linner et al 1996; Cabot et al 1997, 2001; Lyons and Blalock 1997; Kamphuis et al 1998; Blalock 1999; Binder et al 2004; Mousa et al 2004). Originally, the role of these opioid peptides was thought to consist of the regulation of local immune function through the neuroendocrine axis (Lolait et al 1986; Oates et al 1988; Rosen et al 1989; Manfredi et al 1995; Kamphuis et al 1998; Blalock 1999). This was supported by the observation that immune cells express opioid receptors (Carr et al 1989). In addition, functional evidence verified that opioids can modulate immune function, including mitogen-induced proliferation, antibody production, and natural killer (NK) activity (Manfredi et al 1995). An additional role for these peptides emerged with the observation that opioid peptides released locally from immune cells can act on opioid receptors expressed on peripheral neurons to exert an antihyperalgesic effect in inflamed tissue (Stein, Hassan, et al 1990; Schafer et al 1994, 1996; Cabot et al 1997, 2001; Cabot 2001; Mousa et al 2001, 2004; Rittner et al 2001; Rittner and Stein 2005).

Immune cells express opioid peptides and their precursor proteins

The mechanism proposed for this immune-derived peripheral antinociception involves recruitment of immune cells to local inflamed areas, where they release endogenous opioid peptides upon stimulation with proinflammatory mediators such as interleukin 1β (IL-1β) and corticotrophin-releasing factor (CRF) as well as sympathetic mediators such as noradrenaline (Stein, Hassan, et al 1990; Schafer et al 1994, 1996; Cabot et al 1997, 2001; Cabot 2001; Mousa et al 2001, 2004; Rittner et al 2001; Binder et al 2004; Rittner and Stein 2005). Numerous leukocytes, including lymphocytes, macrophages, monocytes, and human peripheral blood mononuclear cells, have been reported to contain these opioid peptides (Lolait et al 1986; Smith et al 1986; Zurawski et al 1986; Oates et al 1988; Buzzetti et al 1989; Rosen et al 1989; Stein, Hassan, et al 1990; Hassan et al 1992; Przewlocki et al 1992; van Woudenberg et al 1992; Schafer et al 1994; Linner et al 1996; Cabot et al 1997, 2001; Lyons and Blalock 1997; Kamphuis et al 1998; Blalock 1999; Binder et al 2004; Mousa et al 2004). Generally, both precursor protein as well as opioid peptide expression appear to be increased in immune cells from inflamed tissue or mitogen-stimulated cells compared with quiescent leukocytes (Smith et al 1986; Zurawski et al 1986; Buzzetti et al 1989; Rosen et al 1989; Hassan et al 1992; van Woudenberg et al 1992; Cabot et al 1997, 2001; Lyons and Blalock 1997; Mousa et al 2004). Regulation of POMC in lymphocytes has been reported to be dynamic with both mRNA and opioid peptide levels increased in mitogen-stimulated cells, while POMC expression in macrophages was of a more constitutive nature (Lyons and Blalock 1997). In addition, the predominant opioid-containing cell type appears to be dynamically regulated depending on the progression of the inflammatory cascade (Rittner et al 2001). Increased complexity is additionally suggested by an observation that immune cells from inflamed lymph nodes contain relatively less opioid peptides compared with cells from noninflamed lymphatic tissue, while at the same time opioid peptide content in local inflamed tissue is increased (Cabot et al 1997). This observation is consistent with a model of peripheral opioid antinociception whereby immune cells migrate to inflamed tissue in a site directed manner, release these opioid peptides, and then return to lymphatic tissues depleted of peptide (Figure 2) (Cabot et al 1997).

Opioid peptides involved in peripheral antinociception

END and ENK have been proposed to be the most important opioid peptides involved in immune-derived opioid antinociception. Antibodies to both END and ENK could reverse the antihyperalgesic effect observed after release of these peptides from immune cells was stimulated using CRF or IL-1β (Stein, Hassan, et al 1990; Cabot et al 2001). Although DYN and PDYN levels were generally reported to be low, this opioid peptide is likely to be of some

significance in mediating peripheral antinociception as antiDYN has been reported to inhibit IL-1β-induced analgesia (Przewlocki et al 1992; Cabot et al 2001). In addition, expression of both the opioid peptide and its precursor were observed to be increased significantly in immune cells during inflammation, although absolute levels were low (Cabot et al 2001). The exact role of DYN in peripheral antinociception remains to be elucidated.

All three opioid peptides have been proposed to be contained within vesicular structures similar to those observed in neurons. The release of these endogenous opioid receptor ligands is Ca^{2+} dependent and can be evoked by stimulation with high K^{+} (Manfredi et al 1995; Cabot et al 1997, 2001). Ultrastructural studies have confirmed the presence of END in secretory granules in macrophages, monocytes, granulocytes, and lymphocytes within immune cells from inflamed tissue (Mousa et al 2004). These granules were grouped in small vesicular structures within the cytoplasm, while larger vesicles were observed to possess extended processes ready for exocytosis (Mousa et al 2004).

Numerous papers have reported that immune cells contain opioid peptides and increasing functional evidence is being accumulated to suggest that these endogenous opioid receptor ligands can be released in local inflamed tissues by leukocytes to alleviate inflammatory hyperalgesia (Lolait et al 1986; Cabot et al 1997; Kamphuis et al 1998; Binder et al 2004; Mousa et al 2004). In early inflammation, the degree of antinociception evoked through these locally released opioid peptides relies heavily upon the number of opioid receptors expressed in these tissues rather than the number of opioid peptide-containing immune cells recruited to the inflamed site (Brack, Rittner, et al 2004c). Analgesia, mediated through the release of endogenous opioid peptides, may become increasingly important as the number of opioid receptors expressed increases with the progression of inflammation (Zollner et al 2003; Brack, Rittner, et al 2004c).

Inflammation and the migration of circulating leukocytes

Leukocyte migration and adhesion molecules

For opioid-containing immune cells to elicit antinociception by releasing their endogenous opioid peptide load in local inflamed tissue, these cells must first migrate to these tissues.

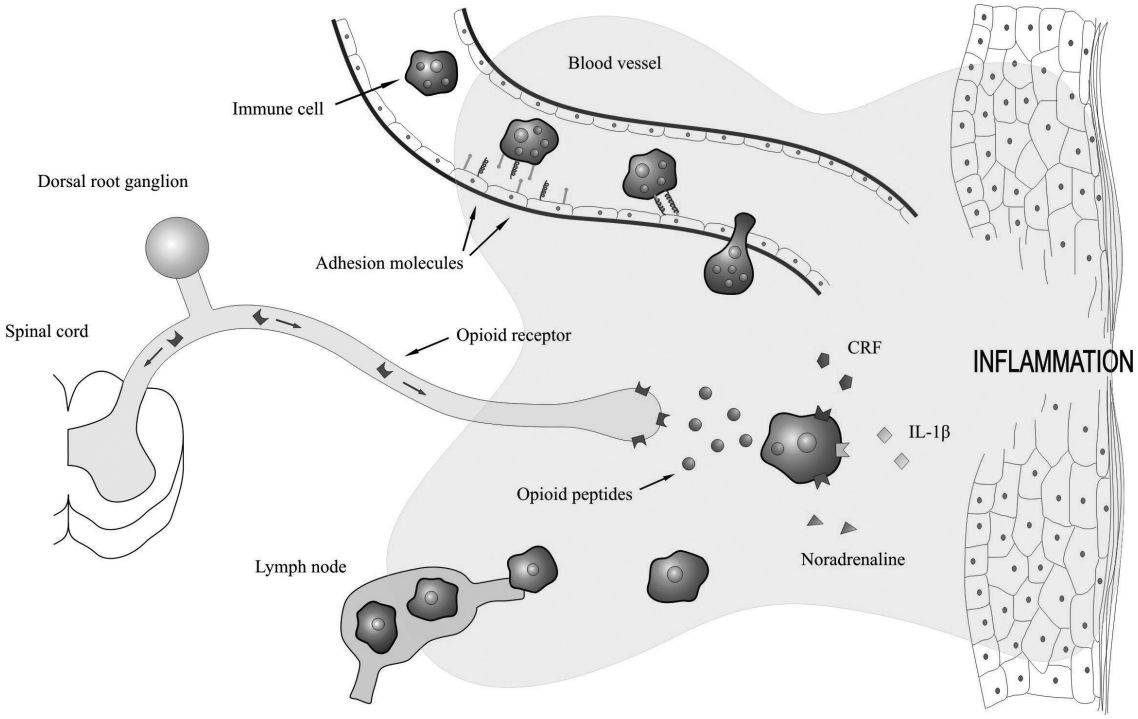


Figure 2 Endogenous opioid peptides are released by immune cells to reduce inflammatory pain. Adhesion molecules expressed on immune cells and inflamed endothelium coordinate the migration of circulation immune cells into inflamed tissue. The proinflammatory mediators corticotropin-releasing factor (CRF) and interleukin-1β (IL-1β), as well as the sympathetic neurotransmitter, noradrenaline, stimulate immune cells to secrete their opioid peptides. These peptides activate opioid receptors located on the peripheral ends of sensory neurons and effectively reduce inflammatory pain. Immune cells, devoid of their opioid contents, then continue their passage to neighbouring lymph nodes.

"Homing" of immune cells to inflamed sites is a complex process and involves numerous inflammatory mediators, chemoattractants, and adhesion molecules. The extravasation of leukocytes into inflamed tissue is enabled by the interaction of adhesion molecules expressed on leukocytes and vascular endothelium. These adhesion molecules comprise the carbohydrate binding selectins, immunoglobulins (Ig) such as ICAM and PECAM, and the integrin family of α and β subunit heterodimers (Wahl et al 1996). The upregulation of adhesion molecules and the engagement of leukocytes in the extravasation process are promoted by various inflammatory mediators and chemoattractants such as interleukin-1 (IL-1), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), thrombin, histamine, RANTES (Regulated upon Activation Normal T cell Expressed and Secreted), monocyte chemotactic peptide (MCP), and macrophage inflammatory peptides (MIP) (Springer 1994; Wahl et al 1996; Villarreal et al 2001). Passing leukocytes are initially tethered by the action of selectins. P- and E-selectins are expressed on vascular endothelium and L-selectins are constitutively expressed on leucocytes (Brown 1997). Once loosely tethered by selectins, leukocytes roll along the inflamed endothelium, which promotes the upregulation of leukocyte integrins and further adhesion (Springer 1994; Butcher and Picker 1996). Leukocyte integrins bind to the immunoglobulins expressed on endothelium; a firm interaction that results in the arrest of circulating leukocytes (Springer 1994; Butcher and Picker 1996). Leukocyte arrest is followed by diapedesis of the leukocyte, through relaxed endothelial junctions and into the inflamed tissue. These processes enable a targeted and leukocyte subset selective migration at the site of inflammation (Springer 1994; Butcher and Picker 1996).

Presentation of opioid-containing immune cells in inflamed tissue

In the rat paw model, the induction of inflammation with FCA results in a significant increase in opioid-expressing leukocytes in the inflamed tissue (Cabot et al 1997; Rittner et al 2001). Assessment with fluorescence-activated cell staining indicates that approximately 20% of immune cells that infiltrate inflamed tissue contain endogenous opioid peptides (Rittner et al 2001; Machelska et al 2002). At the early stages of inflammation, opioid-expressing immune cells are predominantly from a neutrophil lineage, representing 66% of opioid-containing cells. At later stages, monocytes and macrophages are prominent, representing 73% of opioid-expressing leukocytes (Rittner et al 2001; Schmitt et al 2003). Other studies have shown that activated lymphocytes are the prevalent opioid-containing immune cell type in the later stages of inflammation (Cabot et al 1997; Mousa et al 2001). This discrepancy is likely due to the use of different immunoreactive staining procedures. Opioid-expressing cells roughly reflect general immune cell type representation at different stages of inflammation (Rittner et al 2001).

Immune-derived opioids reduce inflammatory pain

Stress-induced analgesia during inflammation in peripheral tissue

Stressful stimuli trigger potent analgesia mediated by endogenous opioid systems in the CNS (Yamada and Nabeshima 1995). The response to stress is characterized by stimulation of the hypothalamic–pituitary–adrenocortical axis resulting in the sequential secretion of CRF and adrenocorticotrophic hormone (ACTH); activation of the sympathetic nervous system, which releases noradrenaline from the adrenal medulla and sympathetic nerve endings; and the activation of endogenous opioid systems in the CNS (Yamada and Nabeshima 1995). In rats with inflammation of the hind paw however, stress-induced analgesia is mediated predominantly by endogenous opioids that bind to peripheral opioid receptors to effectively reduce inflammatory pain (Stein, Gramsch, et al 1990).

A common technique for evoking stress-induced analgesia is cold water swim stress (CWS). In the rat paw model, CWS induces sudden antinociception that manifests as significant increases in pain thresholds in the inflamed paw (Stein, Gramsch, et al 1990). This analgesia is most potent immediately following the insult and subsides within 10 to 15 minutes (Stein, Gramsch, et al 1990). The antinociceptive effect of CWS is dose-dependently reversible by opioid receptor antagonists, which suggests that antinociception is opioid receptor-mediated. At the early stages of inflammation (6 hours after induction with FCA), CWS-evoked antinociception is completely blocked by systemically acting doses of naloxone and partially blocked by peripherally selective antagonists, which demonstrates a central and peripheral component (Machelska et al 2003). However, as inflammation becomes chronic (described in this study as 4 days post FCA), peripherally selective antagonists completely abolish CWS-evoked anti-

nociception, indicating that analgesia is mediated exclusively by opioid receptors on peripheral sensory nerves innervating the inflamed paw (Machelska et al 2003). The use of antibodies against specific endogenous opioid peptides and selective opioid receptor antagonists reveal that at the early stages of inflammation, the peripheral component of CWS-evoked analgesia is mediated by END, ENK, and DYN, which activate MOR, DOR, and KOR (Machelska et al 2003). At later stages of inflammation (as observed at 4 days post FCA), antinociception is delivered wholly by END, activating peripheral MOR and DOR (Stein, Gramsch, et al 1990; Machelska et al 2003).

Endogenous opioids are released from leukocytes

CWS-evoked analgesia in the rat paw depends on the local action of CRF and noradrenaline, evidenced by reversal following local injection of CRF receptor antagonists and adrenergic receptor antagonists respectively (Schafer et al 1996; Binder et al 2004). CRF, noradrenaline, and the proinflammatory cytokine, IL-1β, evoke the release of opioid peptides from leukocytes. Immune cell suspensions extracted from rats with inflamed hind paws exhibit receptor-specific and concentration-dependent opioid release when exposed to CRF, noradrenaline, and IL-1β (Schafer et al 1996; Cabot et al 1997, 2001; Binder et al 2004; Mousa et al 2004). CRF induces the release of END, ENK, and DYN (Cabot et al 2001); noradrenaline releases END (Binder et al 2004; Mousa et al 2004); and IL-1β releases END and DYN (Cabot et al 2001). An upregulation of receptors for these mediators on immune cells participating in an inflammatory response indicating that inflammation consolidates opioid release mechanisms (Zoukos et al 1994; Mousa et al 1996). Separate administration of CRF, noradrenaline, and IL-1β to the inflamed rat paw evokes potent dose-dependent and pharmacologically reversible analgesia (Binder et al 2004; Schafer et al 1994). This antinociceptive effect is the result of local action at the inflamed site as equivalent doses administered systemically are ineffective (Schafer et al 1994; Binder et al 2004). Antibodies against opioid peptides and opioid receptor antagonists abolish the analgesic effects of CRF, noradrenaline, and IL-1β, confirming that the observed analgesia is mediated by endogenous opioids that activate opioid receptors (Figure 2) (Schafer et al 1994; Binder et al 2004). Further evidence supporting immune cells as the source of opioid peptides is provided by findings that the administration of CRF, noradrenaline, and IL-1β into noninflamed paws does not evoke an antinociceptive effect (Schafer et al 1994; Binder et al 2004). The degree of analgesia induced by exposure to CWS increases proportionally with the presentation of opioid-containing immune cells in the inflamed hind paw (Rittner et al 2001; Machelska et al 2003).

Impaired immune cell function leads to impaired endogenous analgesia

Numerous studies have reported that impaired immune cell function is associated with diminished opioid analgesia, which is consistent with the model where immune cells release opioid peptides in inflamed tissue to elicit antinociception (Stein, Hassan, et al 1990; Przewlocki et al 1992; Schafer et al 1994; Machelska et al 1998, 2003; Mousa et al 2000; Brack et al 2004, 2004a, 2004b; Hermanussen et al 2004). This effect has been described for immuno-modulatory agents that affect immune cell trafficking as well as numerous treatments that compromise the functional integrity of leukocytes (Stein, Hassan, et al 1990; Przewlocki et al 1992; Schafer et al 1994; Machelska et al 1998, 2003; Mousa et al 2000; Brack et al 2004, 2004a, 2004b; Hermanussen et al 2004).

Immune cell trafficking

Fucoidin, which binds to selectins and blocks leukocyte "rolling", has been reported to impair analgesia induced by CWS and CRF (Machelska et al 1998). This impaired antinociception was paralleled by a decrease in END content as well as a decrease in the number of END-containing immune cells in inflamed tissue (Machelska et al 1998). Similarly, intracellular adhesion molecule-1 (ICAM-1) has been found to be upregulated in inflamed tissue and appears to contribute to the recruitment of opioid-containing cells to these local microenvironments as antiICAM-1 decreased CWS- and CRF-induced analgesia (Machelska et al 2002). Other adhesion molecules, including P-selectin, platelet-endothelial adhesion molecule-1 (PECAM-1), and L-selectin, are also upregulated in inflammation, and these increased expression levels correlate with increased END levels (Mousa et al 2000). However, while the exact role of these adhesion molecules in endogenous opioid analgesia remains to be elucidated, results indicate that PECAM-1 is not required for endogenous opioid analgesia (Machelska et al 2004). The same study confirmed functional integrity

of selectins and integrins as essential for immune-derived antinociception (Machelska et al 2004).

In contrast to adhesion molecule-mediated leukocyte trafficking, chemokine-mediated recruitment of polymorphonuclear cells may target opioid-containing leukocytes more specifically, as immune cells containing opioid peptides have been reported to be mostly chemokine receptor (CXCR2)-positive (Brack, Rittner, et al 2004b). Accordingly, a blockade of the keratinocyte-derived chemokine (KC) and macrophage inflammatory protein-2 (MIP-2) decreased the number of opioid-containing leukocytes in inflamed tissue and abolished CRF-induced antinociception in the inflamed rat paw (Brack, Rittner, et al 2004b).

Effect of immunosuppressants on immune-derived antinociception

Further evidence for the importance of the functional integrity of opioid-containing immune cells in endogenous analgesia stems from the observation that the immunosuppressant Cyclosporine A (CsA) can dose-dependently block CWS-, IL-1- and CRF-induced peripheral analgesia (Stein, Hassan, et al 1990; Schafer et al 1994; Hermanussen et al 2004). The reduced paw pressure threshold that has been observed in inflamed paws of CsA-treated animals was mirrored by a decrease in the number of infiltrating lymphocytes and could be reversed by administration of activated lymphocytes (Hermanussen et al 2004). Nonspecific immunosuppression induced by whole body irradiation also decreased stress-induced antinociception in inflamed paws, while more specific depletion of monocytes/macrophages by liposomal clodronate similarly decreased stress-induced endogenous opioid antinociception without affecting exogenous opioid analgesia (Przewlocki et al 1992; Brack et al 2004).

Clinical evidence for association between impaired immune function and increased pain

Reduced END content in immune cells has clinically been reported in numerous painful conditions, including arthritis, Crohn's disease, fibromyalgia, migraine, and tension headache (Wiedermann et al 1992, 1994; Panerai et al 2002). There is little evidence to suggest that opioid content in circulating immune cells significantly contributes to analgesia. A reduction in opioid content of these cells may compromise the local delivery of opioid peptides to inflamed tissues by migrating immune cells and thus impair endogenous analgesic mechanisms in these patients. In line with this observation, pain associated with disease or inflammation may be increased in immunocompromised patients (Merine et al 1987). A positive correlation between immunosuppression and increased pain has been reported, and in AIDS patients in particular, a decreased CD4+ count appears to be a risk factor for developing painful pathologies such as polyneuropathy (Lebovits et al 1994; Wyatt and Fishman 1994; Lefkowitz 1996; Hewitt et al 1997; Schifitto et al 2002). These observations highlight the importance of the immune system and release of endogenous opioid peptides for antinociception.

Immunosuppressive effects of exogenous opioids

Exogenous opioids such as morphine remain the most effective treatment for acute and chronic pain. There is evidence suggesting that opioids have an immunosuppressive action as well as clinically apparent side effects, such as respiratory depression, constipation, tolerance, and dependence (Risdahl et al 1998). The implications of opioid-evoked immunosuppression are particularly relevant during the postoperative period when pain and susceptibility to infection are high; for sufferers of chronic pain and drug users who administer opioids for extended periods; and for patients with immunosuppressive disease such as AIDS, transplant patients, and the elderly, who are predisposed to opportunistic infections.

Evidence for opioid-evoked immunosuppression

Much of the data that suggests opioids have immunosuppressive effects come from epidemiological studies with drug users. These individuals are reported to have markedly increased incidence of skin and soft tissue infections, pulmonary infections, endocarditis, skeletal infections, malaria, and viral hepatitis, although it is accepted that interpretation of these findings is confounded by compromised lifestyle practices associated with this population (Hussey and Katz 1950; Scheidegger and Zimmerli 1989; Risdahl et al 1998). In healthy nonaddicted individuals, morphine treatment for obstetric procedures resulted in the reactivation of herpes simplex virus (Crone et al 1988), supporting an immunosuppressive action. Animal models of opioid administration and infection show increased mortality rates in opioid treated animals with

experimentally-induced sepsis and *Toxoplasma gondii* infection, reversible by opioid receptor antagonists (Chao et al 1990; Roy et al 1999). Morphine-dependent swine, challenged with herpes virus, exhibited enhanced pneumonia secondary to herpes virus infection, although these animals also exhibited a paradoxical decrease in clinical signs associated with neurological disease (Risdahl et al 1993). In mice inoculated with *Streptococcus pneumonia*, chronic morphine treatment delayed neutrophil recruitment, increased bacterial burden, and increased mortality (Wang et al 2005). Various commonly measured immune parameters are significantly suppressed following opioid exposure in drug users, healthy human subjects, and animal models. These include suppressed NK cell activity (Carr et al 1993; Yeager et al 1995; Sacerdote et al 1997), macrophage phagocytic function (Rojavin et al 1993), cytokine production (Bhargava et al 1994; Sacerdote et al 1997), lymphocyte proliferation (Bhargava et al 1994; Sacerdote et al 1997), and spleen and thymus weight and cellularities (Fuchs and Pruett 1993; Bhargava et al 1994). In experiments with opioid receptor knock out mice, mutant mice did not exhibit the abnormal immune parameters that were observed in wild type mice following chronic morphine treatment, indicating that opioid receptors mediate morphine-induced immunosuppression (Gaveriaux-Ruff et al 1998). These findings collectively indicate that exposure to exogenous opioids impairs resistance to infection and suppresses immune function.

Centrally mediated opioid-evoked immunosuppression

There is evidence that opioid-evoked immunosuppression is mediated by opioid receptors in the CNS. Systemic administration of morphine significantly reduced lymphocyte proliferation ex vivo but the peripherally restricted N-methylmorphine did not, suggesting that a central mechanism mediates immunosuppression (Hernandez et al 1993). Furthermore, the central administration of both morphine and N-methylmorphine effectively inhibited lymphocyte proliferation. Several studies have identified the periaqueductal gray (PAG) matter of the mesencephalon as a site of morphine-induced opioid receptor-mediated immunosuppression. Injection of morphine into the PAG is reported to suppress ex vivo macrophage nitric oxide (NO) production, NK cell activity, lymphocyte proliferation, and leukocyte IL-2 production (Gomez-Flores et al 1999; Gomez-Flores and Weber 1999). In rats with FCA-induced inflammation of the hind paw, concomitant intrathecal morphine administration selectively attenuated the presentation of END-positive leukocytes in the inflamed paw without affecting total immune cell numbers (Schmitt et al 2003). This result led to the hypothesis that arresting pain transmission in the CNS signals a reduced need for immune-derived opioid peptides in the inflamed tissue (Schmitt et al 2003), and provides further evidence for centrally mediated opioid immunomodulation.

Opioids activate receptors on immune cells and suppress immune activity

In addition to centrally-mediated immunosuppression, the expression of opioid receptors on immune cells suggest that opioids may modulate immune function by direct action on these cells (Bidlack 2000). This is supported by findings that in vitro exposure to morphine resulted in concentration-dependent and antagonist-reversible inhibition of macrophage phagocytic function (Rojavin et al 1993. Opioids also directly suppress macrophage production of proinflammatory cytokines IL-1, IL-6, TNF-α (Alicea et al 1996), and impair monocyte chemotaxis in response to cytokines MIP-1α, MCP-1, and RANTES, through opioid receptor-mediated heterologous desensitization of chemokine receptors (Grimm et al 1998). Morphine directly upregulates immune cell Fas receptors that trigger apoptosis of the cell when activated by the ligand FasL (Yin et al 1999). In this way, morphine administered in vivo apparently primes leukocytes for elimination by apoptosis and suppresses the immune response (Yin et al 1999).

Opioid stimulation of immune cell function

In contrast to findings that opioids exert an immunosuppressive action, there is evidence that some opioids, particularly endogenous opioid peptides, stimulate aspects of immune function. In vitro experiments demonstrate that endogenous opioids such as END and ENK enhance human NK cell activity (Mathews et al 1983); increase IL-2 production in the lymphoid cell line EL-4 (Bessler et al 1990); increase leukocyte surface expression of compliment and immunoglobulin receptors (Menzebach et al 2003); increase leukocyte oxidative burst activity (Menzebach et al 2003); and augment macrophage tumoricidal activity (Hagi et al 1994). In vivo administration of ENK reportedly improves the survival rate of mice infected with herpes simplex virus and inhibits tumor growth (Faith et al 1987).

The immunostimulatory properties of endogenous opioids suggest that their release by immune cells in inflamed tissue not only controls pain but also stimulates immune function.

Clinical implications for pain control

Systemic administration of opioids is clinically associated with numerous side effects. While immunomodulatory effects of opioids can have clinical implications as described above, most dose-limiting adverse events, such as respiratory depression, sedation, and tolerance are mediated through activation of central opioid receptors (Kieffer and Gaveriaux-Ruff 2002). A logical consequence is the attempt to target peripheral opioid receptors more specifically. Numerous studies have demonstrated that peripheral opioids can provide effective analgesia (Stein et al 1989; Likar et al 2001; Truong et al 2003; Furst et al 2005; Pacheco and Duarte 2005). The immunosuppressive effect of opioid receptor agonists may contribute to peripheral opioid analgesia as it has been speculated that a local anti-inflammatory effect contributed to prolonged analgesia produced by intra-articular morphine (Stein et al 1999).

Strategies to target peripheral opioid receptors specifically include the development and use of peripherally active opioid receptor agonists, such as loperamide, asimadoline, and frakefamide, which can usually penetrate the blood brain barrier poorly, or local administration of centrally active opioids as well as treatments aimed at reducing degradation of locally released peptides (Barber et al 1994; DeHaven-Hudkins et al 1999; Chen et al 2001; Zajaczkowska et al 2004; Modalen et al 2005). Peripherally selective opioids have met with some clinical success, although most work has been carried out with peripherally selective KOR agonists such as fedotozine (Delvaux 2001). Despite promising pilot studies, both centrally mediated adverse events as well as peripheral side effects arising from systemic administration of opioid agonists, including constipation, could not be completely excluded (Delvaux 2001; Eisenach et al 2003; Riviere 2004). Thus, research aimed at the development of opioid receptor agonists that do not cross the blood brain barrier and are peripherally selective, but can be administered orally to achieve peripheral opioid analgesia remains ongoing. Targeted delivery of opioids locally to the site of pain could avoid the problems of peripherally selective opioid receptor agonists which retain the potential to cause side effects mediated through opioid receptor activation in the periphery. Side effects include nausea and vomiting arising from stimulation of opioid receptors in the chemo-receptor trigger zone, and constipation from opioid modulation of GI function. Accordingly, while local administration of centrally active opioid receptor agonists can be effective, limiting factors are problems arising from the required routes of administration, such as intra-articular injection (Kizilkaya et al 2004).

Historically, immunosuppression has been used as an analgesic strategy in numerous inflammatory conditions such as rheumatoid arthritis (Lipsky 1983). In light of recent evidence suggesting that endogenous opioid peptides are released locally from immune cells to elicit antihyperalgesia (Stein, Hassan, et al 1990), current clinical practices are likely to be challenged as our understanding of the complex interaction between the immune system and pain control increases. The role of the immune system in analgesia is likely to be delicately balanced depending on whether infiltrating cells are proinflammatory and proalgesic or their major role is an antiinflammatory and analgesic one. In situations where endogenous antinociception exceeds hyperalgesia mediated by immune cells, immuno-suppression of modulation of immune cell trafficking might increase pain. This hypothesis is supported by the observation that intra-articular naloxone lead to an increase in postoperative pain and analgesic consumption (Stein et al 1993).

Approaches could target immune cell trafficking to increase immune-mediated endogenous opioid anti-nociception and thus achieve analgesia, which lacks the side effects usually associated with systemic administration of opioid receptor agonists. The immigration of opioid-containing immune cells could be specifically encouraged by utilising chemokines or adhesion molecules. An increase in antinociceptive and decrease in proalgesic cell migration would complement current immunosuppressive treatment strategies. Endogenous opioid analgesia could be further enhanced by increasing infiltrating analgesic cell numbers or by increasing opioid peptide production in these cells. While stimulation of immune cell production using granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF) has not met with the expected increase in endogenous analgesia to date, gene therapy targeting endogenous opioid peptide production in peripheral neurons has had some success and may be applied to opioid-peptide containing immune cells in future (Braz et al 2001; Brack, Rittner, et al 2004a; Duplan et al 2004; Chuang et al 2005).

A further approach to enhance peripheral opioid analgesia includes inhibition of degradation of endogenous

opioid peptides. Using this approach, enhanced antinociception was achieved in a rodent model of thermal pain by administration of enkephalin-degrading enzyme inhibitor prodrugs (Chen et al 2001). In addition to enhanced antinociception, an additional benefit of this approach to achieve analgesia is the relative lack of immunosuppressive effects of endogenous opioid peptides, which would render these compounds superior to exogenous opioids in certain patient groups. Accordingly, modifications that would enable dosing with these endogenous peptides by eliminating instability and poor bioavailability of the compounds could be the beginning of a new class of analgesic opioid agonists (Dhanasekaran et al 2005)

The clinical implications of the complex interactions between the immune system and pain control are especially pertinent to immunocompromised patients. An impairment in immune cell function may lead to decreased endogenous antinociception and thus increased pain (Merine et al 1987). In AIDS patients in particular, undertreatment of pain has been reported to be common (Lebovits et al 1994), which gives greater significance to our understanding of how endogenous opioid antinociception might be altered in immunocompromised patient groups. Future studies directed at investigating changes in immune cell trafficking and endogenous opioid analgesia in these patients may provide clinical strategies to more effectively treat pain.

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