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Peripheral Mechanisms of Pain and Analgesia

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Abstract

This review summarizes recent findings on peripheral mechanisms underlying the generation and inhibition of pain. The focus is on events occurring in peripheral injured tissues that lead to the sensitization and excitation of primary afferent neurons, and on the modulation of such mechanisms. Primary afferent neurons are of particular interest from a therapeutic perspective because they are the initial generator of noxious impulses traveling towards relay stations in the spinal cord and the brain. Thus, if one finds ways to inhibit the sensitization and/or excitation of peripheral sensory neurons, subsequent central events such as wind-up, sensitization and plasticity may be prevented. Most importantly, if agents are found that selectively modulate primary afferent function and do not cross the blood-brain-barrier, centrally mediated untoward side effects of conventional analgesics (e.g. opioids, anticonvulsants) may be avoided. This article begins with the peripheral actions of opioids, turns to a discussion of the effects of adrenergic co-adjuvants, and then moves on to a discussion of pro-inflammatory mechanisms focusing on TRP channels and nerve growth factor, their signaling pathways and arising therapeutic perspectives.

Keywords

Peripheral analgesia; Opioid receptors; Adrenergic receptors; Nerve growth factor (NGF); Inflammation and cytokines; TRPV1; Primary afferents; Pruritus

1. INTRODUCTION

Tissue destruction, abnormal immune reactivity and/or nerve injury are frequently associated with an inflammatory response. Within peripheral damaged tissue (such as skin, muscles,

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joints, viscera), primary afferent neurons transduce noxious mechanical, chemical or heat stimuli into action potentials. The cell bodies of these neurons are located in the trigeminal and dorsal root ganglia (DRG) and give rise to myelinated (Aδ) and small-diameter unmyelinated axons (C-fibers). The latter are particularly sensitive to capsaicin, a ligand at the transient receptor potential vanilloid-1 (TRPV1) channel, and are considered the dominant fibers in clinical pain. After synaptic transmission and modulation within the primary sensory neuron and spinal cord, nociceptive signals reach the brain, where they are finally perceived as "pain", within the context of cognitive and environmental factors (Woolf and Salter, 2000).

For many years attention was focused on the characterization of proinflammatory and proalgesic effects elicited by the myriad of mediators occurring in injured tissue. Concurrently, however, endogenous mechanisms counteracting pain and inflammation are mounted. In the periphery such effects are produced by interactions between leukocyte-derived opioid peptides and opioid receptors on peripheral endings of primary afferent neurons, by antiinflammatory cytokines and/or by endocannabinoids (Rittner et al., 2008; Stein et al., 2003). This review will begin with the localization, trafficking and function of peripheral opioid receptors, production and release of opioid peptides from inflammatory cells, as well as analgesia, tolerance, antiinflammatory and wound-healing effects brought about by peripherally acting opioids. Thereafter, interactions with adrenergic receptors and proalgesic molecules such as ion channels and growth factors will be discussed.

2. PERIPHERAL OPIOID ANALGESIA – BASIC AND THERAPEUTIC ASPECTS

Peripheral sensory neurons express opioid receptors and opioid peptides, and the function of these neurons can be modulated by endogenous opioids derived from immune cells or by opioid drugs. This scenario has evolved from studies on mechanisms of inflammatory pain and its inhibition. Opioids are the most powerful drugs for severe pain but their use is hampered by side effects such as respiratory depression, nausea, clouding of consciousness, constipation, addiction and tolerance (Zöllner and Stein, 2007). Thus, the development of opioid drugs lacking such effects has always been a major goal in pain research. The discovery of opioid receptors on sensory nerves has now put this goal within reach. Moreover, in the course of these investigations modulatory opioid effects on inflammation and wound healing were detected (Tegeder and Geisslinger, 2004). These latter effects have sparked intense interest in light of the pressing need for novel anti-inflammatory therapies (Ledford, 2007). Following studies on the local application of conventional opioids in peripheral damaged tissue, a new generation of opioid drugs unable to pass the blood-brain-barrier is now emerging, thus avoiding centrally mediated unwanted effects (Brower, 2000; Riviere, 2004; Stein et al., 2003; Vanderah et al., 2008). Endogenous opioid peptides binding to peripheral opioid receptors have been identified within skin and subcutaneous tissue, particularly in inflammatory cells. This has led to new directions of research, for example the selective targeting of opioid peptide-containing cells to sites of injury, the augmentation of opioid synthesis by gene transfer and the inhibition of inflammation by peripherally acting opioids (Machelska, 2007; Rittner et al., 2008; Stein et al., 2003).

2.1. Peripheral Opioid Receptors

2.1.1. Opioid Receptor Types—Early binding studies and bioassays defined three main types of opioid receptors in the central nervous system: mu- (MOR), delta- (DOR) and kappa- (KOR) receptors. Additional receptor types were proposed (e.g., sigma, epsilon, orphanin) but are currently not considered "classical" opioid receptors (Kieffer and Gaveriaux-Ruff, 2002). Molecular cloning resulted in the identification of three opioid receptor genes and allowed for the study of individual opioid receptor types with regard to pharmacological profile, intracellular effector coupling, anatomical distribution, and regulation of expression. Opioid receptors belong to the family of seven transmembrane G-protein coupled receptors (GPCR)

and show 50–70% homology between their genes (Evans et al., 1992; Kieffer et al., 1992; Meng et al., 1993; Wang et al., 1993). Additional pharmacological subtypes may result from alternative splicing, posttranslational modifications or receptor oligomerization. Opioid receptors are expressed by central and peripheral neurons, by neuroendocrine (pituitary, adrenals), immune, and ectodermal cells (Zöllner and Stein, 2007).

2.1.2. Signal Transduction and Recycling—The signaling pathways of opioid receptors are well characterized. After the ligand binds at the receptor, conformational changes allow intracellular coupling of mainly $G_{i/o}$-proteins to the C-terminus of opioid receptors. At the G_{α} subunit GDP is replaced by GTP and dissociation of the trimeric G-protein complex into G_{α} and $G_{\beta\gamma}$ subunits ensues. Subsequently these subunits can inhibit adenylyl cyclase and thereby cyclic adenosine monophosphate (cAMP) production, and/or directly interact with K^+, Ca^{2+} and other ion channels in the membrane. Ion-channels are mainly regulated via $G_{\beta\gamma}$ -subunits (Herlitze et al., 1996). All three opioid receptors modulate various N, T- and P/Q-type Ca^{2+} channels, suppress Ca^{2+} influx and the excitation and/or neurotransmitter release in many neuronal systems. A prominent example is the inhibition of substance P (a pronociceptive and proinflammatory neuropeptide) release from central and peripheral terminals of sensory neurons (Kondo et al., 2005; Yaksh, 1988). At the postsynaptic membrane, opioid receptors mediate hyperpolarization by opening K^+ channels, thereby preventing excitation and/or propagation of action potentials (Zöllner and Stein, 2007). Various enzymes such as phosphokinase C and GPCR kinases can phosphorylate opioid receptors, leading to increased affinity for intracellular arrestin molecules. Arrestin-receptor complexes lead to opioid receptor desensitization by preventing G-protein coupling and promote internalization via clathrin-dependent pathways (Law et al., 2000). Recycling of opioid receptors to the plasma membrane promotes rapid resensitization of signal transduction, whereas targeting to lysosomes leads to proteolytic downregulation. It was suggested that GPCR-associated sorting proteins modulate lysosomal sorting and functional downregulation (Whistler et al., 2002). Additional opioid-modulated pathways involve *N*-methyl-D-aspartate receptors, mitogen-activated protein kinase and phospholipase-C (Zöllner and Stein, 2007).

2.1.3. Opioid Receptors on Peripheral Sensory Neurons—In the late 1980s evidence began to accumulate that the antinociceptive effects of opioids can be mediated by peripheral opioid receptors located on sensory neurons (Bartho et al., 1990; Stein, 1995; Stein et al., 1990b). Opioid receptors are expressed in small-, medium- and large-diameter DRG neurons (Buzas and Cox, 1997; Chen et al., 1997; Coggeshall et al., 1997; Gendron et al., 2006; Mansour et al., 1994; Rau et al., 2005; Silbert et al., 2003; Wang and Wessendorf, 2001; Zhang et al., 1998a; Zhang et al., 1998c), they are coexpressed with prototypical sensory neuropeptides such as substance P and calcitonin-gene-related peptide (CGRP) (Li et al., 1998; Minami et al., 1995; Mousa et al., 2007a; Mousa et al., 2007b; Ständer et al., 2002; Zhang et al., 1998b; Zhang et al., 1998c), they are transported to the peripheral nerve terminals (Hassan et al., 1993; Li et al., 1996; Mousa et al., 2001) and they are coupled to $G_{i/o}$ proteins that inhibit adenylyl cyclase and modulate ion channels (Stein and Zöllner, 2008; Zöllner et al., 2003). The decrease of Ca^{2+} currents, but not the modulation of K^+ channels, appears to be a major mechanism for the inhibiton of sensory neuron functions (Akins and McCleskey, 1993). Recently, G protein-coupled inwardly rectifying K^+ channels and mu-opioid receptors were colocalized on sensory nerve endings in the epidermis (Khodorova et al., 2003), but no direct evidence of functional coupling or modulation of K^+ channels in DRG neurons has been provided so far. Activation of opioid receptors also suppresses pronociceptive modulation of tetrodotoxin-resistant Na^+ and nonselective cation currents (Gold and Levine, 1996; Ingram and Williams, 1994), P2X receptor-mediated currents (Chizhmakov et al., 2005), as well as TRPV1 currents via $G_{i/o}$ and the cAMP pathway (Chizhmakov et al., 2005; Endres-Becker et al., 2007). As a result, opioid agonists can attenuate inflammation-induced increases in the excitability of primary afferent

neurons and the release of proinflammatory neuropeptides (substance P, CGRP) from central and peripheral terminals (Junger et al., 2002; Stein et al., 2003). Particularly within injured tissue, these events lead to antinociceptive and antiinflammatory effects.

2.1.4. Plasticity of Peripheral Opioid Receptors

2.1.4.1. Ontogeny and phylogeny of opioid receptor expression in primary afferents: The ontogeny of opioid receptors has been examined in the central and peripheral nervous system during pre- and postnatal development. Using radioligand binding and in-situ hybridization techniques, MOR, DOR and KOR expression was found to be distinct at all ages. The expression of delta- lags behind that of mu- and kappa-receptors in mouse and rat brain (Spain et al., 1985; Zhu et al., 1998). However, in mouse DRG neurons the first opioid receptor expressed is the delta-receptor at embryonic day 12.5 (E12.5), followed by the mu- (E13.5) and the kappa-receptor (E17.5) (Zhu et al., 1998). In rat, a greater proportion of rat DRG neurons immunoreactive for mu- and delta-receptors was found before postnatal day 7 (P7) than at P21 (kappa was not examined) (Beland and Fitzgerald, 2001). Moreover, during the first postnatal week both opioid receptors were detected in cells of all sizes but by P21 expression was restricted to small and medium diameter cells, suggesting a selective downregulation in non-nociceptive neurons (Beland and Fitzgerald, 2001). Interestingly, there are also phylogenetic differences in opioid receptor expression in the spinal cord and DRG across mammalian species. In monkey and human, delta-receptors are highly localized to primary afferents compared to rodents (Mennicken et al., 2003). In mouse, the postnatal expression of MOR in a subset of DRG neurons appears to be regulated by the transcription factor Runx1 (Chen et al., 2006).

2.1.4.2. Influence of Inflammation: Painful inflammation of peripheral tissue (of varying duration) has been most extensively studied as a regulatory stimulus of opioid receptor plasticity in adult sensory neurons. Both the systemic and the local application of MOR, DOR and KOR agonists elicits significantly more pronounced analgesic effects in injured than in noninjured tissue of animals and humans (Stein, 1995; Stein et al., 2003; Vanderah et al., 2008). This intriguing finding has stimulated extensive research into the underlying mechanisms.

Peripheral inflammation can induce differential upregulation of opioid receptor mRNA and protein in DRG neurons. In complete Freund's adjuvant (CFA)-induced paw inflammation, MOR mRNA displays a biphasic upregulation (at 2 h and 96 h), whereas mRNA for DOR remains unchanged, and KOR mRNA shows a peak at 12 h (Pühler et al., 2006; Pühler et al., 2004). In parallel, MOR and KOR binding is upregulated. The upregulation is related to neuronal electrical activity (Pühler et al., 2004), to cytokine production in the inflamed tissue (Pühler et al., 2006), and may be mediated by cytokine-induced binding of transcription factors to opioid receptor gene promoters (Kraus et al., 2001). Not surprisingly, a short-lasting (30 min) inflammatory stimulus (intraperitoneal acetic acid) does not change opioid receptor expression on sensory nerve terminals (Labuz et al., 2007). Thus, the expression of opioid receptors is dependent on receptor type and the duration of inflammation. MOR is most extensively studied and is consistently shown to be upregulated (Ballet et al., 2003; Ji et al., 1995; Mousa et al., 2002; Pühler et al., 2004; Shaqura et al., 2004; Zhang et al., 1998a; Zöllner et al., 2003). It was shown that the upregulation of mu-opioid binding sites in DRG is due to an increase in both the number of neurons expressing MOR and the density of MOR per neuron; the affinity of opioid agonists to MORs remained unchanged (Zöllner et al., 2003). In addition, G-protein coupling of opioid receptors in DRG neurons is augmented by subcutaneous inflammation (Shaqura et al., 2004; Zöllner et al., 2003).

Bradykinin, a typical inflammatory mediator, was found to stimulate the trafficking of intracellular delta-receptors to the plasma membrane of cultured DRG neurons (Patwardhan et al., 2005). Furthermore, bradykinin pretreatment of these neurons led to more potent inhibition of CGRP release and of cAMP accumulation by MOR- and DOR-agonists (Berg et al., 2007a; Patwardhan et al., 2005). The MOR-agonist effect was dependent on integrins colocalized with MORs in the DRG membrane (Berg et al., 2007b). Similarly, painful paw inflammation and activation of sensory neurons by capsaicin were shown to enhance membrane recruitment as well as ligand-induced internalization of delta-receptors in DRG neurons (Gendron et al., 2006; Zhang et al., 2006).

Subsequent to the opioid receptor upregulation in DRG, the peripherally directed axonal transport of opioid receptors is augmented (Hassan et al., 1993; Ji et al., 1995; Mousa et al., 2001; Pühler et al., 2004). The axonal transport is stimulated by cytokines and nerve growth factor produced within the peripheral inflamed tissue (Jeanjean et al., 1995; Mousa et al., 2007a) and results in increased density of opioid receptors at peripheral nerve terminals (Stein et al., 1990b). Inflammation is also accompanied by a sprouting of opioid receptor-bearing peripheral sensory nerve terminals (Mousa et al., 2001) and by a disrupted perineural barrier facilitating the access of opioid agonists to their receptors (Antonijevic et al., 1995). In addition, low pH can increase opioid agonist efficacy, presumably by altering the interaction of opioid receptors with G-proteins (Rasenick and Childers, 1989; Selley et al., 1993; Vetter et al., 2006). All of these mechanisms likely contribute to the increased antinociceptive efficacy of opioids in inflamed tissue. In line with these findings, clinical studies have shown that the proximal perineural application of opioids along intact (noninjured) nerves (e.g. axillary plexus) does not reliably produce analgesic effects (Picard et al., 1997).

2.1.4.3. Influence of Nerve Damage: Mechanical nerve injury resulting in neuropathic pain is another condition influencing opioid receptors in sensory neurons. Different animal models (e.g. partial nerve ligation, axotomy) have been examined with variable results. For example, at 2 days and 14 days after chronic constriction injury of the sciatic nerve (a partial ligation with preferential ischemic degeneration of large myelinated but relative preservation of unmyelinated fibers) MOR protein was upregulated in DRG and accumulated proximal and distal to the lesion, indicating anterograde and retrograde axonal transport. At 14 days mu-receptors were also increased in distal small surviving axons and in small sprouting axons at and distal to this lesion (Truong et al., 2003). Similarly, on day 14 following partial sciatic nerve ligation an upregulation of delta-receptors was shown in DRG and in sciatic nerve (Kabli and Cahill, 2007) and 14 days after partial saphenous nerve ligation an upregulation of mu-receptors was found in DRG and in paw skin (Walczak et al., 2005). A few studies found downregulation of MOR in DRG at 7 (Rashid et al., 2004) or 16 days after partial sciatic nerve ligation (Pol et al., 2006), as well as after peripheral axotomy (Zhang et al., 1998c). For KOR, a significant upregulation of its mRNA was observed ipsilaterally in the DRG of mice that had developed mechanical allodynia following spinal nerve transection but not in animals that did not display pain behaviors (Sung et al., 2000). Although more studies are needed, there is evidence that the expression of opioid receptors in nociceptive fibers may increase after nerve injury making themselves more susceptible to opioid analgesics.

2.1.4.4. Sympathetic Neurons: Opioid receptor expression in sympathetic postganglionic neurons has also been suggested. However, neither opioid receptor mRNA nor protein has been detected in such neurons (Coggeshall et al., 1997; Mousa et al., 2007b; Ständer et al., 2002; Wenk and Honda, 1999). Moreover, chemical sympathectomy with 6-hydroxydopamine did not change the expression of opioid receptors in the DRG or the peripheral analgesic effects of MOR, DOR and KOR agonists in a model of inflammatory pain (Zhang et al., 1998a; Zhou et al., 1998).

2.2. Endogenous Opioid Peptides

The endogenous ligands of opioid receptors are derived from the three precursor proteins proopiomelanocortin (POMC), proenkephalin (PENK) and prodynorphin. Appropriate processing yields the major representative opioid peptides β-endorphin, Met-enkephalin and dynorphin A, respectively. These peptides and their derivatives exhibit different affinities and selectivities for the mu- (β-endorphin, Met-enkephalin), delta- (enkephalins, β-endorphin) and kappa- (dynorphin) receptors. Two additional endogenous opioid peptides have been isolated from bovine brain: endomorphin-1 and endomorphin-2. Both peptides are considered highly selective mu-receptor ligands but their precursors are unknown to date (Fichna et al., 2007).

2.2.1. Opioid Peptides in Sensory Neurons—Evidence for the presence of opioid peptides in sensory neurons began to accumulate in the 1980s. Several reports demonstrated immunoreactive dynorphins (Gibbins et al., 1987; Przewlocki et al., 1983; Sweetnam et al., 1986; Weihe et al., 1985) and enkephalins (Bergström et al., 2006; Przewlocki et al., 1983; Quartu and Del Fiacco, 1994) in DRG and in peripheral sensory neurons. These peptides were shown to be transported towards central and peripheral nerve terminals and were demonstrated in cutaneous nerves (Carlton and Coggeshall, 1997; Crowe et al., 1994; Gibbins et al., 1987). With regard to mRNA, only one study detected PENK mRNA in intermediate-sized DRG neurons (Pohl et al., 1994) but neither PENK nor prodynorphin mRNA were found in DRG of normal or polyarthritic rats by others (Calza et al., 1998). Endomorphins were also described in sensory nerves (Martin-Schild et al., 1998; Mousa et al., 2002; Pierce et al., 1998). It was suggested that these opioid peptides can exert (auto-) modulation of sensory nerve function but direct evidence has not been provided so far.

2.2.2. Opioid Peptides in Immune Cells—The discovery that opioid receptors on sensory nerves are upregulated during subcutaneous inflammation prompted the search for endogenous ligands within inflamed tissue. POMC-related opioid peptides have been found in leukocytes of many vertebrates and invertebrates (Smith, 2003). Earlier studies described several truncated POMC mRNAs but more recently a full-length transcript encoding all three POMC exons was shown in rat mononuclear leukocytes (Lyons and Blalock, 1997; Sitte et al., 2007). POMC transcripts containing the signal sequence necessary for correct routing into the regulated secretory pathway are upregulated in lymphocytes from rats with painful paw inflammation (Sitte et al., 2007) and the enzymes (prohormone convertases, carboxypeptidase) required for proteolytic processing of POMC are expressed in leukocytes (Mousa et al., 2004). PENK mRNA, Met-enkephalin and the appropriate enzymes for posttranslational processing of PENK have also been detected in human and rodent leukocytes (LaMendola et al., 1997; Vindrola et al., 1994). Deletion of the gene coding for PENK resulted in the complete absence of Met-enkephalin both in the brain and in T-cells, strongly indicating that this peptide derives from the same precursor in the nervous and immune systems (Hook et al., 1999). In addition, dynorphin and endomorphins have been demonstrated in immune cells (Chadzinska et al., 2005; Mousa et al., 2002). Opioid peptide-containing cells include granulocytes, monocytes/macrophages and lymphocytes (Cabot et al., 1997; Labuz et al., 2006; Mousa et al., 2001; Przewlocki et al., 1992; Rittner et al., 2001; Rittner et al., 2007a; Zöllner et al., 2008). Recently, T-lymphocytes were postulated to mediate analgesia via β-endorphin expression in the visceral system (Verma-Gandhu et al., 2006) and keratinocyte-derived β-endorphin was proposed to mediate peripheral antinociception in noninflamed skin (Ibrahim et al., 2005; Khodorova et al., 2003).

2.2.3. Migration of Opioid Containing Cells to Inflamed Tissue—The recruitment of leukocytes from the circulation into inflammatory sites involves a well-orchestrated set of events. This begins with rolling along the endothelial cell wall mediated predominantly by selectins. Then leukocytes are activated by chemokines that are released from endothelial and

inflammatory cells and are presented on the endothelium. This leads to the upregulation and increased avidity of integrins which mediate the firm adhesion of leukocytes to endothelial cells via e.g. intercellular adhesion molecule-1 (ICAM-1). Finally, leukocytes transmigrate through the endothelium mediated by e.g. platelet-endothelial cell adhesion molecule-1 (PECAM-1) (von Andrian and Mackay, 2000).

In inflamed rat paws L-selectin, integrin β_2 and the CXC chemokine receptor 2 (CXCR2) are co-expressed by opioid-containing leukocytes (Brack et al., 2004c; Machelska et al., 2004; Mousa et al., 2000). Pretreatment with a selectin blocker, antibodies against ICAM-1, against the integrins α_4 and β_2, or against the chemokines CXCL1 and CXCL2/3 substantially decreases the number of opioid-containing immune cells accumulating in the inflamed tissue (Brack et al., 2004c; Machelska et al., 2004; Machelska et al., 1998; Machelska et al., 2002). In addition, this cell recruitment is dependent on neurokinin-1 receptors (Rittner et al., 2007b) and might be regulated by adhesion to neurons (Hua et al., 2006). Finally, the migration of opioid-containing leukocytes into injured tissue appears to be modulated by central mechanisms. For example, intrathecally administered morphine, at a dose that produces analgesia, decreases the number of β-endorphin containing leukocytes in inflamed rat paws (Schmitt et al., 2003). This was confirmed in a clinical study using epidural analgesia in patients undergoing surgery (Heurich et al., 2007). Thus, an effective central inhibition of pain apparently signals a reduced need for recruitment of opioid-containing cells to injured tissues.

2.2.4. Release of Opioid Peptides from Immune Cells—As in the pituitary, corticotropin-releasing factor (CRF) and interleukin (IL)-1β can stimulate secretion of opioid peptides from leukocytes in a receptor-specific and calcium-dependent manner (Cabot et al., 1997; Cabot et al., 2001; Schäfer et al., 1994). Several other mediators have been recognized as potent releasing agents of opioid peptides from immune cells. For example, activation of CXCR2 on granulocytes leads to release of β-endorphin and Met-enkephalin, which is dependent on inositol triphosphate receptor-triggered release of Ca^{++} from the endoplasmic reticulum, (partially) on phosphoinositol–3-kinase and on p38 mitogen-activated protein kinase (Rittner et al., 2007a; Rittner et al., 2006a). Furthermore, noradrenaline stimulates release of β-endorphin from leukocytes in an adrenergic receptor-specific manner (Binder et al., 2004; Kavelaars et al., 1990; Mousa et al., 2004). The endogenous source of noradrenaline are sympathetic nerve fibers located in proximity to these cells (Binder et al., 2004). Opioid peptide containing immune cells coexpress adrenergic receptors, chemokine receptors, as well as CRF- and IL-1β-receptors (Binder et al., 2004; Mousa et al., 2003; Mousa et al., 1996). Moreover, these cells package opioids into vesicular structures that are translocated to the membrane upon stimulation (Mousa et al., 2004; Rittner et al., 2007a; Zöllner et al., 2008). In granulocytes these structures have been identified as primary (azurophil) granules (Rittner et al., 2007a). Thus, opioid release from immune cells is consistent with the regulated secretory pathway, similar to neuroendocrine cells.

2.3. Modulation of Pain and Inflammation via Peripheral Opioid Receptors

2.3.1. Peripherally Acting Opioid Agonists—Earlier attempts to demonstrate peripheral opioid analgesia in noninjured tissue produced controversial results but subsequent studies in models of pathological pain were more successful (Stein, 1993; Stein et al., 2003). In models of peripheral inflammation, the local injection of low, systemically inactive doses of MOR, DOR or KOR agonists produced analgesia that was dose-dependent, stereospecific and reversible by selective opioid antagonists (Stein, 1993; Stein et al., 1989). Potent antinociception was also shown in models of nerve damage and of visceral, thermal, bone and cancer pain (Baamonde et al., 2005; Guan et al., 2008; Junger et al., 2002; Obara et al., 2007; Stein et al., 2003; Stein and Zöllner, 2008). In addition, anti-inflammatory effects were demonstrated in different models of somatic and visceral inflammation. Possible underlying

mechanisms include a reduced release of proinflammatory neuropeptides or cytokines, and a diminished expression of adhesion molecules (Chakass et al., 2007; Philippe et al., 2003; Stein et al., 2001; Straub et al., 2008; Tegeder and Geisslinger, 2004). These findings stimulated the development of novel opioid ligands acting exclusively in the periphery without central side-effects (Bileviciute-Ljungar et al., 2006; DeHaven-Hudkins and Dolle, 2004; Fürst et al., 2005; Riviere, 2004). A common approach was the use of hydrophilic compounds to reduce their capacity for crossing the blood-brain-barrier. Among the first generation of compounds were the mu-agonist loperamide (originally known as an antidiarrheal drug) and the kappa-agonist asimadoline (Machelska et al., 1999). For example, in a recent study employing a model of neuropathic pain, systemically (subcutaneously) applied loperamide dose-dependently reversed mechanical allodynia by activation of peripheral mu-receptors (Guan et al., 2008). Peripheral restriction has been pursued with novel arylacetamide (ADL10–0101) and morphinan-based (nalfurafine, also called TRK-820) compounds, and more recently, with peptidic kappa-agonists (CR665, also called FE200665) (Riviere, 2004; Stein et al., 2003; Vanderah et al., 2008; Vanderah et al., 2004). Several studies indicate that a large proportion (about 50 – 80 %) of the analgesic effects produced by systemically administered opioids can be mediated by peripheral opioid receptors (Craft et al., 1995; Fürst et al., 2005; Labuz et al., 2007; Reichert et al., 2001; Shannon and Lutz, 2002). In addition, human studies indicate that opioid agonists that do not readily cross the blood-brain barrier are beneficial in patients with visceral and neuropathic pain (Eisenach et al., 2003; Mangel et al., 2008; Wallace et al., 2006) and can have the same analgesic efficacy as conventional opioids (Hanna et al., 2005; Tegeder et al., 2003; van Dorp et al., 2008). Thus, the analgesic efficacy of peripherally active opioids may be utilized under conditions of acute and chronic pain with the benefit of reduced adverse central nervous system side effects.

2.3.2. Exogenous Stimulation of Opioid Release from Inflammatory Cells—When injected into inflamed subcutaneous tissue, all the releasing agents mentioned above (see 2.2.4.) can produce antinociceptive effects. In addition, CRF (Hargreaves et al., 1989), IL-6 and tumor necrosis factor-α (Czlonkowski et al., 1993) injected into inflamed tissue produce opioid-mediated analgesia. Depending on the stage and type of inflammation, these effects are mediated by different opioid peptides (Binder et al., 2004; Brack et al., 2004c; Labuz et al., 2006; Machelska et al., 2003; Mousa et al., 2003; Schäfer et al., 1994). Immunosuppression with cyclosporine A, depletion of granulocytes, blockade of chemokines (CXCL1, CXCL2/3), or anti-selectin and anti-ICAM-1 treatments significantly reduce opioid-containing cells and antinociception (Brack et al., 2004c Rittner, 2006, CXCR2; Machelska et al., 1998; Machelska et al., 2002; Schäfer et al., 1994). Conversely, the impaired antinociception following immunosuppression can be restored by transfer of allogenic lymphocytes (Hermanussen et al., 2004) or granulocytes (Rittner et al., 2006a).

In noninflamed tissue, however, cytokines such as IL-1α, IL-1β, IL-6 and tumor necrosis factor-α were found to induce hyperalgesia (Cunha and Ferreira, 2003). Also, several chemokines were described to induce pain or to decrease analgesic effects of other compounds (Oh et al., 2001; Szabo et al., 2002). Noradrenaline had no effect or increased pain behavior (Binder et al., 2004). The most obvious explanation for these findings is that noninflamed tissue does not hold opioid-producing cells. Hence, short of immune cells bearing their receptors, these agents now act on different targets, e.g. neurons or blood vessels. It is therefore not surprising that a given agent can produce different effects depending on the presence or absence of inflammatory cells. Interestingly, the selective recruitment of granulocytes does not elicit pain responses, suggesting that these cells are more important in the inhibition than in the generation of hyperalgesia (Rittner et al., 2006b). Another recent finding in noninflamed tissue is that activation of keratinocytes by endothelin- and cannabinoid agonists can lead to release of β-endorphin, which then acts on opioid receptors on primary afferent neurons to inhibit nociception (Ibrahim et al., 2005; Khodorova et al., 2003).

2.3.3. Endogenous Stimulation of Opioid Release from Inflammatory Cells— Stress is a natural stimulus triggering inhibition of pain (Terman et al., 1984; Willer et al., 1981). In rats with unilateral hindpaw inflammation stress, induced by cold water swim elicits potent antinociception in inflamed but not in the contralateral noninflamed paws (Machelska et al., 2003; Stein et al., 1990a). Whereas at early stages of the inflammatory response (several hours) both peripheral and central opioid receptors contribute, at later stages (several days) endogenous analgesia is mediated exclusively by peripheral opioid receptors (Machelska et al., 2003; Stein et al., 1990a; Stein et al., 1990b). Thus, peripheral opioid mechanisms of pain control become more prevalent with the duration and severity of inflammation. The most prominent opioid peptide involved is β-endorphin, but Met-enkephalin, dynorphin and endomorphins also contribute (Labuz et al., 2006; Machelska et al., 2003; Stein et al., 1990a). Endogenous triggers of swim stress-induced analgesia are locally produced CRF and sympathetic nerve-derived catecholamines (Binder et al., 2004; Machelska et al., 2003; Schäfer et al., 1996).

Stress-induced analgesia can be abolished by cyclosporine A, whole body irradiation or by depletion of monocytes/macrophages (Brack et al., 2004a; Przewlocki et al., 1992; Stein et al., 1990b). Since L-selectin, integrin β_2 and the chemokine receptor 2 (CXCR2) are expressed by opioid-containing leukocytes (Brack et al., 2004c; Machelska et al., 2004; Mousa et al., 2000), pretreatment with selectin blockers, antibodies against ICAM-1, integrins or against the chemokines CXCL1 and CXCL2/3 substantially decreases the number of opioid-containing cells and abolishes endogenous peripheral opioid analgesia (Brack et al., 2004c; Machelska et al., 2004; Machelska et al., 1998; Machelska et al., 2002). Stress-induced analgesia is also decreased by blockade of neural cell adhesion molecule (NCAM), presumably by preventing the adhesion of opioid-containing cells to peripheral nerves in inflamed tissue (Hua et al., 2006). Thus, adhesion molecules apparently modulate pain via extravasation of opioid-containing immune cells and/or their adhesion to sensory neurons. In addition, the migration of opioid-containing cells and endogenous analgesia within peripheral injured tissue appear to be influenced by central mechanisms (see above) (Heurich et al., 2007; Schmitt et al., 2003).

Importantly, in models of inflammation (Sitte et al., 2007) and bone cancer (Baamonde et al., 2006), as well as in humans undergoing knee surgery (Stein et al., 1993), the local injection of opioid receptor antagonists into injured tissue was shown to exacerbate pain. This strongly indicates that opioid peptides are continuously released and counteract hyperalgesia elicited by the many known proinflammatory agents present in inflammation (Rittner et al., 2008). Thus, even though hyperalgesia typically prevails in inflamed tissue, this hyperalgesia would be much more severe if opioid peptides were not present and tonically released at the same time.

A future challenge is to identify factors that increase homing of opioid-containing cells to injured tissue. For example, hematopoetic growth factors mobilized granulocytes in the blood but produced only a minor increase in the number of opioid-containing leukocytes in inflamed paws, and no change of CRF- or stress-induced antinociception (Brack et al., 2004b). Increasing the recruitment of opioid-containing cells with local injections of CXCL2/3 did not result in stronger antinociception either. Most probably this was a result of the relatively low number of neuronal opioid receptors at the respective (early) stage of tissue injury (Brack et al., 2004b). Indeed, previous studies had shown that intrinsic analgesia increases with the duration of inflammation, in parallel with the number of opioid-containing leukocytes, with the number of peripheral opioid receptors and with the efficacy of opioid receptor-G-protein coupling in sensory neurons (Mousa et al., 2001; Rittner et al., 2001; Zöllner et al., 2003).

2.3.4. Tolerance—Long-term opioid treatment can result in the eventual loss of opioid receptor-activated function (i.e. desensitization). Three mechanisms are associated with

desensitization of G-protein coupled receptors (GPCR): (1) receptor phosphorylation, (2) receptor internalization and/or sequestration, and (3) receptor downregulation (i.e. a reduced total number of receptors). Opioid receptors are substrates for second messenger kinases (e.g. protein kinase C) and for GPCR kinases. Opioid receptor phosphorylation by these kinases increases the affinity for arrestin molecules. Arrestin-receptor complexes sterically prevent coupling between receptor and Gproteins and promote internalization via clathrin-dependent pathways (Law et al., 2000). Agonist-induced internalization of the receptor via the endocytic pathway has been thought to contribute directly to tolerance by decreasing the number of opioid receptors on the cell surface. However, more recent studies have shown that morphine fails to promote endocytosis of opioid receptors in cultured cells (Eisinger et al., 2002) and native neurons (Sternini et al., 1996), although it is highly efficient in inducing tolerance in vivo (Hanninen et al., 1996). Moreover, increased endocytosis and recycling of opioid receptors was shown to dramatically decrease opioid tolerance (Koch et al., 2005). These findings led to the current concept that desensitization and receptor internalization prevent the development of tolerance.

Experimental studies on tolerance are often performed in the absence of painful tissue injury, which precludes extrapolation to the clinical situation. This distinction may be important given recent evidence that rats undergoing prolonged treatment with morphine do not develop signs of tolerance at peripheral mu-opioid receptors in the presence of painful paw inflammation. Although internalization of MOR was significantly increased in the DRG neurons from these animals, G-protein coupling of MOR as well as inhibition of cAMP accumulation were preserved. However, opioid receptor internalization and signaling were reduced and tolerance was restored when endogenous opioid peptides in inflamed tissue were removed by antibodies or by depleting opioid-producing granulocytes, monocytes and lymphocytes with cyclophosphamide (Zöllner et al., 2008). Thus, the continuous availability of endogenous opioids in inflamed tissue apparently increases recycling and preserves signaling of mu-receptors in sensory neurons, and thereby counteracts the development of peripheral opioid tolerance. These findings suggest that the use of peripherally acting opioid agonists for the prolonged treatment of inflammatory pain is not necessarily accompanied by tolerance. Evidence for this has recently been provided using the kappa-agonist asimadoline in patients with diarrhea-predominant irritable bowel disease where a significant reduction in pain and other symptoms was observed, and persisted over a treatment period of 3 months (Mangel et al., 2008).

2.4. Therapeutic Issues and Research Perspectives

Peripheral mechanisms of opioid analgesia have gained recognition in the clinical setting. Opioid receptors have been demonstrated on peripheral terminals of sensory nerves in human synovia (Mousa et al., 2007b; Stein et al., 1996), dermal and epidermal nerve fibers (Ständer et al., 2002) and dental pulp (Jaber et al., 2003). That such receptors mediate analgesia has been amply demonstrated in patients with various types of pain (e.g. in chronic rheumatoid arthritis and osteoarthritis, oral mucositis, bone pain, complex regional pain syndrome, after dental, laparoscopic, urinary bladder and knee surgery) (Azad et al., 2000; Dionne et al., 2001; Kopf et al., 2006; Sawynok, 2003; Stein et al., 2003). One of the most extensively studied and most successful applications is the intraarticular injection of morphine into inflamed knee joints (2004; Kalso et al., 2002; Likar et al., 1997; Stein et al., 1999; Stein et al., 1991). Novel peripherally restricted kappa-agonists have been investigated in humans with chronic painful pancreatitis and neuropathic pain (Eisenach et al., 2003; Wallace et al., 2006) and morphine derivatives that do not cross the blood-brain barrier (morphine-6-glucuronide) have proven equieffective to morphine at pain relief but with much fewer side effects (Hanna et al., 2005; Tegeder et al., 2003; van Dorp et al., 2008).

Opioid peptides are found in human subcutaneous and synovial cells, mast cells, granulocytes, lymphocytes and macrophages. The predominant peptides are β-endorphin and Met-enkephalin, but dynorphin and endomorphins were also detected (Heurich et al., 2007; Likar et al., 2004; Likar et al., 2007; Mousa et al., 2007b; Rittner et al., 2007a; Stein et al., 1993; Stein et al., 1996; Straub et al., 2008). Furthermore, in patients undergoing knee surgery, blocking intraarticular opioid receptors by the local administration of naloxone resulted in significantly increased postoperative pain (Stein et al., 1993). These findings suggest that in a stressful (e.g. postoperative) situation, opioids are tonically released in inflamed tissue and activate peripheral opioid receptors to attenuate clinical pain. In addition, CRF receptors are co-expressed with β-endorphin in synovial inflammatory cells and the intraarticular application of CRF can transiently reduce postoperative pain (Likar et al., 2007). Apparently, endogenous immune cell-derived opioids do not interfere with exogenous agonists since intraarticular morphine is an equally potent analgesic agent in patients with and without opioid-producing inflammatory synovial cells (Likar et al., 2004; Stein et al., 1996). Similar to results obtained in animal studies (Zöllner et al., 2008) this suggests that immune cell-derived opioids may prevent the development of tolerance at peripheral opioid receptors.

These findings provide new insights into intrinsic mechanisms of pain control and open novel strategies to develop drugs and alternative approaches to treatment of pain and inflammation. Immunocompromised patients (e.g. in AIDS, cancer, diabetes, multiple sclerosis) frequently suffer from painful neuropathies. These can be associated with intra- and perineural inflammation, with reduced intraepidermal nerve fiber density and/or with low $CD4^+$ lymphocyte counts (Polydefkis et al., 2003). Thus, it may be interesting to investigate the opioid production/release and the migration of opioid-containing leukocytes in these patients. The important role of adhesion molecules and chemokines in the trafficking of opioid-containing cells indicates that anti-adhesion or anti-chemokine strategies for the treatment of inflammatory diseases may, in fact, carry a significant risk to exacerbate pain. It would be highly desirable to identify stimulating factors and strategies that selectively attract opioid-producing cells, augment opioid peptide production and/or increase peripheral opioid receptor numbers in damaged tissue. Studies using various gene therapeutic approaches are currently underway (Beutler et al., 2005; Kyrkanides et al., 2007; Mata et al., 2002; Pohl et al., 2003). A further interesting question is whether immune-derived opioid peptides and exogenous opioids interact in a synergistic fashion. Undoubtedly, peripherally acting opioid agonists would be most attractive for their lack of central side effects (respiratory depression, nausea, dysphoria, addiction, tolerance) and their lack of the typical adverse effects of nonsteroidal anti-inflammatory drugs (gastric erosions, ulcers, bleeding, diarrhea, renal toxicity, thrombo-embolic complications) in order to improve the standard of patient care in the management of acute and chronic pain.

3. INTERACTIONS BETWEEN ADRENERGIC AND OPIOID RECEPTORS ON PRIMARY AFFERENT NEURONS

3.1. Synergistic Interactions Between α_2-Adrenergic and Opioid Receptors

As described above, α_2-Adrenergic and opioid receptors (α_2ARs and ORs) mediate diverse physiological functions, including analgesia. Although many approaches have been investigated in attempts to overcome adverse side affects associated with the prolonged use of opioids, several reports have described enhanced opiate-mediated spinal antinociception following coadministration of low doses of α_2AR agonists. Furthermore, it has been shown extensively through both behavioral (Hylden and Wilcox, 1983; Monasky et al., 1990; Ossipov et al., 1990a; Ossipov et al., 1990b; Ossipov et al., 1990c; Roerig et al., 1992; Stevens et al., 1988) and electrophysiological (Omote et al., 1990; Sullivan et al., 1987; Wilcox et al., 1987) methods that co-activation of α_2ARs and ORs produces synergistic interactions in the

spinal cord, although the mechanisms underlying this phenomenon have yet to be characterized. This property may be important in managing chronic, opioid-insensitive pain because synergy-enabled decreases in dose may mitigate the unwanted side effects observed clinically. Furthermore, hyperalgesia induced by spinal administration of opioids could be minimized if co-administered adrenergic agonists allow reduced opioid doses (Gardell et al., 2002). Therefore, understanding the molecular mechanisms involved in the synergistic interactions of these receptors is of both clinical and theoretical importance in the development of more efficacious therapies for pain management.

3.2. Receptor Localization and the Occurrence of Synergy

Similar to the three known opioid receptor genes, three α_2AR subtypes, α_{2A}, α_{2B}, and α_{2C}, have been cloned (for review see (Aantaa et al., 1995; MacDonald et al., 1997; Philipp et al., 2002)). Agonists acting at these six receptor subtypes are thought to share common signal transduction systems mediated primarily through inhibitory G proteins, the activation of which inhibit pain transmission in the spinal cord. Previous literature suggests that synergy will be observed between agonists acting at the following receptor pairs: DOR/α_{2A}AR (Stone et al., 1997), DOR/α_{2C}AR (Fairbanks et al., 2002), MOR/α_{2A}AR (Stone et al., 1997) and MOR/α_{2C}AR (Fairbanks et al., 2000). In addition, synergistic interactions between subtypes of receptors within the same family (OR/OR or α_2AR/α_2AR) have been reported (Daniels et al., 2005; Graham et al., 2000; Malmberg and Yaksh, 1992; Omote et al., 1990; Porreca et al., 1992; Portoghese et al., 1987; Vaught et al., 1981; Vaught and Takemori, 1979; Ward and Takemori, 1983). While these receptors may synergize *in vivo*, the mechanisms by which synergy occurs may not be the same for all pairs, in part due to differential localization in the central nervous system. It has been shown that the primary localization of the α_{2A}AR in the rat spinal cord is on the terminals of capsaicin-sensitive, SP-containing primary afferent fibers (Stone et al., 1998). In contrast, the majority of α_{2C}ARs were shown not to be of primary afferent origin, not strongly colocalized with SP, and likely expressed by a subset of spinal interneurons (Stone et al., 1998). MORs and DORs also appear to localize in separate compartments in the spinal cord. Whereas MORs have been shown to localize on both the terminals of primary afferent fibers and on second-order spinal neurons (Arvidsson et al., 1995), DORs have been detected primarily in terminals of primary afferent fibers (Arvidsson et al., 1995; Cheng et al., 1997; Dado et al., 1993; Zhang et al., 1998b) with only a small subset of DORs found on neuronal plasma membranes (Cahill et al., 2001). These data suggest that, under steady state conditions, minimal membrane-bound DORs are required for cellular function and that the majority are located presynaptically in an intracellular “reserve” capacity, awaiting targeting to the plasma membrane in response to physiological changes.

It has been proposed that synergy between receptors sharing signal transduction pathways cannot occur if the receptor populations are anatomically co-localized because of competition for and/or saturation of intracellular signaling pathways (Honore et al., 1996). For example, it was demonstrated that co-activation of OR and α_2AR in individual locus coeruleus (LC) neurons fails to produce a synergistic interaction (Stone and Wilcox, 2004). LC neurons are known to co-express MOR and α_{2A}AR, the activation of which results in increased K^+ conductance and membrane hyperpolarization due to their convergence on a common set of K^+ channels (Aghajanian and Wang, 1987). Therefore, despite evidence that MOR and α_{2A}AR synergize in behavioral tests of analgesic efficacy (Stone et al., 1997), they do not interact in a system where they are co-localized and converge on a signaling pathway. In contrast to this, α_{2A}ARs and DORs, which co-localize in spinal cord terminals, have been shown to produce powerful analgesic synergy behaviorally (Stone et al., 1997). These receptor subtypes have both been shown to localize in primary afferent terminals (Fields et al., 1980; Lamotte et al., 1976; Stone et al., 1998) and mediate at least part of their analgesic effect through presynaptic inhibition of release of substance P (SP) and calcitonin gene-related peptide

(CGRP), which are often stored in the same large dense-core vesicles (LDCV) (Merighi et al., 1988). This inhibition of nociceptive neuropeptide release is primarily mediated through receptor coupling to pertussis toxin-sensitive G_i/G_o proteins, activation of which can decrease transmitter release by inhibiting adenylyl cyclases (Makman et al., 1988) and voltage-gated Ca^{2+}channels (Holz et al., 1989). Conversely, DOR and α_{2A} AR coupling to Gi has also been shown to mobilize IP_3-sensitive Ca^{2+} stores through a signal transduction pathway that involves activation of PLC by $G_{\beta\gamma}$ subunits released from agonist-induced dissociation of the G_i heterotrimer (Dorn et al., 1997; Yoon et al., 1999). Recent evidence has suggested that ORs can couple not only to G_i/G_o, but to a variety of G proteins. For instance, opioids have been shown to produce analgesia by activation of PLA_2 and have been shown to act through various G proteins to activate phospholipase C (PLC), mobilizing Ca^{++}, activating PKC and enhancing presynaptic voltage-gated, ATP-gated and Ca^{++}-gated K^+-channel activity (for reviews see (Aantaa et al., 1995; Connor and Christie, 1999; Law et al., 2000; Millan, 1999; Millan, 2002). In agreement with this differential G protein coupling, it was recently shown that agonist activation of DORs can result in LDCV exocytosis, leading to functional DOR insertion in the plasma membrane through G_q coupling (Bao et al., 2003). These data fit well with the localization studies placing the majority of DORs intracellularly, needing either DOR agonist binding or other G-protein coupled stimuli to traffic receptors to the plasma membrane. α_2ARs have also been shown to exhibit differential coupling to G proteins. Although all α_2ARs couple to the inhibitory G proteins G_i/G_o, there is evidence indicating α_2AR interactions with other G proteins, including G_s and $G_{q/11}$ (Aantaa et al., 1995), leaving open the possibility of trafficking mechanisms similar to that of DOR.

3.3. The Possibility of Heterodimerization

One explanation for the synergism observed between these two receptors co-localized in a single subcellular compartment (i.e. primary afferent terminals) is the formation of heterodimeric complexes accounting for the change in G protein coupling. It is now recognized that GPCRs can form oligomeric complexes as well as act as monomeric cell-surface receptors. For example, it has been shown that α_{2A}ARs and MORs can form functional heterodimer complexes on the plasma membrane of transfected cells in culture as well as native neurons (Jordan et al., 2003). Not only do heterodimers form, but these complexes can mediate conformational changes via cross-talk that propagates from one receptor to the other, leading to changes in function (Vilardaga et al., 2008). An interesting aspect of these heterodimeric associations is the possibility of novel pharmacological properties distinct from either component receptor alone. Recent studies suggest that opioid-induced tolerance and physical dependence are mediated through physical association of MOR and DOR as heterodimers. Studies using bivalent ligands combining a mu agonist and a delta antagonist produced analgesia with 50-fold greater potency than intravenous morphine while also suppressing both physical dependence and tolerance (Daniels et al., 2005). This phenomenon is particularly interesting for DOR/α_2 synergy because GPCR dimerization may facilitate transport of receptors to the cell surface as well as facilitate G protein coupling and activation (Brock et al., 2007; Kaupmann et al., 1998; Milligan, 2007). Thus, the generation of novel properties upon dimerization may play a key role explaining synergistic interactions between receptor pairs that are found to co-localize.

3.4. Clinical Implications

Many issues still need to be addressed to determine the dominant mechanisms underlying synergistic interactions. Subcellular localization studies combined with a more detailed analysis of the specific signaling pathways involved are still needed. These questions are important because synergistic pairs may have substantial clinical applications, particularly for the treatment of special conditions, including cancer and neuropathic pain (Eisenach et al., 1994; Eisenach et al., 1995; Eisenach et al., 1989), where the use of morphine and other opioids

has provided inconsistent or unsatisfactory long-term relief (Arner and Meyerson, 1988; Coombs et al., 1984; Siddall et al., 1994).

4. OPIOIDS AND WOUND HEALING

4.1. Cytokines and Exogenous Opioids

An area of particular importance to the subject of peripheral opioid action relates to opioid-cytokine interactions in the setting of peripheral inflammation including inflammation occurring in the tissue surrounding surgical wounds. Surgical wounds represent archetypical inflammatory lesions having all stigmata of acute inflammation: increased blood flow, edema, increased temperature, pain and loss of function. The inflammatory nature of surgical wounds is highly complex and changes over time as healing proceeds from coagulation through an inflammatory phase followed by revascularization and finally remodeling of the local tissue. Cytokine functioning in wound models has been studied for many years though typically not for the purpose of understanding pain. Rather, cytokine regulation of various aspects of the healing process has been the focus of most laboratories, e.g. fibroblast proliferation, neovascularization, re-epithelialization, etc.

4.2. Time Course of Incisional Sensitization

There is reason to think that cytokines liberated in the settings of peripheral inflammation and incision contribute to pain, albeit by poorly defined mechanisms, and that exogenous opioids modulate these effects. However, the direction and magnitude of opioid effects on peripheral cytokine levels has not been entirely consistent between reports. For example, Nelson et al. used a model of skin inflammation and sensitization by exposing rats to a topical preparation of 2,4-dinitrofluorobenzene (DNFB) (Nelson and Lysle, 2001). When skin samples were harvested after DNFB exposure in rats pre-treated with either saline or morphine, these investigators found sharply higher levels of IL-6 mRNA 3–12 hours after DNFB application in the morphine pre-treated animals. There was no change, however, in levels of the anti-inflammatory cytokine IL-10 over this same period. Using the carrageenan model of peripheral inflammation, Pourpak et al. initiated an inflammatory reaction in the hind paws of mice with or without pretreatment with various doses of systemic morphine (Pourpak et al., 2004). The results were complex in that low doses of morphine (< 3 mg/kg) actually enhanced hind paw edema, an index of local inflammation, while larger doses (> 5 mg/kg) had the opposite effect. The high dose anti-edema effects were somewhat unexpectedly associated with enhanced serum IL-1β levels. On the other hand Fecho et al using a similar carrageenan model observed that while morphine did reduce carrageenan-induced hind paw edema, there was no effect of this opioid on serum levels of IL-1β, IL-6 or TNFα (Fecho et al., 2007). Many factors may have affected the reported results including the specific model, the timing of the assays versus induction of inflammation and perhaps the specific types of opioids used.

Because of its relevance to perioperative pain management, the hind paw incisional model has been used to study pain mechanisms and analgesic efficacy. The model has been described for both rats and mice (Brennan et al., 1996; Pogatzki and Raja, 2003). In general, both thermal and mechanical nociceptive thresholds drop immediately after the incisions are made, and these changes resolve after 4–7 days. The analgesic effects of opioids have been studied using this model, although little work has focused on the peripheral effects of opioids specifically (Zahn et al., 1997). Recently the pattern of liberation of several cytokine inflammatory mediators and nerve growth factor (NGF) in peri-incisional skin were described (Clark et al., 2006; Wu et al., 2007). Incision area cytokine production has been studied using homogenates for protein and mRNA quantification. Immunohistochemical and in situ hybridization techniques have further refined our understanding of cell type specific effects. Consistent with other inflammatory lesions, incisions induce the rapid but transient production of many cytokines

including several with independent support for participation in pro-nociceptive processes such as IL-1β, IL-6, NGF and TNFα. The time course for the liberation of these mediators is variable. However, several of the interleukins such as IL-6 and keratinocyte-derived cytokine (KC) seem to have very early peak levels (within 2 hours) followed by rapid resolution. Others like IL-1β and NGF have somewhat more sustained increases.

4.3. Dermal Cytokines and Pain

While a good deal is known about the profile of cytokine production, less is known about the relative contributions made by each to specific inflammatory processes associated with incision or other types of inflammation. In fact, cytokines tend to be both pleiotrophic and redundant. Thus, in order to understand the importance of opioid effects on these peripheral mediators, we need to know which are involved in nociception. This issue has been addressed directly by several laboratories by injecting small quantities of cytokine and related mediators into the dermis of the paws and following the ensuing sensitization. For example Cunha et al. administered picogram to low nanogram quantities of IL-1β, KC, TNFα and other mediators intradermally in mice (Cunha et al., 2005). These caused rapid, profound and sustained mechanical sensitization. Similar results have been reported for IL-8, IL-12, IL-15 and IL-18 (Cunha et al., 2000; Verri et al., 2008; Verri et al., 2006; Verri et al., 2005). In addition, the skin injection of NGF is well known to cause nociceptor sensitization (Ma and Woolf, 1997; Taiwo et al., 1991). An alternative strategy to the injection of the cytokines themselves to implicate specific mediators in nociceptor sensitization has been the injection of antisera and interleukin receptor blockers to reduce nociception caused by locally produced mediators. Experiments involving anti-NGF (Banik et al., 2005), anti-IL-6 (Summer et al., 2008), and IL-1 receptor antagonist (Cunha et al., 2000) indicated that all three mediators contribute to nociceptor sensitization in models of inflammation.

4.4. Acute Regulation of Incisional Cytokines by Exogenous Opioids

Analyses of the direct effects of opioids on cytokines in tissue surrounding incisional wounds indicate that cytokine production is inhibited in a dose-dependent manner. For these studies, animals were treated systemically with morphine immediately prior to hind paw incision (Clark et al., 2007). Morphine pretreatment reduced the production of IL-1β, IL-6, G-CSF and other cytokines. Interestingly, morphine was more potent in inhibiting cytokine production relative to its antinociceptive effect. The investigators went on to ask where the relevant site of action for morphine was in reducing wound area cytokine levels.

Immunohistochemical studies from both incisional and excisional wound experiments demonstrate that the keratinocyte layer as well as infiltrating immune cells produce cytokines after tissue injury (Clark et al., 2007; Engelhardt et al., 1998; Kondo et al., 2002; Roy et al., 2008). In the incisional experiments by Clark et al., very distinct epidermal keratinocyte staining for cytokines was observed following incision.

Moreover, MOR, DOR and KOR were all up-regulated in keratinocytes during the healing process (Cheng et al., 2008). As noted above, both of these cell populations (keratinocytes and infiltrating neutrophils) express functional opioid receptors. Thus, opioid effects on one or both populations of cells might explain the effects of acutely administered morphine on cytokine production.

Overall, however, we have a poor understanding of the relative contribution of opioid effects on keratinocytes versus neutrophils in wounds. Studies directly examining exogenous opioid effects on keratinocyte cytokine production are lacking. Neutrophils, the predominant immunocyte found in wounded skin for the first few days after injury, also express opioid receptors as well as produce opioid peptides. Thus morphine might have direct effects on

neutrophil cytokine production. In addition to direct effects on neutrophil cytokine production, opioids can also affect neutrophil migration into wounded tissue (Choi et al., 1999; Miyagi et al., 2000; Wang et al., 2005; Yossuck et al., 2008). However the dose required morphine for inhibiting neutrophil migration are far in excess of those required to significantly reduce wound area cytokine levels (Clark et al., 2007).

4.5. The Chronic Morphine Issue

The management of pain in persons chronically consuming opioids is known to be highly problematic. Lowered pain theresholds and exaggerated pain are commonly reported by patients who use opioids chronically prior to their procedures (Angst and Clark, 2006; Cohen et al., 2008). Several mechanisms have been proposed to account for these changes observed in patients on chronic opioids, including enhanced descending facilitation, reduced spinal cord neurotransmitter reuptake and increased peripheral nociceptor sensitization. Studies of the spinal cord tissue of rats, suggests that after the chronic administration of opioids intrathecally (Johnston et al., 2004) or systemically (Raghavendra et al., 2002) that the expression of IL-1β, IL-6 and TNFα are significantly increased, probably in glial cells even in the absence of opioid withdrawal. Though it is difficult to extrapolate from these central effects to the periphery, these observations suggest the intriguing hypothesis that chronic morphine exposure increases cytokine production in peripheral tissues. Consistent with this hypothesis, recent studies suggest a significant increase of NGF and TRPV1 channels in peripheral nociceptive neurons after chronic administration of morphine (Vanderah T.W. and Porreca F. unpublished observations). However, no information is available measuring indices of inflammation in the wounds of opioid naïve versus opioid consuming patients.

Investigators have addressed the question as to whether exogenous opioids administered in repeated or chronic fashion elicit effects distinct from those observed after single administration. For example, early experiments involving the repeated local injection of the MOR-selective agonist D-Ala^2,N-Me-Phe^4,Gly^5-ol-enkephalin (DAMGO) into rat hind paw skin demonstrated that nociceptive sensitization can in fact be observed in this setting (Aley et al., 1995). Local plastic events involving the protein kinase C (PKC) pathway seem to be involved in this type of response (Aley and Levine, 1997). It was subsequently demonstrated that animals chronically treated with opioids, including morphine, displayed exaggerated nociceptive sensitization after hind paw incision (Li et al., 2001; Liang et al., 2008).

One proposed mechanism for these observations involves an increase in the local production of inflammatory mediators at the site of incision in animals chronically treated with an opioid relative to opioid-naïve animals. Using the hind paw incision model, Liang et al studied the relative abundance of several cytokines in the peri-incisional skin of mice chronically pre-treated with either saline or morphine (Liang et al., 2008). Although the relative levels of cytokines in the skin prior to incision were not different between the two groups, several cytokines including IL-1β, IL-6, TNFα, and G-CSF were found in increased abundance in the peri-incisional skin of animals that were chronically pre-treated with morphine; the level of another cytokine, KC, was similar between the two groups. No differences in wound area neutrophil infiltration were noted. Administration of pentoxiphylline, a broad inhibitor of cytokine production, following chronic morphine treatment reduced the local abundance of these mediators following skin incision and also eliminated the post-incisional hypersensitivity in these mice. Together, these data suggest that exogenous opioids modulate the local production of inflammatory cytokines, and possibly other inflammatory mediators, in the vicinity of incisional and perhaps other inflammatory wounds. The mechanisms controlling these effects are unclear but could involve actions on either resident keratinocytes or infiltrating neutrophils. Additional questions related to opioid receptor subtype involvement, second

messenger system participation and modulation by peripheral nerves remain largely unexplored.

5. TRPV1 ION CHANNELS IN PAIN AND ITCH

One of the mechanisms leading to the sensitization of peripheral sensory nerves by cytokines and inflammatory mediators likely involves the functional modulation of ion channels such as TRPV1. TRPV1 is a ligand-gated channel activated by capsaicin, protons and heat and is expressed in nociceptors but not in other peripheral neurons (Caterina et al., 1997; Oh, 2006; Oh et al., 1996). Due to its activation and expression profile, TRPV1 has been implicated as an important mediator of inflammatory pain. Indeed, some types of thermal pain during inflammation are reduced in TRPV1-deficient mice (Caterina et al., 2000; Davis et al., 2000). TRPV1 is also activated by many endogenous compounds including endocannabinoids such as anandamide and NADA (De Petrocellis et al., 2004; Zygmunt et al., 1999). Metabolites of arachidonic acid produced by lipoxygenases (LO) such as 12-hydroperoxyeicosaenoic acid (12-HPETE) have been shown to activate TRPV1 (Hwang et al., 2000). The activation of TRPV1 by 12-HPETE can be blocked by capsazepine suggesting that 12-HPETE acts at the capsaicin binding site. This was confirmed in a competition binding assay where 12-HPETE was shown to bind TRPV1 with greater affinity than capsaicin (Shin et al., 2002). Additionally, by comparing the three-dimensional structures of 12-HPETE and capsaicin, it was found that the overall length and contour of the two chemicals are very similar and functional residues such as dipolar groups are at very close proximity with each other (Hwang et al., 2000). This evidence clearly suggests that 12-HPETE is another endogenous activator of TRPV1. But what could be the upstream signal to the LO/TRPV1 pathway?

Bradykinin is a mediator that also plays an important role in acute inflammatory pain and sensitization. Although, bradykinin has long been known to excite sensory neurons, the mechanisms underlying this effect appear to vary as a function of cell and tissue type (Wood and Docherty, 1997). For example, in nodose ganglion neurons, bradykinin activates Ca^{2+} dependent Cl^{-} channels (Oh and Weinrich, 2004). Because bradykinin can stimulate phospholipase A2 (PLA2) to produce arachidonic acid, the bradykinin signaling pathway was chosen for testing the utilization of the LO/TRPV1 pathway in somatic sensory neurons. Indeed, currents induced by bradykinin in cultured sensory neurons are blocked by the TRPV1 antagonist capsazepine, suggesting that bradykinin activates TRPV1 in sensory neurons (Shin et al., 2002). Furthermore, excitation of cutaneous C-fibers or Ca^{2+}-influx induced by bradykinin is markedly attenuated by inhibitors of PLA2, LO, and TRPV1 (Shin et al., 2002). The concentration of the LO product 12-HETE is markedly increased after bradykinin application in cultured sensory neurons. These results strongly suggest that bradykinin excites sensory neurons via the PLA2/LO/TRPV1 pathway.

However, other pathways such as the phospholipase C/protein kinase C pathway that sensitizes TRPV1 after phosphorylation should also be considered.

Itch (pruritus) is an unpleasant sensation that produces the desire to scratch or rub the skin to provide relief. Atopic dermatitis often causes severe pruritus with the result that many patients suffer from depression or sleep deprivation (Hashiro and Okumura, 1997; Sheehan-Dare et al., 1990). Among the numerous itch-causing substances, histamine is the best studied. Intradermal injection of histamine induces itch (Heyer et al., 1997; Magerl et al., 1990; Schmelz et al., 1997) and also excites peripheral sensory nerve fibers (Schmelz et al., 1997; Schmelz et al., 2003). Histamine activates a subset of C-fibers that differ from nociceptors because some small unmyelinated nerve fibers that respond to noxious heat and mechanical stimulation are insensitive to histamine (Handwerker et al., 1991). Interestingly, polymodal C-fibers are mostly insensitive to histamine (Handwerker et al., 1991). It is now well known that itch signals ascend

to the brain through specific conduction pathways that differ from the central nociceptive conduction pathways (Andrew and Craig, 2001; Sun and Chen, 2007). Although histamine's pruritic action has been well characterized, its molecular mechanism for inducing itch signals in sensory nerve fibers has remained largely unknown.

Evidence that histamine also uses the PLA2/LO/TRPV1 pathway to excite sensory neurons was recently reported (Shim et al., 2007). Because histamine is another inflammatory mediator known to stimulate PLA2, the PLA2/LO/TRPV1 pathway was a candidate for the excitation of sensory neurons. As expected, the histamine-induced inward current is inhibited by capsazepine, indicating that histamine activates TRPV1 in cultured sensory neurons. Shim and coworkers further demonstrated that histamine causes inward currents in human embryonic kidney cells only when TRPV1 and histamine receptor subtype 1 are cotransfected (Shim et al., 2007). In Ca^{2+} imaging experiments, histamine causes Ca^{2+} influx in cultured sensory neurons that are isolated from wild type mice but not from $TRPV1^{-/-}$ mice. More importantly, application of histamine causes robust scratching behaviors in wild-type mice that are reversed by PLA2, LO and TRPV1 inhibitors (Shim et al., 2007). The scratching behavior induced by histamine was significantly reduced in $TRPV1^{-/-}$ mice. These results clearly suggest that histamine causes itch sensation by the excitation of sensory neurons via stimulation of the PLA2/LO/TRPV1 pathway. Elucidation of the histamine signaling pathway may be helpful for developing anti-pruritogenic substances to treat patients with atopic dermatitis and other forms of pruritus. These findings suggest a mechanism that may be targeted in the development of new therapeutic strategies for the treatment of inflammatory pain and itch.

Thus, it appears that TRPV1 is present both in nociceptors and itch fibers, and mediates bradykinin-induced nociception and histamine-induced itch sensations. So how can one type of channel mediate both nociception and itch? This is likely not due to differences in the channel itself but due to its expression in different types of fibers that transmit nociceptive or itch signals in peripheral sensory nerves. It is generally accepted that painful stimuli can inhibit itch sensation via central pathways. Thus, when both nociceptive and itch conducting pathways are activated, the nociceptive pathway apparently predominates – inhibiting the transmission of signals associated with itch. This possibility may underly the phenomenon whereby scratching the affected skin relieves the sensation of itch.

6. NERVE GROWTH FACTOR AND PAIN

Although nerve growth factor (NGF) is a classic neurotrophin that is essential for the development and survival of embryonic sympathetic neurons and sensory neurons (Levi-Montalcini and Angeletti, 1968), its importance in the pain field is based largely on its ability to produce hyperalgesia by increasing the sensitivity of nociceptive sensory neurons. This increase in sensitivity of sensory neurons augments firing in response to noxious stimuli, thereby causing hypersensitivity. Indeed, in adult animals much evidence supports the notion that NGF acts as an inflammatory mediator (Levi-Montalcini et al., 1996; McMahon, 1996). It is produced and released at the site of inflammation in response to tissue injury (Halliday et al., 2004; Woolf et al., 1994) by a number of cell types (Woolf et al., 1996).

Exogenous administration of NGF produces hypersensitivity to noxious stimuli in animal models of tissue injury (Amann et al., 1995; Lewin et al., 1994), whereas pretreatment with antibodies to NGF or a fusion protein attached to a modified TrkA receptor attenuates inflammation-induced hypersensitivity (McMahon et al., 1995; Woolf et al., 1994). The NGF-induced hypersensitivity results largely from direct actions on small diameter sensory neurons. A subset of small diameter sensory neurons expresses the TrkA NGF receptors and a majority of neurons express the p75 receptor (Averill et al., 1995; Verge et al., 1992). Acute exposure of isolated sensory neurons to NGF increases the sensitivity of these neurons as indicated by

a number of endpoints, including a reduced threshold of thermal excitation (Rueff and Mendell, 1996), an increase in capsaicin current (Shu and Mendell, 1999), an increase in capsaicin-induced influx of calcium (Bonnington and McNaughton, 2003), an increase in the number of action potentials elicited by a ramp of depolarizing current (Zhang and Nicol, 2004), and an increase in capsaicin-evoked release of CGRP (Bowles et al., 2006; Malcangio et al., 2000).

One established mechanism for NGF-induced sensitization of sensory neurons results from a posttranslational modification of activity at the TRPV1 channel. Nerve growth factor binding to its tyrosine kinase receptor (TrkA) can activate phospholipaseγ (PLCγ) which cleaves phosphatidylinositol–4,5-biphosphate (PIP2) to produce inositol 1,4,5-trisphosphate and diacylglycerols (Chao, 2003; Huang and Reichardt, 2003). These second messengers increase release of intracellular calcium and activate protein kinase C (PKC), respectively, and PKC activation has been shown to cause an increase in TRPV1 channel activity (Vellani et al., 2001) and an increase in capsaicin-evoked peptide release from sensory neurons (Barber and Vasko, 1996). Inhibiting PLC blocks the ability of NGF to augment heat-induced currents in sensory neurons (Galoyan et al., 2003). Nerve growth factor binding to the TrkA receptor also increases activity of PI3 kinases which can convert PIP2 to PIP3 and can activate another kinase, AKT (Chao, 2003; Huang and Reichardt, 2003). Several studies have shown that NGF-induced sensitization of capsaicin responses is blocked by PI3 kinase inhibitors (Bonnington and McNaughton, 2003; Zhu and Oxford, 2007; Zhuang et al., 2004).

Activation of TrkA also is linked to the ras/MEK/ERK pathway, and some studies have shown that ERK inhibitors block the actions of NGF on TRPV1 (Zhu and Oxford, 2007; Zhuang et al., 2004), whereas others do not (Bonnington and McNaughton, 2003; Shu and Mendell, 2001). Thus, unresolved questions exist regarding the intracellular signaling pathways that mediate NGF-induced sensitization. It seems likely that multiple signaling pathways are involved in NGF-induced sensitization and that different endpoints of NGF-induced sensitization may involve different signaling pathways. Furthermore, the conditions by which NGF binding to TrkA activates the various downstream signaling pathways remains to be determined. Since cross-talk exists between signaling pathways, additional studies are needed to clearly establish which signaling pathways are essential for NGF-induced increases in TRPV1 activity and which signaling pathways are secondary.

Recent studies also suggest an additional mechanism for NGF-induced sensitization of capsaicin-induced excitability: the ability of the growth factor to alter trafficking of TRPV1 to the plasma membrane (Stein et al., 2006; Zhang et al., 2005). The increase in trafficking may be associated with the formation of a complex between TRPV1 and PI-3 kinases (Stein et al., 2006) although causal studies have yet to be performed. The increase in trafficking also is attenuated by the Src kinase inhibitor, PP-2 (Zhang et al., 2005) suggesting the involvement of Src kinases in NGF-induced sensitization. NGF also can sensitize sensory neurons through the production of second messengers. For example, by binding to the p75 receptor on sensory neurons, NGF has been shown to activate sphingomyelinase (Dobrowsky et al., 1994) which liberates ceramide from sphingomyelins. Ceramide, in turn, can be converted to sphingosine, sphingosine-1-phosphate, or ceramide-1-phosphate, and these second messengers alter activity of various kinases and phosphatases.

In embryonic sensory neurons, the ability of NGF to increase the number of action potentials elicited by a ramp of depolarizing current is attenuated by blocking sphingomyelinase, suggesting that liberation of ceramide is an important mechanism for NGF-induced sensitization of sensory neurons. It is interesting to note that ceramide-1-phosphate increases the activity of phospholipase A2, the enzyme that liberates arachidonic acid (the rate- limiting step in prostaglandin synthesis), whereas S-1-P is involved in the ability of cytokines to increase expression of cyclooxygenase 2 (Pettus et al., 2005). Thus, increasing the production of these

sphingolipids could result in an increase in prostaglandin production, which in turn can result in sensitization of sensory neurons (see below).

Although acute exposure to NGF alters sensitivity of sensory neurons to thermal responses and to capsaicin through post-translational modification, long-term exposure to NGF also can alter the excitability of sensory neurons presumably by increasing the expression of proteins that are involved in excitability. Indeed, previous studies have shown that long-term exposure of sensory neurons to NGF increases the expression of TRPV1 (Bron et al., 2003; Ji et al., 2002), bradykinin receptors (Petersen et al., 1998), purinergic receptors (Ramer et al., 2001) and sodium channels (Fjell et al., 1999). This increase in expression of molecules that excite sensory neurons can augment the responsiveness of these neurons to various inflammatory mediators, and this could constitute an important mechanism for maintaining peripheral sensitization during inflammation. Long-term exposure of sensory neurons to NGF also increases the expression of putative nociceptive transmitters such as substance P and CGRP (Lindsay and Harmar, 1989) which could result in an increase in the amount of transmitter released after a depolarizing stimulus. An increase in the release of neurotransmitters from sensory nerve endings in the spinal cord can contribute to enhanced nociception and to central sensitization, whereas an increase in release from peripheral endings contributes to neurogenic inflammation.

Based on the work reviewed above, it is clear that NGF causes acute sensitization of sensory neurons through a number of signaling cascades and that it also can increase expression and trafficking of molecules that mediate excitability of sensory neurons. The question remains whether there are other mechanisms by which NGF mediates excitability of sensory neurons. One intriguing prospect is to examine whether NGF alters the ability of sensory neurons to respond to other inflammatory mediators. Because small diameter sensory neurons are acutely sensitized by the pro-inflammatory prostaglandins PGE2 and PGI2 (Svensson and Yaksh, 2002), one obvious question is whether NGF-induced sensitization could be mediated at least in part by an interaction with prostaglandins. There is evidence in the literature that NGF can increase the production of prostaglandins in mast cells (Marshall et al., 1999; Murakami et al., 1997). Moreover, as discussed above, NGF can increase production of sphingolipids which, in turn, can increase prostaglandin synthesis. This leads to speculation that components of NGF-induced sensitization of sensory neurons could be mediated by the production of prostaglandins, although studies to test this notion directly have not been performed.

Recent work also suggests that inflammatory mediators (including NGF) might also alter intracellular signaling by prostaglandins. It has long been appreciated that the acute sensitizing actions of prostaglandins are mediated largely by the ability of EP and IP prostaglandin receptors to increase intracellular cAMP and thus activate protein kinase A (Ferreira and Nakamura, 1979; Hingtgen et al., 1995; Lopshire and Nicol, 1998; Southall and Vasko, 2001). Levine and co-workers, however, have evidence to suggest that inflammation results in a change in the downstream signaling pathway that mediates PGE_2-induced hyperalgesia (Aley et al., 2000). Their work shows that, under conditions of inflammation, the sensitizing actions of prostaglandin are attenuated by inhibition of PKC rather than PKA. The discovery of two additional effectors for cAMP, the guanine nucleotide exchange factors that are activated by cAMP or Epacs (de Rooij et al., 1998), provides a link between EP receptors coupled to Gs and other downstream signaling pathways including PLC epsilon and small GTP-ases such as Rap (Bos, 2006). Levine and co-workers propose that Epac activation is the intracellular mechanism by which increasing cAMP produces hyperalgesia during inflammation (Hucho et al., 2005) since the Epac selective agonist produces hyperalgesia and activates PKC epsilon. In a similar manner, Huang and her co-workers demonstrated that Epac activation in sensory neurons after inflammation mimics the sensitizing actions of PGE_2 on ATP currents, whereas inhibiting Epac attenuates the actions of PGE_2 (Wang et al., 2007).

The potential for inflammation to alter prostaglandin signaling has important implications. Indeed, the ability of PGE_2 to sensitize sensory neurons does not downregulate with inflammation or with chronic exposure to the eicosanoid. For example, we have shown that the ability of PGE_2 to augment capsaicin-evoked release of neurotransmitters from sensory neurons is not altered by chronic exposure of neurons to PGE_2 despite a significant reduction in PGE_2 receptor binding (Southall et al., 2002). Thus, it is intriguing to speculate that the lack of PGE_2 desensitization could be secondary to a shift in intracellular signaling, and NGF or other inflammatory mediators could cause the shift.

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