Mini-Symposium

Pain and Poppies: The Good, the Bad, and the Ugly of Opioid Analgesics

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Treating pain is one of the most difficult challenges in medicine and a key facet of disease management. The isolation of morphine by Friedrich Sertürner in 1804 added an essential pharmacological tool in the treatment of pain and spawned the discovery of a new class of drugs known collectively as opioid analgesics. Revered for their potent pain-relieving effects, even Morpheus the god of dreams could not have dreamt that his opium tincture would be both a gift and a burden to humankind. To date, morphine and other opioids remain essential analgesics for alleviating pain. However, their use is plagued by major side effects, such as analgesic tolerance (diminished pain-relieving effects), hyperalgesia (increased pain sensitivity), and drug dependence. This review highlights recent advances in understanding the key causes of these adverse effects and explores the effect of chronic pain on opioid reward.

Significance Statement

Chronic pain is pervasive and afflicts >100 million Americans. Treating pain in these individuals is notoriously difficult and often requires opioids, one of the most powerful and effective classes of drugs used for controlling pain. However, their use is plagued by major side effects, such as a loss of pain-relieving effects (analgesic tolerance), paradoxical pain (hyperalgesia), and addiction. Despite the potential side effects, opioids remain the pharmacological cornerstone of modern pain therapy. This review highlights recent breakthroughs in understanding the key causes of these adverse effects and explores the cellular control of opioid systems in reward and aversion. The findings will challenge traditional views of the good, the bad, and the ugly of opioids.

Opioids and pain: scope of the problem

It is estimated that 20–30% of Americans suffer from chronic pain, which is similar to that reported in Canada, Australia, and European countries (Blyth et al., 2001; Breivik et al., 2006; Johannes et al., 2010; Schopflocher et al., 2011). Equally striking is that chronic pain is among the most common forms of chronic illness afflicting individuals younger than 60 years of age (O'Connor, 2009). Chronic pain is also a major cause of disability (Manchikanti et al., 2013), and it is the cardinal feature of a diverse spectrum of diseases, including arthritis, migraine, cancer, metabolic disorders, and neuropathies. Treating pain in these diseases is notoriously difficult and often requires opioids, the most potent class of drugs used for controlling pain. Opioids are particularly effective for treating acute moderate-to-severe pain after surgery or trauma, and they are quintessential drugs in a physician's pharmacological toolbox for managing chronic pain. In 2012, physicians in the United States wrote >259 million opioid prescriptions, which equates to one bottle of pills for every adult American (according to the Centers for Disease Control and Prevention). Consumption of prescription opioids is highest in the United States and Canada, and, in these countries, opioid use for managing pain continues to grow (Gomes et al., 2014). Long-term opioid exposure can result in the development of analgesic tolerance, the hallmark feature of which is a loss in pain-

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relieving effect. In an effort to overcome tolerance, current strategies involve the use of either higher (and more frequent) doses of opioids or switching to more potent opioid agonists. However, these strategies have limited success because tolerance often reestablishes over time, leaving patients without adequate pain control. The need for escalating amounts of opioids puts patients at risk of severe sides effects, including dependence and respiratory distress. In the United States, an estimated 2.1 million Americans suffer from opioid substance use disorders (according to the National Institute of Drug Abuse), and 44 deaths a day are attributed to opioid overdose (according to the Centers for Disease Control and Prevention). There is also a higher incidence of opioid prescription medication addiction that has led to an increase in heroin use and a nearly quadrupling of death rate from overdose between 2002 and 2013 (Okie, 2010). Prescription, diversion, and illicit use of opioid therapeutics have emerged as major societal concerns in recent years (Compton and Volkow, 2006; SAMHSA, 2011). Although the effect of opioid tolerance and dependence has gained considerable attention, the problem of opioid-induced hyperalgesia—characterized by a paradoxical increase in pain sensitivity—is a less recognized consequence of opioid use. Despite the serious side effects that hinder long-term opioid use, there remains a strong reliance on this class of drugs for pain management. Therefore, it is imperative that we understand the underlying causes of these adverse effects to improve the utility of opioids in treating pain. In this review, we focused our efforts on critically evaluating the evidence that platelet-derived growth factor receptor-β (PDGFR-β), adenosine, and microglia are key cellular substrates in opioid analgesic tolerance, hyperalgesia, and neuropathic pain. We also present emerging evidence that chronic pain fundamentally alters reward circuitry, which has direct implications for the ability of opioids to alleviate the emotional component of pain and for their abuse potential. This article is a summary of topics covered in a mini-symposium. For a more comprehensive review of the subject, the reader is referred to other articles on this topic (Marchand et al., 2005; Beggs et al., 2012; Williams et al., 2013; Bourinet et al., 2014; Cahill et al., 2014; Grace et al., 2014; Fields and Margolis, 2015; Mélik Parsadaniantz et al., 2015).

Opioid receptors, analgesia, and the CNS

Opioids exert their effects through interaction with the superfamily of G-protein-coupled opioid receptors: μ, δ, and κ. All three receptor subtypes share extensive structural homology and couple to pertussis toxin-sensitive guanine nucleotide binding $G_{i/o}$-proteins to inhibit adenylyl cyclase, activate potassium conductance, suppress calcium conductance, and inhibit neurotransmitter release (Feng et al., 2012). Opioid receptors are expressed throughout the CNS and PNS on key nodes within the pain pathway. In the CNS, opioid receptors are highly localized in subcortical regions of the brain (thalamus, periaqueductal gray, rostral ventromedial medulla, and locus ceruleus) from which descending pain-modulating pathways originate and in the dorsal horn region of the spinal cord, an area important for the relay of nociceptive input to the brain and a primary site of action for the analgesic effects of opioids (Fields, 2004; Fig. 1). In the spinal dorsal horn, opioid receptors are expressed primarily in laminae I and II, with the proportion of μ, δ, and κ receptors comprising 70, 20, and 10%, respectively (Gouardères et al., 1985; Morris and Herz, 1987; Stevens et al., 1991). These receptors are localized on presynaptic afferent fibers (Lamotte et al., 1976; Gamse et al., 1979), interneurons (Kemp et al., 1996), and postsynaptic projection neurons (Zieglgänsberger and Bayerl, 1976). Additional opioid-like receptors have been identified in the CNS, including the opioid receptor like-1 (ORL-1) receptor (Meunier et al., 1995; Fioravanti and Vanderah, 2008). Unlike classical opioid receptors, the ORL-1 receptor is insensitive to naloxone, a nonselective opioid receptor antagonist. Evidence suggests that opioids can also bind and activate the toll-like receptor 4 (TLR4), an innate immune pattern-recognition receptor (Hutchinson et al., 2010; Jacobsen et al., 2014). The importance of these nonclassical opioid receptors in the modulation of pain and in the negative effects associated with opioid use is beyond the scope of the current review (Largent-Milnes and Vanderah, 2010; Pasternak and Pan, 2013; Convertino et al., 2015).

PDGFR-β: novel role in opioid tolerance and neuropathic pain

Opioid receptor activation triggers a complex cascade of signaling events. A major downstream consequence of μ receptor signaling is the activation of receptor tyrosine kinases, which are critically implicated in an array of cellular processes (Lemmon and Schlessinger, 2010). Wang et al. (2012) discovered recently in rats that chronic morphine treatment results in the activation of PDGFR-β, a receptor tyrosine kinase that is activated by μ receptors (Belcheva et al., 2001). Activation of the PDGFR-β receptor, in turn, modulates μ receptor function (Belcheva et al., 2001). Key to their finding was that chronic morphine treatment induced PDGFR-β phosphorylation, which was abrogated by imatinib, a Food and Drug Administration-approved inhibitor of PDGFR used clinically to treat cancer (Wang et al., 2012). Spinal or subcutaneous administration of imatinib significantly blocked the development of morphine analgesic tolerance and reversed established tolerance. Although imatinib prevented tolerance, when rats were challenged with morphine alone after repeated doses of morphine and imatinib, the animals were all tolerant to antinociceptive effects of morphine. This suggests that imatinib did not directly inhibit the processes that cause tolerance, but rather it bypassed these cellular adaptations in restoring the analgesic effects of morphine. The results indicate that imatinib could be effective as an adjuvant therapy to limit or reverse morphine analgesic tolerance.

The mechanism responsible for morphine-induced activation of the PDGFR-β was identified to be dependent on the release of PDGF-B, a selective agonist of the PDGFR-β receptor. Rodents treated with a PDGFR-β–Fc chimeric protein, which scavenges released PDGF-B, suppressed the development of morphine tolerance and restored analgesia in morphine tolerant rats, similar to the actions of imatinib. Conversely, administration of PDGF-B induced robust tolerance in opioid-naive animals, indicating that PDGFR-β signaling is both necessary and sufficient to cause morphine analgesic tolerance. However, the importance of PDGF-B extends beyond morphine tolerance to include a role in neuropathic pain (pain arising from damage or dysfunction of the nervous system), which is often refractory to the analgesic effects of opioids. Donica et al. (2014) reported that morphine, when administered together with imatinib, reversed mechanical allodynia in nerve-injured rats, suggesting that this pathway may be responsible for dysfunction of opioid effects in neuropathic pain states. In contrast, mechanical allodynia was not affected when morphine or imatinib was administered alone (Donica et al., 2014). Collectively, these studies provide the first lines of evidence that PDGFR-β signaling is a critical overlapping mechanism in the etiology of morphine tolerance and neuropathic pain. A major question arising from these studies is, what is the critical cell type(s) through which PDGFR-β signaling gates mor-

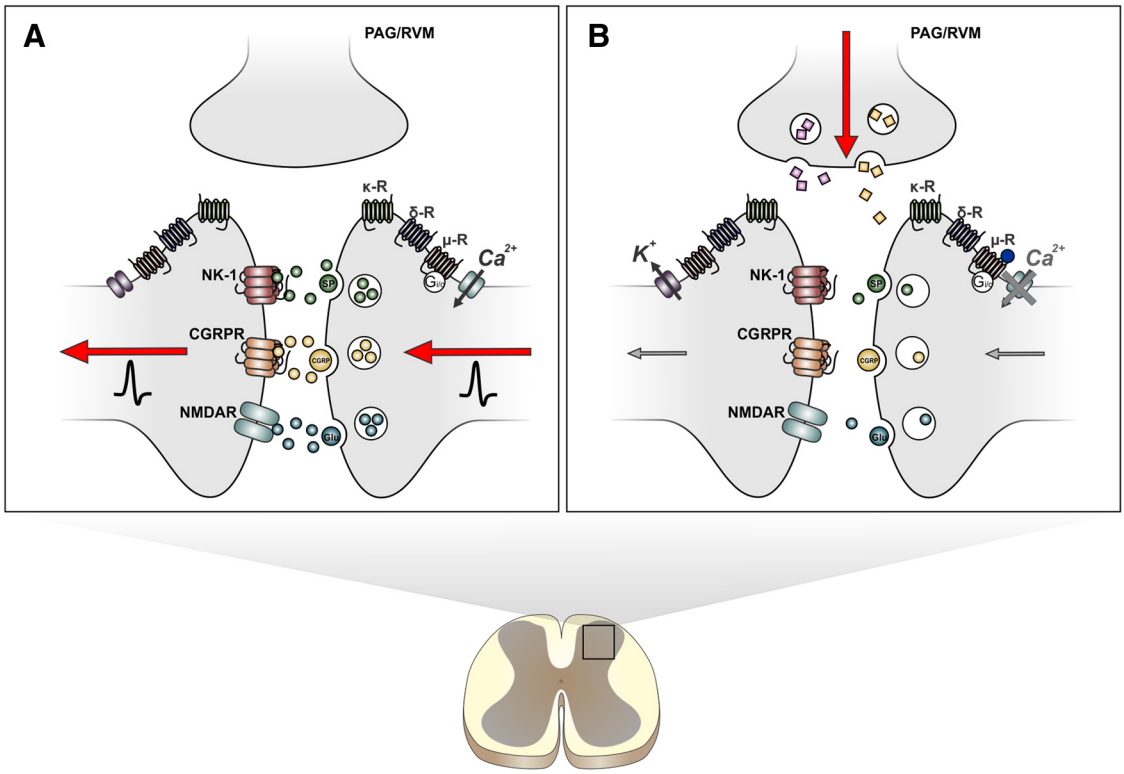

Figure 1. Spinal mechanisms of opioid analgesia. ***A***, In the spinal dorsal horn, nociceptive signals encoded by action potentials trigger the release of nociceptive transmitters, such as substance P (SP), calcitonin gene-related peptide (CGRP), and L-glutamate (Glu). Second-order projection neurons then relay this information from the spinal cord to the brain, where the information is disseminated and decoded to produce the emotional and sensory experiences of pain. ***B***, Opioids produce their analgesic effects by inhibiting spinal nociceptive transmission. On presynaptic nerve terminals, opioids reduce cAMP signaling and suppress activity of voltage-gated calcium channels, which inhibits release of nociceptive transmitters. On postsynaptic neurons, opioids activate inward potassium channels to cause hyperpolarization of ascending projection neurons. Opioids also produce analgesia by activating descending inputs from the periaqueductal gray (PAG) and rostral ventromedial medulla (RVM), which are key components of the descending inhibitory pain pathways. In these supraspinal structures, opioid-mediated disinhibition of GABAergic interneurons leads to activation of monoamine-containing descending neurons that suppress nociceptive transmission in the spinal dorsal horn. CGRPR, Calcitonin gene-related peptide receptor; NK-1, neurokinin-1 receptor.

phine tolerance and neuropathic pain? Moreover, there are mechanistic similarities between the development of opioid tolerance and the occurrence of pain hypersensitivity associated with nerve injury, such as NMDA receptor phosphorylation and gliosis (Mao and Mayer, 2001; Wen et al., 2011). Whether there is overlap between these signaling cascades and PDGFR remains unresolved.

Adenosine, adenosine receptors, and interactions with opioids

Adenosine is an essential molecular building block for the prototypical energy source molecule ATP. However, adenosine in its own right is an important, biologically active molecule that regulates neuronal and non-neuronal function in the CNS and PNS. Released in response to trauma, seizure, inflammation, pain, and a host of other conditions, adenosine triggers compensatory homeostatic and neuromodulatory responses that protect against neuronal damage (Cunha, 2001). Extracellular adenosine can be derived through a series of catalytic steps. First, the cell surface ectonucleoside triphosphate diphosphohydrolase [cluster of differentiation (CD) 39] rapidly hydrolyzes ATP or ADP to AMP, which is then further broken down by ecto-5′-nucleotidase (CD73) to produce adenosine (Robson et al., 2006; Bonan, 2012). Another source of adenosine comes from intracellular pools released by ectonucleoside transporters (ENTs) expressed on neurons and glia (Bonan, 2012). The removal of extracellular adenosine is controlled by ENT uptake (when extracellular adenosine concentration exceeds intracellular concentration), adenosine deaminase deamination, and intracellular phosphorylation by adenosine kinase (Blackburn and Kellems, 1996; Spychala et al., 1996; Thorn and Jarvis, 1996; King et al., 2006). It is generally accepted that adenosine kinase is the "upstream coordinator" of adenosine receptor (AR) signaling, which is mediated by the P1 family of G-protein-coupled receptors: A_1, A_{2A}, A_{2B}, and A_3 AR subtypes (Fredholm et al., 2005, 2011; Boison et al., 2010).

The first clues that adenosine may modulate pain and regulate opioid analgesia came from the demonstration that systemic administration of an adenosine analog is antinociceptive (Vapaatalo et al., 1975) and that methylxanthines (which block ARs) inhibits morphine analgesia (Ho et al., 1973a,b). Evidence soon emerged that the spinal cord is a key site of action for adenosine modulation of pain (Post, 1984) and that morphine administration in rats readily evokes the release of adenosine in the spinal cord (Sweeney et al., 1987). This release has also been reported in human subjects after intrathecal injection of fentanyl, a potent μ receptor agonist (Eisenach et al., 2004). It is now clear that spinal analgesia produced by adenosine or morphine administration depends critically on the adenosine receptor subtype A_1AR (Poon and Sawynok, 1998; Wu et al., 2005; Zhang et al., 2005). However, adenosine may be playing both the roles of "good cop" and "bad cop" because its activity has also been implicated in the unwanted side effects associated with chronic opioid use (Ahlijanian and Takemori, 1985; Contreras et al., 1990; Germany et al., 1990). For example, opioid withdrawal increases

adenosine acting at A_1AR in the ventral tegmental area (VTA) and substantia nigra, resulting in an inhibition of GABA release and thereby altering reward processing (Bonci and Williams, 1996; Shoji et al., 1999; Matsui et al., 2014). Chronic opioid administration can also decrease extracellular levels of adenosine and impair A_1AR signaling in the brainstem (Nelson et al., 2009).

Attempts at targeting the adenosine system in search for better analgesics have focused on A_1AR and A_2ARs but with limited success because of negative side-effect profiles (Zylka, 2011; Boison, 2013). Other attempts aimed at increasing endogenous adenosine with recombinant ectonucleotidases (Sowa et al., 2010) or adenosine kinase inhibitors (Kowaluk et al., 2000) have also proved disappointing. Attention has now shifted to A_3AR as a possible cellular target for developing novel analgesics (Little et al., 2015). A_3ARs are expressed throughout the CNS in neurons, astrocytes, and microglia, and their expression is upregulated by inflammatory cytokines (Abbracchio et al., 1997; Lopes et al., 2003). Importantly, these receptors are expressed in critical pain signaling areas such as the spinal dorsal horn and the rostral ventral medulla (Little et al., 2015), as well as in the reward pathways of the VTA and ventral striatum (D.S., unpublished observations). Small-molecule A_3AR agonists that are potent, selective, and orally bioavailable (e.g., IB-MECA [*N*6-(3-iodobenzyl)-adenosine-5′-*N*-methyl-uronamide] and MRS5698 [(1*S*,2*R*,3*S*,4*R*,5*S*)-4-[6-[[(3-chlorophenyl)methyl]amino]-2-[2-(3,4-difluorophenyl)ethynyl]-9*H*-purin-9-yl]-2,3-dihydroxy-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide]) have been developed (Jacobson, 1998; Tosh et al., 2012) and tested in Phase II/III clinical trials for the treatment of cancer and various autoimmune disorders. These trials have reported favorable safety profiles (Fishman et al., 2002; Silverman et al., 2008; Stemmer et al., 2013). Moreover, a recent study by Little et al., (2015) found that selective A_3AR agonists are potent, stand-alone agents that effectively attenuate chronic neuropathic pain without disrupting normal nociceptive processing. Tolerance to the analgesic effects of these novel A_3AR agonists also did not develop, and their use did not produce inherent reward. Thus, A_3AR appears to be a promising therapeutic target for developing novel analgesics that may complement, or be used as pharmacological substitutes of, traditional opioid therapy.

Sex, opioids, pain…and microglia

Neurons were considered the principal cell types targeted by opioid analgesics. This neuron-centric view was supported by evidence that repeated opioid exposure alters the fundamental properties of neurons and their circuits within the CNS and PNS and that this altered neuronal output is implicated causally in opioid tolerance, hyperalgesia, and dependence (Guitart and Nestler, 1989; Christie, 1991). However, the vast majority of cells within the nervous system are not neurons but rather glia (Banati, 2003). It is estimated that glia make up 70–90% of cells, with microglia comprising ~10% of the total glial population in the adult CNS (Kreutzberg, 1996; Aguzzi et al., 2013; Kettenmann et al., 2013). Conventionally regarded as macrophages, microglia were relegated to being passive bystanders that subserve immune and supportive roles for neurons. This perception of microglia has changed radically in the past decade, and it is now apparent that microglia play an active and direct role in a variety of processes, including synaptic remodeling, neuronal excitability, and neurotransmission (Tremblay et al., 2010; Blank et al., 2014; Salter and Beggs, 2014). The role of microglia as cellular detectors and effectors of injury, infection, or disease has also gained considerable traction (Trang et al., 2012; Ji et al., 2013). In this respect, the importance of microglia in the sequelae of neuropathic pain is well established, and it is known that injury to a nerve instigates a stereotypical series of change in microglial morphology, gene expression, function, and number (Hanisch and Kettenmann, 2007; Kettenmann et al., 2011; Trang and Salter, 2012). These changes are also engaged by morphine; in response to chronic morphine treatment, microglia transform from a ramified ("resting") to amoeboid ("activated") state and upregulate expression of the cell-surface markers CD11b and ionized calcium-binding adaptor-1 (Ferrini et al., 2013; Schwarz et al., 2013; Mattioli et al., 2014). These phenotypic changes, triggered by opioid treatment or nerve injury, are the cellular and molecular signatures of microglial "activation." Additional evidence that microglia are important opioid targets comes from reports that they express μ and κ receptors (Chao et al., 1997; Mika et al., 2014). Activation of opioid receptors on microglia induces the release of a myriad of chemokines, cytokines, and neurotrophic factors (Coller and Hutchinson, 2012; Mélik Parsadaniantz et al., 2015). Treatments with nonspecific glial inhibitors (e.g., minocycline, fluorocitrate, or propentofylline) block the opioid-induced release of these signaling molecules and suppress the increase in microglial reactivity (Watkins and Maier, 2003; Cui et al., 2008; Horvath and DeLeo, 2009). The cellular effects of glial inhibitors translate into a reduction in morphine analgesic tolerance, an attenuation of the paradoxical increase in pain sensitivity, and a decrease in dependence (Horvath et al., 2010; Fukagawa et al., 2013). Glial inhibitors have also been found to alleviate pain hypersensitivity after peripheral nerve injury (Raghavendra et al., 2003; Guasti et al., 2009; Grace et al., 2014). Thus, the most parsimonious conclusion is that glia, in particular microglia, play an essential role in both neuropathic pain and the negative effects associated with long-term opioid use.

This conclusion, which is derived from experiments conducted almost exclusively on male rodents, has gained wide acceptance and is perpetuated by the discovery of an ever-increasing number of microglial mechanisms being implicated in chronic pain. However, a study by Sorge et al. (2011) challenged the totality of such a conclusion as it relates to chronic pain mechanisms in male versus female mice. In this study, they reported that involvement of spinal TLR4 in mechanical allodynia was male specific. Because TLR4 in the CNS is expressed primarily on microglia, this initial observation hinted at the possible existence of a divergent microglial pain signaling mechanism being sexually dimorphic. In a more recent study, Sorge et al. (2015) demonstrated that intrathecal administration of glial inhibitors prevented and reversed mechanical allodynia caused by peripheral nerve injury in male, but not in female, mice. Allodynia in female mice was also resistant to selective ablation of spinal microglia by saporin toxin conjugated to macrophage antigen complex-1 and occurred independent of purinergic $P2X_4$ receptor ($P2X_4R$)-mediated release of brain-derived neurotrophic factor (BDNF), which is a core microglia pain signaling pathway identified previously in male rodents (see below) (Coull et al., 2005; Sorge et al., 2015). Together, these findings indicate that microglia are not sufficient for pain hypersensitivity in female mice. Instead, it appears that adaptive immune cells, likely T-lymphocytes, critically mediate pain hypersensitivity in female mice. This sexual dimorphism in mice is consistent with a clear gender bias in humans toward women being the majority of chronic pain patients (Mogil, 2012).

Although there is also evidence for sex differences in analgesic response to opioids and in susceptibility for opioid abuse in humans and rodents (Mogil, 2012; Lee and Ho, 2013), it remains to

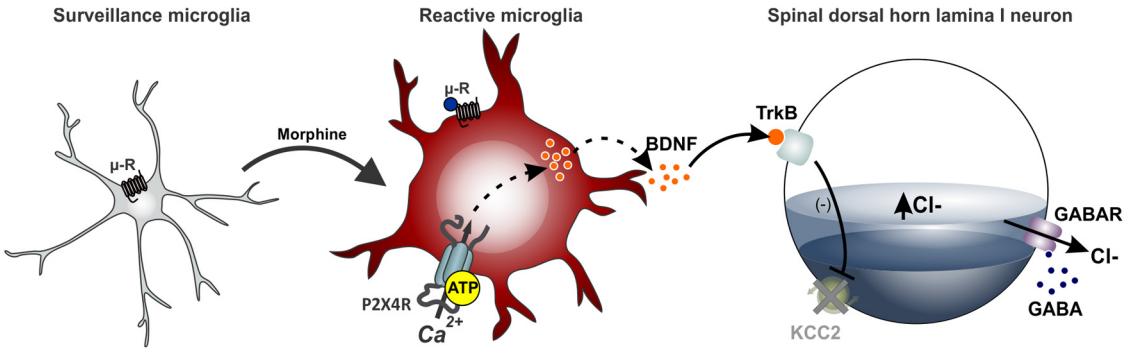

Figure 2. Microglial-mediated disruption of chloride homeostasis in spinal lamina I neurons gates morphine hyperalgesia. In response to chronic morphine treatment, microglia residing in the spinal cord adopt a reactive state characterized by μ receptor-dependent upregulation of $P2X_4R$ expression. Activation of $P2X_4R$ causes the release of BDNF, which signals to downregulate the potassium-chloride cotransport KCC2, resulting in a dysregulation of chloride homeostasis in spinal lamina I pain signaling neurons. The $P2X_4R$–BDNF–KCC2 pathway is not only critical for the paradoxical hyperalgesic effect of morphine but also for pain hypersensitivity after peripheral nerve injury.

be shown whether these differences involve divergent microglial signaling mechanisms. Therefore, we will proceed to discuss a novel microglial signaling mechanism discovered to be essential in the paradoxical pain produced by morphine, with the explicit caveat that these discoveries were made using male rodent subjects. In this context, a recent study by Ferrini et al. (2013) examined the relationship between morphine analgesic tolerance and morphine-induced hyperalgesia; the prevailing view was that tolerance and hyperalgesia reflect a single underlying mechanism. In this study, the authors found that morphine acting on μ receptors drives increased expression of $P2X_4R$ on microglia and that blocking $P2X_4R$ or specifically ablating spinal microglia reversed morphine-induced hyperalgesia without affecting tolerance. Furthermore, morphine treatment caused a $P2X_4R$-dependent release of BDNF from microglia: the release of BDNF signaled to downregulate the Cl^- cotransporter KCC2 on spinal lamina I neurons, leading to a dysregulation of Cl^- homeostasis (Fig. 2). Restoring Cl^- homeostasis by genetically deleting BDNF from microglia reversed morphine-induced hyperalgesia but had no effect on analgesic tolerance. Collectively, these findings dissociate morphine-induced hyperalgesia from analgesic tolerance and suggest that interfering with key nodes in the microglia-to-neuron $P2X_4R$–BDNF–KCC2 pathway suppresses hyperalgesia without affecting morphine analgesia. It is important to note that this pathway was first causally implicated in mechanical allodynia caused by peripheral nerve injury (Tsuda et al., 2003; Coull et al., 2005). Thus, the $P2X_4R$–BDNF–KCC2 pathway is a common microglial mechanism that underlies the hyperalgesic effects of morphine and neuropathic pain (Tsuda et al., 2003; Coull et al., 2005; Keller et al., 2007; Ulmann et al., 2008; Trang et al., 2009; Ferrini et al., 2013). Although this pathway is a major component of the functional alterations in the spinal dorsal horn that results in ongoing pain after chronic morphine treatment or peripheral nerve injury, it is one of several microglial mechanisms thought to contribute to these conditions (Watkins et al., 2001; Clark et al., 2007; Ji, 2010; Zhuo et al., 2011; Berta et al., 2014). How each of these mechanisms converges or diverges and whether sexual dimorphism has an effect on their relative importance has yet to be determined.

Opioid reward and its modulation by chronic opioids and pain

Opioids are potent analgesics that produce both positive and negative reinforcement. The positive reinforcing effects (euphoria, hedonism, or reward) resulting from opioid use are associated strongly with their high potential for abuse, whereas the negative reinforcing effects of opioid use arise from the potent alleviation of the negative sensory and affective/emotional aspects of pain. A canonical neurotransmitter associated strongly with altered mood states and the positive and negative reinforcing effects of opioids is dopamine. The mesocorticolimbic system, which includes the VTA and the nucleus accumbens (NAc, part of the ventral striatum) is responsible for the expression of motivated behaviors, arousal, and reinforcement learning produced by natural (e.g., sex, food) and drug-rewarding stimuli, as well as aversion-based behaviors (Wise, 1989; Koob and Le Moal, 2005; Fields et al., 2007).

Dopaminergic neurons within the VTA are regulated by GABAergic tone via either intrinsic or synaptic input. Activation of opioid receptors expressed on intrinsic and synaptic projection GABAergic neurons causes an increase in dopamine release via disinhibition of the GABA tone. This opioid control of dopamine neurons is gated primarily by opioid receptors expressed on GABAergic terminals arising from the rostromedial tegmental nucleus (RMTg) rather than GABA intrinsic neurons or the NAc medium spiny neurons (Matsui et al., 2014). Additionally, separate GABA afferents are affected in the development of tolerance (RMTg) and withdrawal (NAc), whereas VTA GABAergic intrinsic neurons were not modulated by either state (Matsui et al., 2014). Nevertheless, the VTA is critical for opioid reward because rodents will self-administer intra-VTA morphine, and conditioned place preference to systemically administered morphine is blocked by intra-VTA administration of μ receptor antagonists (David et al., 2008; Mazei-Robison and Nestler, 2012; Fields and Margolis, 2015). These data together suggest that opioid receptors in the VTA are "sufficient" but not "necessary" for opioid-induced reward.

Recent studies suggest that VTA dopamine neurons are organized topographically according to output projection targets and that such organization may be relevant to their activation by appetitive and aversive stimuli (Lammel et al., 2012). Whether opioids modulate specific VTA dopaminergic output projections remains an important unresolved question. Similar to the VTA, the NAc shows topographical organization with hedonic and aversive "hot and cold spots" (McCutcheon et al., 2012; Berridge and Kringelbach, 2013). The rostrodorsal quadrant of the medial NAc shell contains a specialized opioid hedonic hotspot that mediates an increase in "liking" after μ receptor activation. In contrast, a distinct suppressive cold spot in the caudal half of the shell was identified in which opioid stimulation reduced sucrose pos-

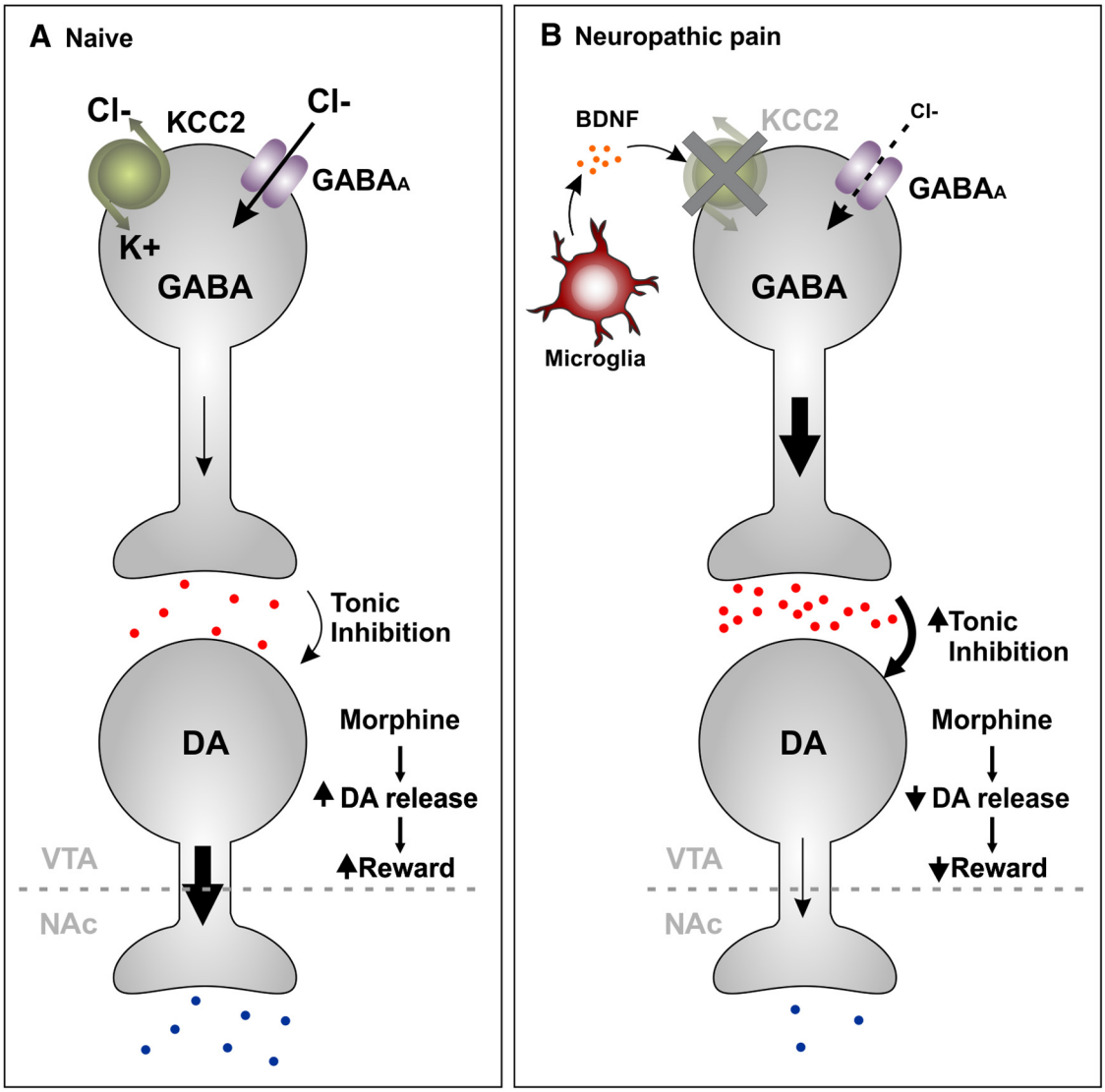

Figure 3. Model of microglial-mediated altered reward circuitry. As a consequence of chronic opioid exposure or chronic pain, microglial activation occurs in many areas of the CNS, including brain regions involved in reward such as the VTA. It is hypothesized that gliosis triggers a reorganization of reward circuitry such that microglia change E_{GABA} in GABAergic neurons within the VTA. ***A***, In the naive state, these neurons tonically inhibit mesolimbic dopaminergic neurons projecting to the NAc. ***B***, In chronic pain states, activated microglia release BDNF. This in turn causes disruption of E_{GABA} via downregulation of the K^+/Cl^- transporter KCC2 protein levels and activity. The loss of KCC2 causes a disruption of Cl^- homeostasis in GABAergic neurons, resulting in these neurons being more depolarized. Consequently, activation of $GABA_A$ receptors on these GABAergic neurons results in their depolarization rather than hyperpolarization because of a net inward anion (Cl^-/HCO_3^-) current (that is normally outward). An increase in the excitability of GABAergic neurons results in an increase in GABA release and augmentation of the inhibitory tone on dopaminergic neurons, leading to less dopamine release in the NAc. DA, Dopamine.

itive liking reactions (Castro and Berridge, 2014). These results demonstrate the anatomical heterogeneity of the NAc and the importance of this region in opioid reward.

Reward produced by intra-VTA administration of opioids is blunted or absent after chronic opioid administration. This effect correlates with an increase in VTA GABAergic currents (Madhavan et al., 2010) and an increase in the frequency of spontaneous miniature IPSCs within the VTA dopaminergic neurons (Bonci and Williams, 1997). This is consistent with the report that electrically evoked VTA dopamine output to the NAc is decreased 24 h after cessation of chronic morphine (Mazei-Robison et al., 2011). Importantly, repeated drug exposure causes a decrease in the rewarding effect of the drug (reward tolerance), and this leads to an escalation of drug intake, as seen in humans (O'Brien, 1997). As noted previously, similarities exist between chronic opioid use and chronic pain. This is also true in the mesolimbic system. Similar to dependent states, morphine-stimulated dopamine release is attenuated in animals with chronic pain (Ozaki et al., 2002). Taylor et al. (2015b) replicated this finding and also reported that cocaine-induced, but not amphetamine-induced, dopamine release was attenuated in chronic pain animals. The impaired mesolimbic dopamine response in chronic pain is supported by a recent human functional magnetic resonance imaging study that found dramatically reduced reward-stimulated activity in the VTA of fibromyalgia patients (Loggia et al., 2014).

Although the mechanisms underlying the neuronal plastic changes associated with the development and the progression of drug addiction remain unknown, BDNF is implicated in incentive motivation that drives drug-seeking behavior. BDNF is a critical modulator of VTA dopamine neuronal activity in opioid-dependent animals (Vargas-Perez et al., 2009, 2014; Koo et al., 2012). Furthermore, intra-VTA BDNF can produce an opioid-

dependent-like state in naive animals (Laviolette and van der Kooy, 2001; Laviolette et al., 2002, 2004; Vargas-Perez et al., 2009, 2014). BDNF can cause $GABA_A$ function in VTA GABAergic interneurons to shift from inhibition to excitation (Fig. 3). There is evidence in other systems that BDNF can change GABAergic excitability via downregulation of KCC2 (Coull et al., 2005; Ferrini et al., 2013; Gagnon et al., 2013; Ting-A-Kee et al., 2013). Like other areas of the brain, downregulation of KCC2 in VTA $GABA_A$-expressing neurons results in an increase in their excitability. It was identified recently that both chronic pain and chronic administration of morphine causes a loss in the expression of KCC2 in VTA GABAergic neurons that results in a greater tonic inhibition on dopamine neurons (Taylor et al., 2015a,b). The mechanism of how chronic administration of opioids or chronic pain modulates reward circuitry within the VTA remains unresolved, although recent evidence demonstrates that microglial activation contributes to changes in opioid reward (Taylor et al., 2015a,b). Chronic morphine administration results in significant gliosis in the VTA and NAc (Narita et al., 2006; Hutchinson et al., 2009; Schwarz et al., 2013). Similarly, chronic pain in the absence of opioid medication use also causes microglial activation in reward circuitry (Taylor et al., 2015b). Inhibitors of both BDNF and microglia recover the loss of KCC2 function and restores morphine- and cocaine-evoked dopamine release. The functional behavioral consequence of this change in VTA GABAergic tone was evidenced by the loss of reward-related behaviors dependent on mesolimbic dopamine release in the NAc. Hence, intra-NAc cocaine and intra-VTA opioid conditioned place preference was blunted in chronic pain animals. Again, microglial inhibitors recovered reward in chronic pain animals but had no effect in pain-naive animals. Together, such findings suggest that microglial activation results in dysfunction of reward reliant on mesolimbic dopamine during ongoing chronic pain or opioid-dependent states. How microglia become activated in the brain after a peripheral nerve injury remains unresolved, and it is unknown whether any of these effects are reversible.

Summary

In 1680, Thomas Sydenham espoused that "Among the remedies which it has pleased almighty God to give to man to relieve his sufferings, none is so universal and so efficacious as opium." Indeed, the potent pain-relieving effects of opioids are unmatched, but it is now understood that along with the "good" can come the "bad": analgesic tolerance, hyperalgesia, dependence, and reward are recognized as potential adverse consequences of opioid use. Attempts to negate the adverse effects of opioids and provide relief to those in pain have been directed mostly at neuronal targets but with only limited success. These strategies were predicated on the misconception that neurons are the sole and principal targets of opioid action. On the contrary, converging lines of evidence indicate that opioids engage a complex constellation of cellular and molecular processes, through which a diversity of cell types interact to produce the negative effects associated with opioid use. Glia–neuron interactions are key to establishing and maintaining altered responsiveness of the CNS to long-term opioid treatment, and, in particular, it is the influence of microglia that is critical. The conceptual shift from microglia as passive bystanders to active effectors of opioid analgesia is a major breakthrough that has reoriented the search for new therapeutic targets. One potential target highlighted in this review is the microglia-to-neuron $P2X_4R$–BDNF–KCC2 pathway, which serves an overlapping role in the pain hypersensitivity caused by repeated morphine treatment and by peripheral nerve injury. This pathway is also important in altering reward circuitry in chronic pain states. Moreover, adenosine and PDGFR-β signaling have emerged as a common mechanism in morphine tolerance and neuropathic pain. The commonalities in mechanisms between opioid tolerance, opioid-induced hyperalgesia, and neuropathic pain provide an exciting opportunity to bridge therapeutic strategies aimed at improving the utility of opioids for treating pain. However, the design of any therapeutic approach must take into account potential mechanistic differences between males and females given the recent evidence for sexual dimorphism in the role of microglia in neuropathic pain. The challenge, from the perspective of pain management, is to exploit this new knowledge to enhance the utility of opioids in treating chronic pain without stifling the potent analgesic properties of this important class of drugs.

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