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Curr Opin Investig Drugs. Author manuscript; available in PMC 2009 July 1.

Published in final edited form as:
Curr Opin Investig Drugs. 2008 July ; 9(7): 726–734.

Neuroimmune interactions and pain: Focus on glial-modulating targets

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Abstract

Chronic pain is the most difficult type of pain to treat. Previously, the development of analgesics has focused on neuronal targets; however, current analgesics are only modestly effective, have significant side effects and do not provide universal efficacy. New strategies are needed for the development of more effective analgesics. Glial cells have integral roles in CNS homeostasis, and chronic pain etiology and progression. In this review, the role of glia in neuropathic pain and opioid administration is described, as well as the potential superior efficacy and wider therapeutic indices provided by drugs that modulate specific glial function via novel targets.

Keywords

Astrocyte; cannabinoid; microglia; minocycline; neuropathic pain; propentofylline

Introduction

An estimated 100 million individuals in the US suffer from chronic pain [1]. Chronic pain is the most difficult pain to treat, especially neuropathic pain, which follows nerve injury. Chronic pain is debilitating and can cause extreme physical, psychological and social disruption for patients. Although opioid agonists may be modestly effective in the treatment of neuropathic pain, tolerance and physical dependence significantly limit their use. Additionally, newer agents, such as calcium channel α_2-δ ligands, tricyclic antidepressants and selective serotonin/norepinephrine reuptake inhibitors, have significant side effects and do not provide universal efficacy [2].

Thus, there is a need for the development of new agents for the treatment and prevention of chronic pain that utilize novel mechanisms, display enhanced effectiveness and possess decreased side-effect profiles. Previous drug development for pain has been predominately focused on altering neuronal function. It is increasingly recognized that glial cells play a major role in maintaining CNS homeostasis, and in disease etiology and progression [3]. Accordingly, attention has shifted to the discovery of glial-modulating agents for the treatment of a range of neurodegenerative diseases, as well as chronic pain. Potential drug targets to modulate glial-

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neuronal function are shown in Figure 1. This review highlights how glia enhance pain states following nerve injury or opioid administration, and how drugs that modulate specific glial function (including migration, proliferation and neurotransmitter expression) via novel targets may provide superior efficacy and wider therapeutic indices.

Glial cells and pain

Glial cells, including parenchymal (resident) microglia, perivascular microglia, astrocytes and oligodendrocytes, constitute > 70% of the total cell population in the brain and spinal cord. Although once considered merely a physical support system for neurons, glial cells have been identified as key neuromodulatory, neurotrophic and neuroimmune elements in the CNS. Microglia (cells of monocytic origin) are the macrophages of the brain, and perform a vast number of immune-related roles [4••]. Microglia are the first cell types to respond to several forms of CNS injury [5••,6••]. The initial signal that triggers microglial reactivity is not fully understood; however, neuronal depolarization and extracellular ion changes following nerve injury may be major stimuli [7]. Alternatively, neuronal signals, such as nitric oxide (NO) or proinflammatory cytokines, may induce this reactivity [8,9]. It has been proposed that TLR4 and CD14 may constitute the 'receptor complex' for these activating signals from neurons [10,11•,12].

During autoimmune inflammation of the nervous system, microglia release and respond to several cytokines, including IL-1, IL-6, TNFα and IFNγ, which are instrumental in astrocytic activation, induction of cellular adhesion molecule expression and recruitment of T-leukocytes into the lesion [10,11•,12]. In addition to synthesizing inflammatory cytokines, microglia may also act as cytotoxic effector cells by releasing harmful substances, including proteases, reactive oxygen intermediates and NO. Perivascular microglia are CNS antigen-presenting cells [4••] and immune modulators that possess surveillance functions [13]. These cells play an important role in the communication of the CNS with the peripheral immune system, especially in pathological conditions [14] such as neuropathic pain [15•]. Perivascular microglia shed or express the surface marker ED2/CD163 [16,17] and secrete anti-inflammatory proteins [18]. The production of these classes of molecules is associated with the resolution of inflammation [19]. Of relevance to chronic pain states, it has been reported that spinal ED2/CD163 expression is reduced following peripheral nerve injury [20•]. Perivascular and parenchymal microglial cells are thought to have an important role in antigen recognition and presentation in the CNS, but their different location and surface markers (parenchymal microglia express CR23/CD11b and Iba1, and perivascular microglia express ED2/CD163) may confer different functions. Perivascular microglial cells are integral to the integrity of the blood-brain barrier and are continually replaced by bone-marrow derived cells under normal conditions; this turnover is accelerated in inflammatory processes [4••,21]. Conversely, parenchymal microglia represent a stable cell pool, which in adult animals is only exceptionally replaced by hematogenous cells, even after recovery from severe brain inflammation. In addition, they surveil, migrate and proliferate in a dynamic and plastic manner [4••,21]. It has been demonstrated that parenchymal reactive microglia (surrounding encephalitis lesions) may express ED2/CD163 in chronically simian immunodeficiency virus-infected macaques with encephalitis [22].

Astrocytes may have a primary role in nociceptive processing, and in the thermal and mechanical hyperalgesia produced by peripheral nerve injury [23]. Astrocytic changes in response to injury include proliferation, hypertrophy and overexpression of glial fibrillary acidic protein (GFAP). Spinal GFAP increases following chronic nerve constriction [24], nerve crush and axotomy [25,26]. There has been considerable focus on the mechanisms of the initiation versus maintenance phases of persistent pain states and it has been proposed that distinct glial populations may have an integral role. Using real time RT-PCR, it was

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demonstrated that peripheral nerve injury induces an early microglial activation, as assessed by ITGAM (integrin,αM[complement, complement 3 receptor 3 subunit]), TLR4 and CD14, followed by a delayed but sustained mRNA GFAP expression [12]. Interestingly, in a more recent study, increases in spinal CR3/CD11b protein, a microglial marker, were observed at days 28 and 42 after nerve injury in the absence of transcript changes [27]. Therefore, the concept that microglia are only involved in initiating chronic pain is oversimplified and requires re-evaluation. Superficial reliance on limited activation markers may not relate to glial functioning and their ability to interact with nociceptive neurons. These findings suggest that the therapeutic window for glial-modulating agents may be broader than originally speculated.

Similarly, it has been proposed that astrocytic expression of MAPK plays a key role in maintaining neuropathic pain [28]. Additionally, Kawasaki *et al* observed a rapid and transient increase in matrix metalloprotease (MMP)-9 in dorsal root ganglia sensory neurons and a delayed increase of MMP-2 in satellite cells and spinal astrocytes after spinal nerve ligation [29]. These studies underscore the value of investigating functional glial properties versus simply observing morphological reactivity.

Opioids – The current gold standard

Opioids are among the most potent analgesic agents available for clinical use and are the gold standard for treating acute, post-surgical and cancer pain; however, their use in neuropathic pain is often limited by the development of analgesic tolerance and unwanted side effects that are unmasked during the resulting dose escalation. Much of the early research on combating opioid tolerance focused on determining potential neuronal mechanisms of tolerance formation and designing opioid analogs with improved tolerance profiles. Beitner-Johnston *et al* demonstrated that naltrexone, a μ-opioid receptor antagonist, limited the development of tolerance to opioids [30]. This finding led to the synthesis of new μ-opioid receptor agonists, mixed κ-receptor agonist and μ-receptor antagonists, and partial μ-receptor agonists, which have all failed to improve upon morphine and thus have only limited value in the treatment of pain. Opioid receptor desensitization involves the NMDA receptor cascade. Preclinical [31, 32] and preliminary clinical studies [33,34] suggest that the blockade of NMDA reduces opioid-induced tolerance; however, large-scale clinical trials using NMDA antagonists in conjunction with opioids to limit tolerance formation have produced disappointing results [35].

Song and Zhao have identified a casual link between glial activation and morphine tolerance [36•]. In accordance with these findings, it has been demonstrated that spinal CR3/CD11b, GFAP (an astrocyte marker) and expression of the cytokines IL-1α, IL-6 and TNFα increase following chronic morphine administration [37•]. Also, chronic subcutaneous and intrathecal morphine administration induces analgesic tolerance within 6 and 3 days, respectively [38]. The burgeoning body of research implicating glia in opioid tolerance led to the investigation of whether selectively targeting glial cells can provide a potential method for attenuating opioid tolerance. Both inhibiting chronic opioid-induced glial reactivity using propentofylline [39] and inhibiting proinflammatory cytokines [40] attenuate morphine-induced tolerance. Minocycline, which inhibits microglial migration [41], attenuates the development of anti-nociceptive tolerance to chronic morphine through inhibition of p38 MAPK in activated microglia [42]. These studies indicate that microglial migration may have a critical role in morphine tolerance, as has been demonstrated in neuropathic pain states [43,44•]. It was discovered that morphine enhances microglial Iba-1 expression [RJ Horvath, unpublished data] and migration [45] toward ADP in a μ-opioid receptor-dependent manner (Figure 2). It was proposed that chronic opioid administration induces microglial reactivity and migration toward the dorsal horn, which leads to increased proinflammatory/algesic factor production and neuronal sensitization. Microglial migration might thus prove to be an attractive pharmacological target to inhibit the induction of opioid tolerance.

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Cannabinoids and neuroimmune interactions

The cannabinoid system regulates and modulates both neuronal and immune functions using at least two protein-coupled cannabinoid receptors (CBRs), CBR_1 and CBR_2. CBR_1s are expressed in the brain, spinal cord and peripheral nerves, and are responsible for the psychotropic effects of cannabinoids [46–50]. Neuronal CBR_1s are synthesized in cells of the dorsal root ganglia and are inserted by axonal transport onto terminals in the periphery [51]. Additionally, CBR_1s are also expressed in microglial cells and may act as immune modulators in the CNS [52–54]. CBR_2 activation produces a host of peripheral immune effects, including regulation of cell migration, inhibition of cell proliferation, reduction of cytokine production, downregulation of surface marker expression and impairment of cell functions [55–57]. CBR_2s are expressed peripherally in immune cells [58] and keratinocytes [59]. CBR_2s also exist in the CNS, mainly in microglia and perivascular microglia cells, in healthy human and rat brains [60]. CBR_2s regulate microglial migration [61] and proliferation [62]. CBR_1 expression is enhanced in the spinal dorsal horn after peripheral nerve injury, an effect that is at least in part mediated by ERK [48]. The acute spinal CBR_1 activation induces anti-nociception, but also causes neurological side effects [20•,63]. In addition, chronic spinal CBR_1 activation induces anti-nociceptive tolerance and hypersensitivity [64]. Interestingly, inflammation, nerve injury and other neuronal insults induce increased CBR_2 expression in microglia, perivascular microglia and astrocytes [20•,65–68]. CBR_2 is expressed in microglia and perivascular microglia (Figure 3), but not in neurons or astrocytes, 4 days after peripheral nerve injury [20•,69]. These findings are interesting because at this time point, microglial cells have an integral role in the development of pain following peripheral nerve injury [70,71]. Furthermore, it has been demonstrated for the first time that perivascular microglial cells seem to be instrumental in the maintenance of pain following L5 nerve transection [20•]. Thus, this cell type provides a potential target for modulating the spinal immune response in neuropathic pain conditions. Others have failed to demonstrate CBR_2 expression in spinal microglial cells and have observed CBR_2 expression mainly in neurons 14 days after peripheral nerve injury [72]. The discrepancy between these results may be due to non-specific antibodies, peripheral nerve injury models or the time after surgery (4 versus 14 days, respectively) used in both studies. It is possible that CBR_2 is expressed in different cells at different time points in neuropathic pain conditions; this differential expression has been observed with ERK following peripheral nerve injury [28].

CBR_2 activation induces anti-nociception [63,72,73]. Spinal CBR_2 activation using the selective CBR_2 agonist JWH-015 (Clemson University; Figure 4) induces anti-nociception while producing a concomitant reduction of spinal Iba-1, and CD11b/CR3 and GFAP expression in postoperative and neuropathic pain models. JWH-015 also increases the anti-inflammatory factor ED2/CD163 in spinal perivascular microglia following peripheral nerve injury [20•]. *In vitro*, JWH-015 inhibits primary microglial migration [EA Romero-Sandoval, unpublished data] and TNFα production [74]. The ability of CBR_2 agonists to reduce macrophage migration [75] and the release of microglial proinflammatory factors [76•] relies on the modulation of ERK1/2.

Additionally, CBR_2 mediates inhibition of the immune response (including a reduction of microglial reactivity) by reducing the number and regulating the function of infiltrating T-leukocytes into the brain of rodent models of multiple sclerosis [57,62,77,78•]. This CBR_2 action is significant because T-leukocyte and macrophage trafficking into the CNS following peripheral nerve injury is instrumental for the maintenance of microglial activation and pain [10,15•]. CBR_2 provides an attractive target that may pharmacologically act to induce a spinal microglial anti-inflammatory phenotype, reduce microglial migration to damaged neurons, and possibly modulate the function and number of infiltrating T-cells in pain conditions.

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Novel glial-modulating drugs and targets

Currently, the total number of specific glial modulators is limited, but includes a broad spectrum of chemical structures and mechanisms, including: (i) fluorocitrate, a non-selective metabolic inhibitor [23]; (ii) minocycline, a tetracycline derivative with selective *in vitro* microglial inhibition [71]; (iii) ibudilast, a non-selective PDE inhibitor [79,80]; (iv) methionine sulfoximine (MSO), an astrocytic glutamine synthetase inhibitor [81]; and (v) propentofylline, a methylxanthine [82,83]. It is important to consider that some of the CNS glial-modulating properties of these agents may be downstream from their primary mechanism of action.

It was first demonstrated in 1991 by Garrison *et al* that lumbar spinal GFAP increases following a sciatic nerve constriction injury [24]. The role of spinal astrocytes in nociceptive processing and hyperalgesia was further demonstrated using fluorocitrate in the chronic constriction injury rodent model [23]; however, due to the potential neurotoxicity of fluorocitrate, its use as a selective glial inhibitor is not favored. More recently, a more selective astrocytic metabolic inhibitor, intrathecal MSO was used to demonstrate that an astroglial glutamate-glutamine shuttle induces central sensitization of nociceptive neurons following peripheral inflammation [81].

Minocycline, a semi-synthetic second-generation tetracycline, is an antibiotic that possesses superior penetration through the blood-brain barrier and into the CNS [84]. Minocycline is a potent inhibitor of microglial reactivity and has no direct action on astrocytes or neurons *in vitro* [85–88]. The anti-inflammatory profile of minocycline is distinct from its antimicrobial action, and minocycline provides effective neuroprotection in experimental brain ischemia [89], in a mouse model of Huntington's disease [90], in traumatic brain injury [91], and following 6-hydroxydopamine and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration [92].

In a rat model of neuropathic pain induced by L5 spinal nerve transaction, systemic administration of minocycline: (i) reversed the development of mechanical allodynia and hyperalgesia, but did not produce an effect on existing behavioral hypersensitivity; (ii) prevented increased spinal microglial CR23/CD11b and GFAP expression in the pre-emptive treatment, but inhibited only microglial reactivity when treatment was started on day 5 after nerve injury; and (iii) inhibited the production of proinflammatory cytokines, such as IL-1β, IL-6 and TNFα, in the L5 lumbar spinal cord in association with the reversal of hyperalgesia and allodynia [71]. In a separate *in vitro* study, minocycline significantly reduced microglial migration to cellular debris, astrocyte-conditioned medium, ADP and algesic mediators, and significantly reduced the expression of CD29 (β_1 integrin), but not CD18 (β_2 integrin) [41]. Minocycline also reduces the effect of extracellular potassium and later decreases microglial Kv1.3 expression [41]. These results indicate a novel effect of minocycline: it inhibits microglial motility by reducing β_1 integrin expression and Kv1.3 channel activity and, later, expression. Reducing microglial trafficking to injured neurons following nerve injury decreases the release of proinflammatory mediators into the synaptic milieu and prevents neuronal sensitization, the pathological counterpart to chronic pain.

Ibudilast is a non-selective PDE inhibitor that decreases CNS CR3/CD11b and GFAP expression following nerve injury or morphine administration. Based on these findings, it has been postulated that the primary mechanism of action of ibudilast is glial modulation, not PDE inhibition as previously thought. The efficacy of ibudilast in rat models of neuropathy has thus far been inconclusive because both transient and sustained effects on behavioral hypersensitivity have been observed [80]; however, ibudilast enhances morphine analgesia while decreasing morphine tolerance and morphine withdrawal symptoms, and thus may have clinical utility as an opioid adjuvant to decrease dose escalation and adverse opioid side effects.

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Finally, the contribution of glia to nerve injury-induced behavioral hypersensitivity has been further probed using propentofylline, an atypical methylxanthine, which previously decreased GFAP expression in a rodent model of ischemia [93]. Systemic or intrathecal administration of propentofylline attenuates spinal GFAP, CR23/CD11b, IL-1β, IL-6 and TNFα expression *in vivo* [27,83,94]. In addition to being neuroprotective and anti-inflammatory, propentofylline reduces glial reactivity, proliferation and migration [27,39,71,81,95•,96,97], [JA DeLeo, unpublished data]. The specific mechanism of its anti-nociceptive effects remains unknown; however, several actions have been proposed. Propentofylline inhibits adenosine transport, as well as the cAMP-specific PDE4 [98–100]. Propentofylline increases cAMP levels, suppresses an activated astrocytic phenotype, enhances a specific glutamate transporter, GLT-1, at the mRNA and protein levels, and increases glutamate uptake *in vivo* and *in vitro* [101,102]. Interestingly, other methylxanthines that inhibit PDE have demonstrated limited efficacy in attenuating existing nerve injury-induced behavioral hypersensitivity [103], [JA DeLeo, unpublished data]. Therefore, the role of PDEs may be limited in driving glial activation and as targets for chronic pain agents.

Conclusions

Within minutes to hours following a peripheral nerve injury, spinal microglia may become reactive by using TLR4/CD14 as a receptor complex to sense changes in the glial/neuronal milieu. This signaling leads to the release of cytokines and growth factors from microglia, and promotes microglial migration and proliferation, which in turn alerts astrocytes to a perturbed synaptic milieu. Astrocytes then produce and release specific sensitizing chemokines, such as monocyte chemoattractant protein, which enable T-leukocytes and/or macrophage migration into the parenchyma of the CNS. This cell migration propagates the immune response and further induces microglial and astrocytic immunocompetence (Figure 1). There are several novel drug targets involved in glial function and the immune response, including: (i) inhibition of glial proliferation and migration, (ii) modulation of perivascular ED2/CD163 and astrocytic glutamate transporter GLT-1, and (iii) interference of proinflammatory interactions between populations of immune and glial cells.

Acknowledgments

The authors would like to acknowledge Matthew S Alkaitis for his excellent editorial assistance, Robert Lee for his work on Figure 1, and NIH/NIDA DA11276.

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•• of outstanding interest

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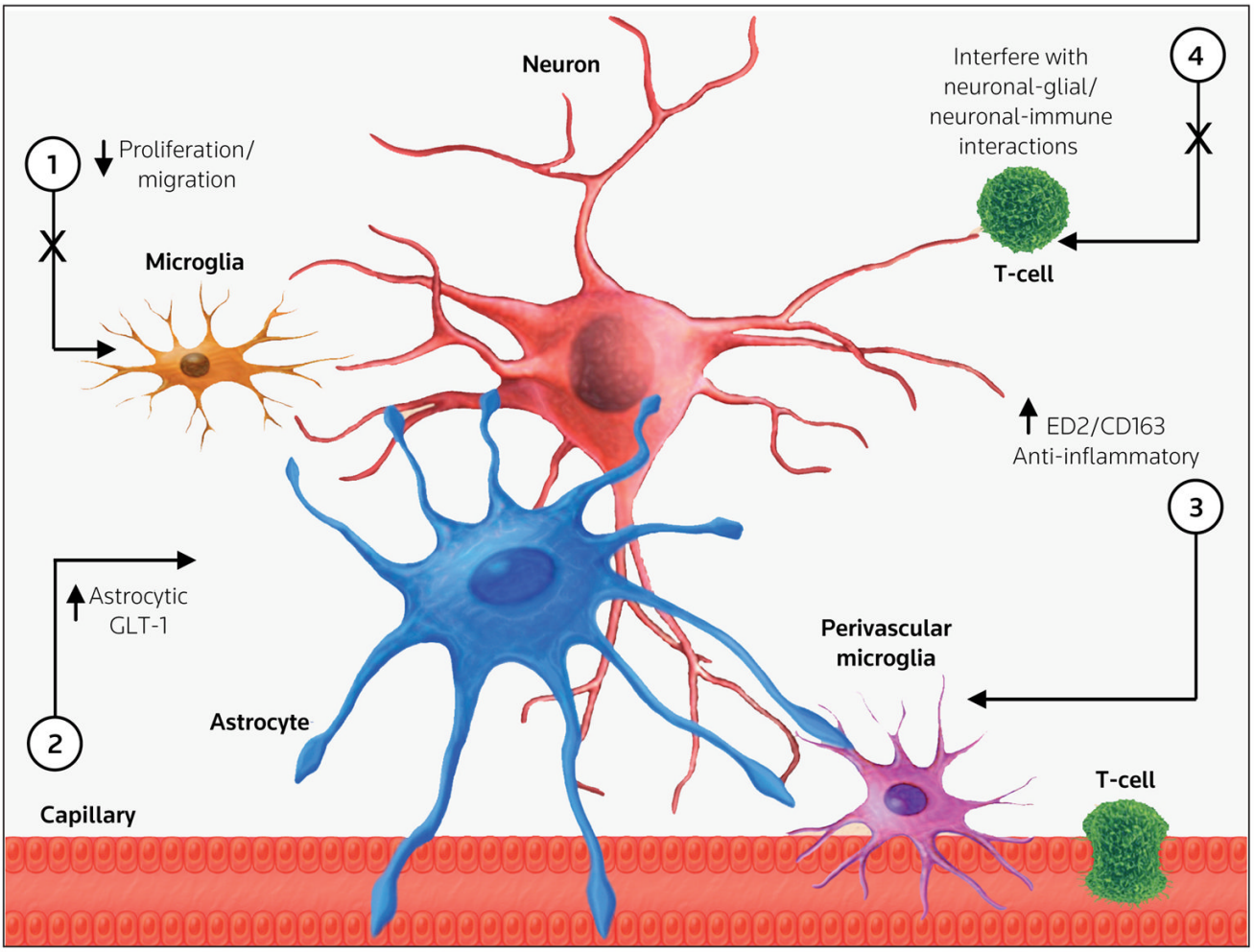

Figure 1. Potential drug targets to modulate glial-neuronal function

The major cellular contributors of chronic pain states and their interface with the microvasculature, including neurons, microglia, astrocytes, perivascular microglia and T-leukocytes are illustrated. Potential drug targets to modulate glial-neuronal function include: (**1**) inhibiting microglial proliferation and migration, (**2**) enhancing astrocytic GLT-1 expression, (**3**) enhancing perivascular ED2/CD163 expression, and (**4**) interfering with specific neuronal-glial and/or neuronal-immune interactions.

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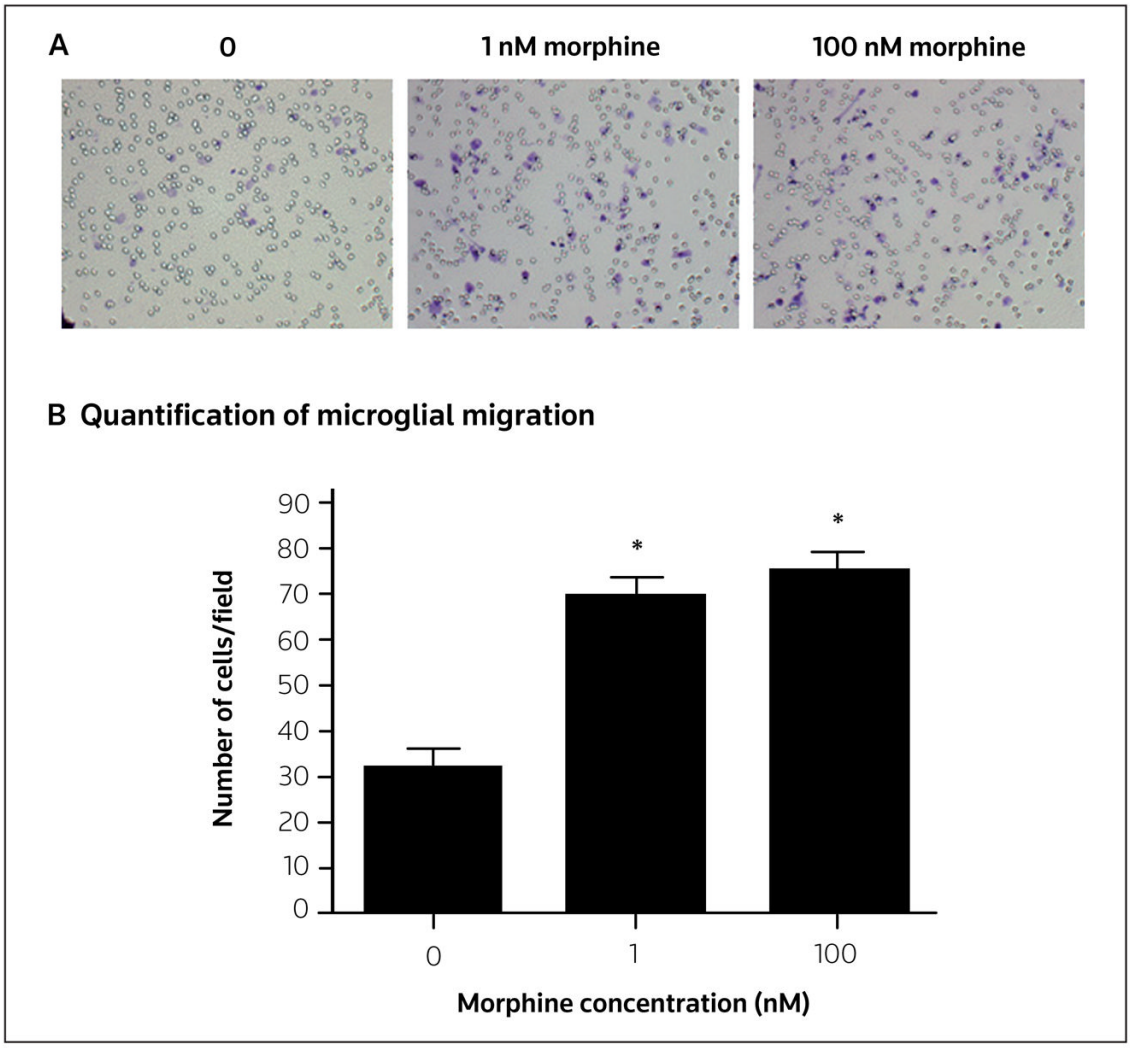

Figure 2. Migration of morphine-treated primary neonatal rat cortical microglia toward ATP

Primary neonatal rat cortical microglia were harvested, incubated with morphine (0, 1 or 100 nM) for 2 h, then allowed to migrate toward ADP (10 μM) for 2 h. (**A**) Images of microglia that had migrated through a porous membrane and were then stained with crystal violet. (**B**) Microglial migration was quantified by counting ten random fields at 40x magnification for each membrane (n = 3 for all treatments). Error bars represent the standard error of the mean. *$p < 0.05$.

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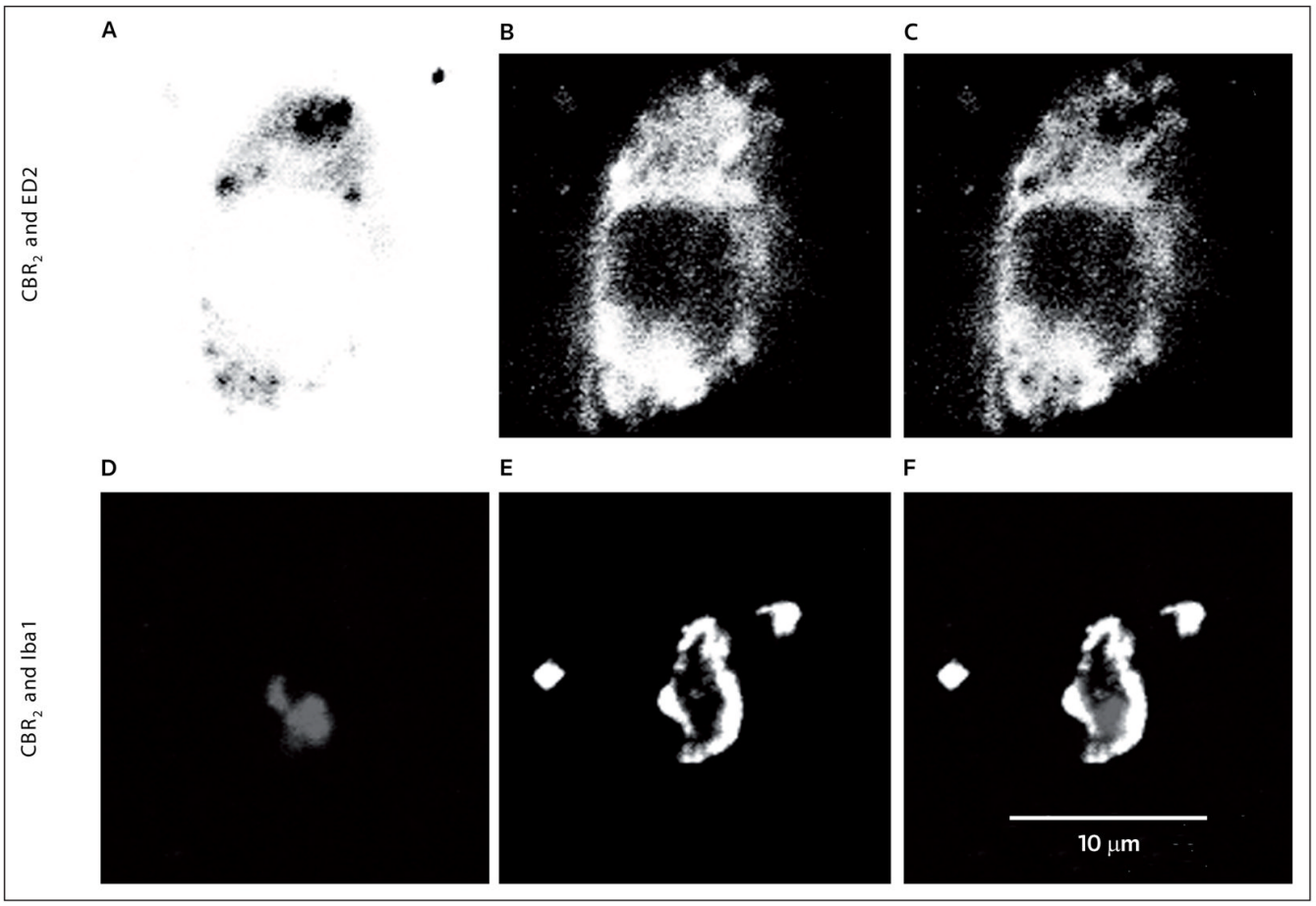

Figure 3. The expression of CBR2 in perivascular and microglia following peripheral nerve injury

CBR_2s are expressed in perivascular microglia (ED2/CD163) and microglia (Iba-1) of dorsal horn L5 spinal cord ipsilateral to L5 nerve transection (4 days after surgery). The top row shows representative confocal images of (**A**) CBR_2 (black), (**B**) ED2/CD163 (white) and (**C**) co-regionalization of both markers in perivascular microglia. The bottom row shows representative confocal images of (**D**) CBR_2 (gray), (**E**) Iba-1 (white) and (**F**) co-regionalization of both markers in microglia. All images were converted to grayscale and the colors in image 'A' were inverted to facilitate the appreciation of the double staining.

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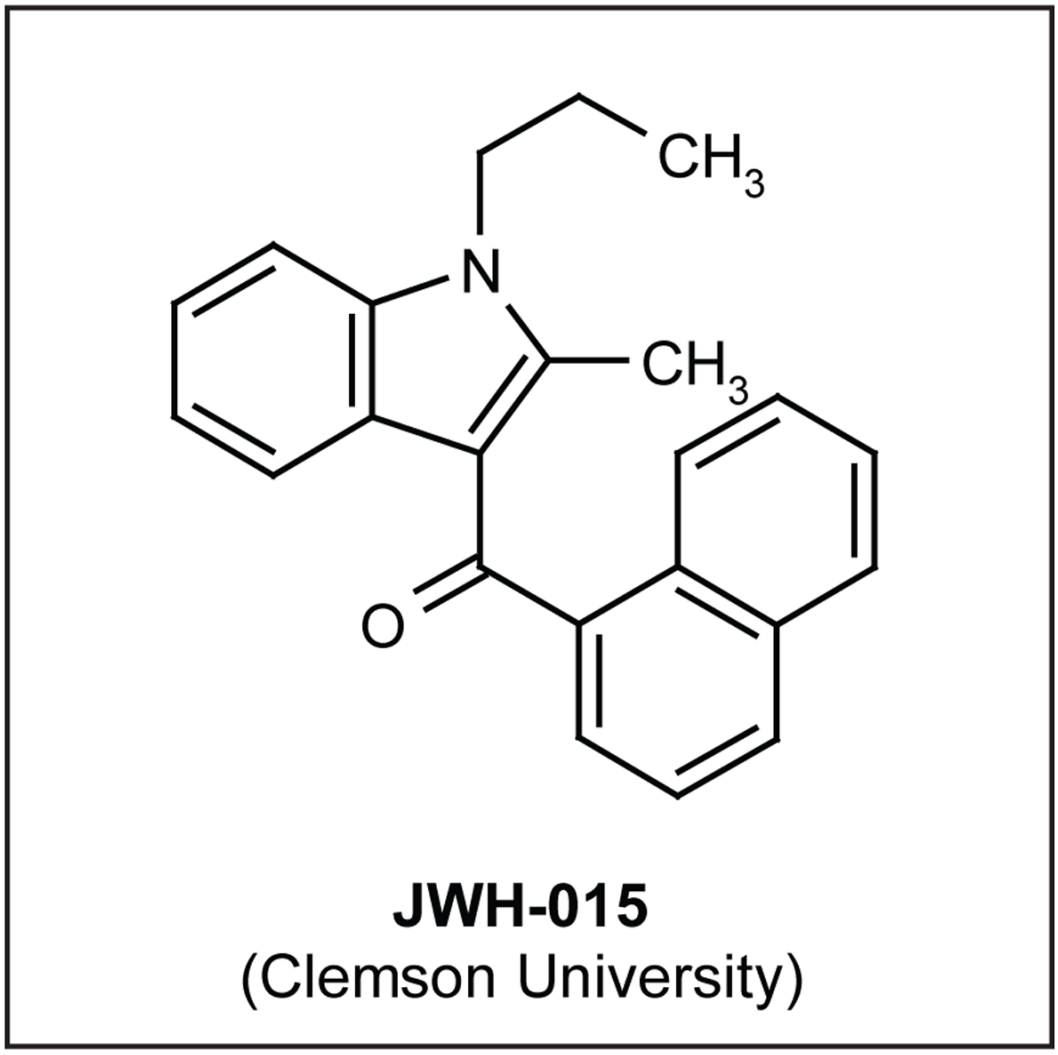

Figure 4.
The structure of JWH-015.