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# Cannabinoid-opioid interactions during neuropathic pain and analgesia

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## Abstract

Opiates and exogenous cannabinoids, both potent analgesics used for the treatment of patients with neuropathic pain, bind to and activate class A G-protein coupled receptors (GPCRs). Several lines of evidence have recently suggested that opioid and cannabinoid receptors can functionally interact in the central nervous system (CNS). These interactions may be direct, such as through receptor heteromerization, or indirect, such as through signaling cross-talk that includes agonist-mediated release and/or synthesis of endogenous ligands that can activate downstream receptors. Interactions between opioid and cannabinoid receptors may mediate many of the behavioral phenomena associated with use of these drugs, including the production of acute antinociception and the development of tolerance and cross-tolerance to the antinociceptive effects of opioid and cannabinoid-specific ligands. This review summarizes behavioral, anatomical, and molecular data characterizing these interactions during the development of neuropathic pain and during antinociceptive treatment with these drugs alone or in combination. These studies are critical for understanding how the receptor systems involved in pain relief are altered during acute or chronic pain, and for designing better antinociceptive drug therapies, such as the combined use of opioid and cannabinoid receptor agonists or selective activation of receptor heteromers, that directly target the altered neurophysiology of patients experiencing pain.

## Introduction

### Neuropathic pain

In the United States alone, over 2 million people suffer chronic and debilitating neuropathic pain as a result of trauma or disease affecting the peripheral or central nervous system (CNS) [1]. Common causes of neuropathic pain include diabetic neuropathy, nerve compression syndromes, postherpetic or trigeminal neuralgia, stroke, multiple sclerosis and spinal cord injury [2]. Clinically, neuropathic pain has both a sensory discriminative component, manifested in part as allodynia (an abnormally painful response to normally innocuous stimuli) and hyperalgesia (an exaggerated response to painful stimuli), and an affective component characterized by heightened anxiety and depression, diminished motivation, and changes in motor control [3,4]. Treatment of neuropathic pain is difficult and controversial; however, both components of neuropathic pain are affected significantly by administration of opiates such as



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morphine and exogenous cannabinoids such as $\Delta^9$tetrahydrocannabinol (THC), compounds that have analgesic and addictive properties [1]. These observations suggest that there are functional interactions between the endogenous cannabinoid and opioid receptor systems, both of which are altered during neuropathic pain [5], in the modulation of nociceptive processing. This review focuses on recent work detailing interactions between these receptor systems during peripheral nerve injury and during production of antinociception. These studies are crucial for understanding the pathophysiological response of the CNS to a peripheral nerve injury and for designing new treatments that will optimize the analgesic effects of receptor-specific ligands while minimizing undesirable side effects such as development of tolerance and reward.

## Common features of opioid and cannabinoid receptor systems

There is considerable behavioral, anatomical and biochemical evidence describing similarities between the opioid receptor system – which includes three subtypes of receptors, mu, delta and kappa (MOR, DOR, and KOR) and the endogenous opioid peptides that can activate these receptors, beta-endorphin, enkephalin, and dynorphin (for review see [6]) – and the cannabinoid receptor system – which comprises the two major cannabinoid receptors, $CB_1R$ and $CB_2R$, and their endogenous ligands, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), both lipid-derived messengers (for review see [7]). Activation of opioid or cannabinoid receptors can produce similar behavioral effects, including antinociception, hypothermia, sedation, hypotension, inhibition of intestinal motility and motor depression [8], suggesting a similar distribution and mechanism of action. Both receptor types are found in several brain regions known to participate in antinociception, including periaqueductal gray (PAG), raphe nuclei and central-medial thalamic nuclei [9], suggesting that they may either act alone, or in tandem to produce antinociception. Furthermore, MOR and $CB_1R$ have been shown to co-localize to the same neurons within the superficial dorsal horn of the spinal cord [10,11], the first site of synaptic contact for peripheral nociceptive afferents, raising the possibility of direct interactions between these receptor types within the same cell.

Opioid and cannabinoid receptors also share similar signal transduction properties. Both are GPCRs that 1) couple to $G\alpha_i$, blocking cAMP production, 2) activate MAP kinases through other second messenger systems, and 3) inhibit neurotransmitter release via inhibition of calcium channels and activation of potassium channels [7,9,12]. Both receptors types are generally found on presynaptic terminals, a location that is consistent with the inhibition of neurotransmitter release [12]. These data raise the intriguing possibility that opioid and cannabinoid receptors function together within the same cell or neuronal circuit to produce antinociception and that modulation of one receptor system may lead to alterations in the activity of the other.

Activation of both opioid and cannabinoid receptors with selective agonists can lead to antinociception in animals experiencing neuropathic pain [5]. Several groups have examined whether both receptor systems are required for the production of antinociception by exogenous drug treatment. THC-induced antinociception was not modified in MOR, DOR, or KOR knockout mice [13], or in MOR-DOR knockout mice [14] while, morphine-induced antinociception was not modified in $CB_1R$ knockout mice [15]. In addition, antinociception induced by DOR or KOR selective agonists was not altered in $CB_1R$ knockout mice [15]. Therefore, selective agonists to opioid and cannabinoid receptors only require the presence of their respective receptor to produce antinociceptive effects. However, in wild-type animals considerable evidence has demonstrated the involvement of the endogenous opioid system in the production of antinociception by exogenous cannabinoid agonist treatment and vice versa, as described below.

## Opioid/cannabinoid interactions in the production of antinociception by exogenous drug treatment

Studies in intact animals have examined opioid/cannabinoid receptor interactions during antinociceptive drug treatment by three main approaches, including: using selective antagonists to one receptor to evaluate alterations in antinociception produced by selective agonists to the other receptor; measuring the development of cross-tolerance to opioid and cannabinoid agonists; and evaluating synergistic interactions between drugs that activate these receptors. Results using receptor antagonists were initially controversial. While naloxone, a general opioid receptor antagonist, was reported to block THC-induced antinociception on the tail-flick test, relatively high doses of naloxone, doses that may have caused off-target effects, were used in these studies [8]. Additional studies done with lower doses of selective antagonists against opioid receptor subtypes or using antisense oligos (to KOR) clarified that MOR and KOR, but not DOR, are involved in the antinociceptive effects of THC [8]. These results imply that treatment with THC or other cannabinoid receptor agonists that do not bind to or activate opioid receptors leads to release of endogenous opioid peptides that activate MOR and KOR. In support of this hypothesis, it has been reported that intrathecal administration of the cannabinoid receptor agonists THC or CP 55,490 leads to dynorphin B release in the spinal cord and that antisera to dynorphin can block THC-induced antinociception [16–19]. Prodynorphin knockout mice show a reduction in THC induced antinociception [12]. The role of opioid peptide release in cannabinoid-mediated antinociception was further supported by a study that demonstrated that inhibitors of opioid degrading enzymes are able to potentiate THC-induced antinociception [20]. Moreover, THC administered over 5 days leads to increases in preproenkephalin expression in the spinal cord, PAG and striatum, increases in prodynorphin gene expression in the spinal cord and increases in proopiomelanocortin (POMC) gene expression in the hypothalamus [21–23]. Together, these data suggest that acute administration of cannabinoid receptor agonists can lead to opioid peptide release and that chronic THC administration increases endogenous opioid precursor gene expression.

Interactions between endogenous cannabinoid ligands, or “endocannabinoids”, and the endogenous opioid system during antinociception have been evaluated using cannabinoid receptor antagonists, or using inhibitors of enzymes that degrade endocannabinoids. The $CB_1R$ antagonist AM251 can reverse morphine-induced, but not DOR agonist-induced, peripheral antinociception in an inflammatory pain model [24] or central antinociception in an acute thermal pain model [25], indicating that endocannabinoid activity may be critical for morphine’s actions. Several groups have reported that the antihyperalgesic effects of inhibitors of fatty acid amide hydrolase (FAAH), the enzyme that degrades AEA, can be blocked by naloxone or nor-BNI, the KOR antagonist [26,27]. These results support the hypothesis that increasing endocannabinoid levels using inhibitors of endocannabinoid degradation elicits antinociception by a similar mechanism as direct activation of $CB_1R$ with THC or CP 55,490 – through the release of endogenous opioid peptides. This effect may not be restricted to $CB_1R$ – one report recently demonstrated that direct activation of $CB_2R$, the cannabinoid receptor generally thought to be localized to the periphery, can induce antinociception via release of endogenous opioid peptides, such as beta-endorphin [28].

## Cross-tolerance between exogenous agonists

A considerable number of studies have examined bidirectional cross-tolerance between opioid and cannabinoid agonists in eliciting antinociceptive effects, though these results are conflicting. One study reported that morphine-dependent animals show decreased antinociceptive responses to THC [29], while others reported that THC or CP 55,490 can potentiate acute antinociception in morphine-tolerant animals [30,31]. These behavioral findings cannot be explained solely based on changes in cannabinoid receptor expression, as $CB_1R$ density in the spinal cord does not change in animals showing cross-tolerance to THC,

and $CB_1R$ density is alternatively reported to decrease [31] or increase [30] in animals showing sensitization to THC. . Other mechanisms such as cross desensitization or G-protein sequestration could account for cross tolerance to THC [32]. Reciprocal studies, evaluating the effects of chronic cannabinoid treatment on opioid receptor expression, have shown that cannabinoid tolerance is associated with a slight increase in MOR protein in several brain areas [33]. This result is somewhat counterintuitive in light of studies showing that antinociception to morphine is reduced in animals made tolerant to the antinociceptive effects of CP 55,490 [12].

Genetic studies have yielded less ambiguous results – tolerance to the antinociceptive effects of THC is not altered in MOR and DOR knockout animals, and tolerance to the antinociceptive effects of morphine is not altered in $CB_1R$ knockout animals [34]. This suggests that tolerance to exogenous opioids does not depend on the presence of cannabinoid receptors and that tolerance to exogenous cannabinoids does not depend on the presence of opioid receptors. However, preproenkephalin knockout animals show a decrease in THC-induced antinociception and a decrease in the development of tolerance to the antinociceptive effects of THC [35]. Thus, both acute antinociception and development of tolerance induced by THC may be associated with endogenous opioid peptide release.

## Synergism between opioid and cannabinoid receptor agonists

Though opioid agonists such as morphine are commonly used for patients with chronic pain, these treatments are limited by the development of tolerance as well as the deleterious side effects of high doses of opioids, including constipation and respiratory depression. Thus, combination treatment of low doses of cannabinoid agonists alongside opioid agonists is particularly attractive. Several groups have reported additive effects between agonists of these two classes, though effects may be compound specific and correct pairing of the most effective combinations is essential [9,36]. For example, CP 55,490 enhances morphine antinociception when administered intrathecally or intraperitoneally while AEA does not [9], likely because it is quickly degraded. Interestingly, opioid receptor protein is upregulated after chronic treatment with low doses of THC and morphine [37], which may underlie the antinociceptive synergism between these two agonists.

## Changes in expression of opioid and cannabinoid receptors during neuropathic pain

Changes in receptor expression, function, and degree of interaction may underlie altered responsiveness to antinociceptive drug treatment during chronic pain syndromes [38]. A number of studies have compared alterations in the expression and function of opioid and cannabinoid receptors during the development of neuropathic pain (see Table 1). While $CB_1R$ levels have been reported to increase in ipsilateral spinal cord [39–41], or in contralateral thalamus [42] after chronic constriction injury, spinal nerve ligation, or spared nerve injury (see Table 2), reports of changes in opioid receptor expression during neuropathic pain vary with the pain model used. Decreases in MOR in the spinal cord and dorsal root ganglion (DRG) are associated with peripheral nerve lesion [38,43,44], while increases at the lesion site or in DRG are observed during inflammatory pain [45]. DOR expression and G-protein coupling are decreased or unchanged in spinal cord and DRG [43,46–49], while KOR expression is reportedly unchanged or slightly increased in these tissues [50]. Together these data suggest changes in receptor expression during neuropathic pain that are pain-model specific. It is interesting to note that $CB_1R$ expression increases while MOR expression decreases in the spinal cord after peripheral nerve lesion suggesting that the increase in $CB_1R$ expression could be due to a compensatory role of this receptor.

### Functional interactions at the cellular level

Evidence from electron microscopy studies has demonstrated co-localization of $CB_1R$ and MOR in the same neuron [10,11,51,52], raising the possibility of direct interactions between these receptors. Consequently, several groups have evaluated functional interactions between opioid and cannabinoid receptors in brain tissues and within *in vitro* systems. Studies using whole brain or cortical membranes found that application of THC leads to an increase in the dissociation of MOR and DOR-specific ligands [53,54], demonstrating that THC can act as an allosteric modulator of opioid receptor binding. Functional interactions between $CB_1R$ and DOR receptors have also been evaluated in N18TG2 cells, which endogenously co-express these receptors. Chronic treatment of N18TG2 cells with either etorphine, an opioid agonist, or DALN, a cannabinoid agonist, leads to agonist-induced desensitization to the respective drug [55]. However, long term exposure to etorphine reduces the ability of DALN to activate cannabinoid receptors – evidence for cross-desensitization [55]. This effect is not reciprocal, as long term exposure to DALN does not reduce the ability of etorphine to activate opioid receptors [55]. These data provide evidence for cross-tolerance between opioid and cannabinoid receptors in an *in vitro* system. Together, these data demonstrate functional and sometimes bidirectional interactions between opioid and cannabinoid receptors within cells known to contain both receptors. These interactions may underlie the cross-tolerance and synergism associated with antinociceptive treatment using combinations of ligands.

## Conclusions and future directions

Opioid and cannabinoid receptor specific agonists are potent analgesics and remain among the more effective treatments for patients with neuropathic pain. However, tolerance and cross-tolerance can develop quickly to the antinociceptive actions of these drugs, limiting their long-term use. The molecular correlates of cross-tolerance remain unclear – changes in receptor density, thought to be markers of receptor responsiveness, are not directly associated with the development of cross-tolerance and may be pain-model specific. Studies showing that treatment with cannabinoid receptor agonists can lead to opioid peptide release and that endocannabinoids are involved in the actions of opioid agonists underscore the synergistic interactions between these two receptor systems. Indeed, combination therapy with acute or subacute doses of opioid and cannabinoid receptor agonists may be an alternative way to circumvent the undesirable side effects of these drugs.

Another strategy is to directly target receptor heteromers, which have emerged as new candidates for antinociceptive therapy. Recent studies have demonstrated that opioid and cannabinoid receptors heteromerize in membranes of co-transfected cells [56], and that $CB_1R$-DOR and $CB_1R$-KOR can form heteromers as well [56]. Signaling responses to a $CB_1R$ agonist are attenuated in the presence of a MOR agonist, and vice versa, in co-transfected cells and in endogenous tissue expressing $CB_1R$ and MOR, indicating that the association between these receptors leads to an antagonistic response. It follows that a bivalent drug that simultaneously activates one receptor protomer while blocking the activity of the other receptor protomer could be an effective analgesic. To date, such compounds have been designed against MOR-DOR or DOR-KOR heteromers [57,58], but not against opioid-cannabinoid receptor complexes; these compounds should be created and tested. Together, the strategies of using combination treatment of opioid and cannabinoid agonists or directly targeting receptor heteromers are two exciting avenues of research that need to be more fully explored, but that have tremendous potential for reducing chronic pain.

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## Table 1

Changes in opioid receptor levels in nervous system during neuropathic pain

| Pain Model | Region examined (all ipsilateral, unless specified) | Receptor | Direction of change |
|---|---|---|---|
| Paw inflammation | Spinal cord, dorsal horn | MOR | ↑[a] [59], ↔[b] [43,60,61] |
| | | DOR | ↔ [43,59–61] |
| | | KOR | ↑ [59], ↔ [43,60,61] |
| | DRG | MOR | ↑ [45], ↔ [43] |
| | | DOR | ↔ [43,45] |
| | | KOR | ↔ [43] |
| Chronic constriction injury | Spinal cord, dorsal horn | MOR | ↑ [62], ↔ [43], ↓[c] [46] |
| | | DOR | ↔ [43,63], ↓ [46,62,64,65] |
| | | KOR | ↑ [62], ↔ [43] |
| | DRG | MOR | ↑ [66,67], ↓ [43] |
| | | DOR | ↔ [63], ↓ [43] |
| | | KOR | ↓ [43] |
| | Sciatic nerve | MOR | ↑ [67] |
| | | DOR | ↔ [63] |
| Lumbar spinal nerve ligation | Spinal cord, dorsal horn | MOR | ↔ [49], ↓ [68] |
| | | DOR | ↓ [64] |
| | | KOR | ↔ [49] |
| Sacral spinal nerve ligation | DRG | KOR | ↑ [50] |
| Sciatic nerve transection | DRG | MOR | ↓ [44] |
| | | DOR | ↓ [64,69] |
| Partial sciatic nerve ligation | Lower midbrain | MOR | ↔ [47] |
| | Spinal cord, dorsal horn | MOR | ↓ [48] |
| | | DOR | ↔ [48] |
| | | KOR | ↔ [48] |
| | DRG | MOR | ↓ [38,48] |
| | | DOR | ↔ [48] |
| | | KOR | ↔ [48] |
| Partial saphenous nerve ligation | Spinal cord, dorsal horn | MOR | ↑ [41] |
| | DRG | MOR | ↑ [41] |

[a] ↑, increase;

[b] ↔, no change;

[c] ↓, decrease

## Table 2

Changes in cannabinoid receptor levels in nervous system during neuropathic pain

| Pain Model | Region examined (all ipsilateral, unless specified) | Receptor | Direction of change |
|---|---|---|---|
| Paw inflammation | Spinal cord, dorsal horn | $CB_2R$ | $\leftrightarrow^b$ [70] |
| Chronic constriction injury | Spinal cord, dorsal horn | $CB_1R$ | $\uparrow^a$ [39] |
| | | $CB_2R$ | ↑ [70] |
| Axotomy of sciatic nerve (tibial branch) | Contralateral thalamus | $CB_1R$ | ↑ [42] |
| Lumbar spinal nerve ligation | DRG | $CB_1R$ | ↑ [40] |
| | | $CB_2R$ | ↑ [70] |
| Partial saphenous nerve ligation | Spinal cord, dorsal horn | $CB_1R$ | ↑ [41] |
| | | $CB_2R$ | ↑ [41] |
| | DRG | $CB_1R$ | ↑ [41] |
| | | $CB_2R$ | ↑ [41] |

[a] ↑, increase;

[b] ↔, no change