Is Paradoxical Pain Induced by Sustained Opioid Exposure an Underlying Mechanism of Opioid Antinociceptive Tolerance?

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Abstract
Opiates are the primary treatment for pain management in cancer patients reporting moderate to severe pain, and are being increasingly used for non-cancer chronic pain. However, prolonged administration of opiates is associated with significant problems including the development of antinociceptive tolerance, wherein higher doses of the drug are required over time to elicit the same amount of analgesia. High doses of opiates result in serious side effects such as constipation, nausea, vomiting, dizziness, somnolence, and impairment of mental alertness. In addition, sustained exposure to morphine has been shown to result in paradoxical pain in regions unaffected by the initial pain complaint, and which may also result in dose escalation, i.e. 'analgesic tolerance'. A concept that has been gaining considerable experimental validation is that prolonged use of opioids elicits paradoxical, abnormal pain. This enhanced pain state requires additional opioids to maintain a constant level of antinociception, and consequently may be interpreted as antinociceptive tolerance. Many substances have been shown to block or reverse antinociceptive tolerance. A non-inclusive list of examples of substances reported to block or reverse opioid antinociceptive tolerance include: substance P receptor (NK-1) antagonists, calcitonin gene-related peptide (CGRP) receptor antagonists, nitric oxide (NO) synthase inhibitors, calcium channel blockers, cyclooxygenase (COX) inhibitors, protein kinase C inhibitors, competitive and non-competitive antagonists of the NMDA (N-methyl-D-aspartate) receptor, AMPA (alpha-amino-3-hydroxy-5-methyl-4 isoxazolepropionic acid) antagonists, anti-dynorphin antiserum, and cholecystokinin (CCK) receptor antagonists. Without exception, these substances are also antagonists of pain-enhancing agents. Prolonged opiate administration indeed induces upregulation of substance P (SP) and calcitonin gene-related peptide (CGRP) within sensory fibers in vivo, and this is accompanied by an enhanced release of excitatory neurotransmitters and neuropeptides from primary afferent fibers upon stimulation. The enhanced evoked release of neuropeptides is correlated with the onset of abnormal pain states and opioid antinociceptive tolerance. Importantly, the descending pain modulatory pathway from the brainstem rostral ventromedial medulla (RVM) via the dorsolateral funiculus (DLF) is critical for maintaining the changes observed in the spinal cord, abnormal pain states and antinociceptive tolerance, because animals with lesion of the DLF did not show enhanced evoked neuropeptide release, or develop abnormal pain or antinociceptive tolerance upon sustained exposure to opiates.
Microinjection of either lidocaine or a CCK antagonist into the RVM blocked both thermal and touch hypersensitivity as well as antinociceptive tolerance. Thus, prolonged opioid exposure enhances a descending pain facilitatory pathway from the RVM that is mediated at least in part by CCK activity and is essential for the maintenance of antinociceptive tolerance.

**Introduction**

Opiate analgesics, such as morphine and fentanyl, are the mainstay of pain management in conditions ranging from acute pain, postoperative pain to chronic pain including cancer pain, and are used as substitution treatment for opioid dependence [1, 2]. These clinical uses of opiates often require opiate treatment for extended periods. However, the use of opioid analgesics for the treatment of many chronic pain states is often offset by the development of tolerance. Antinociceptive tolerance is defined as the decrease in analgesic activity of a drug after a previous exposure to the same or a similar drug [3–6]. Thus, higher doses of the drug are required to elicit the same amount of pain relief. Opioid analgesic tolerance is well recognized experimentally and clinically, and can occur over a period of days to weeks [4–6]. Clinically, the need for increasing doses of opioids in cases of chronic pain is well documented and is a major obstacle to providing adequate pain relief over a long period of time [4, 5, 7].

In animal studies, antinociceptive tolerance is defined as a rightward shift in the antinociceptive effect of morphine challenge in nociceptive assays. This rightward shift in the dose response curve has been demonstrated following repeated daily systemic injections of morphine to mice or rats as well as following repeated intrathecal injections of morphine in rats [8–10]. Prolonged exposure to subdermal slow-release morphine pellets likewise produces a significant shift to the right in the dose-effect curve for subsequent morphine challenge administered either spinally (intrathecally) or supraspinally (intracerebroventricularly) [11, 12]. In addition, prolonged exposure to spinal administration of the mu opioid receptor agonist DAMGO through osmotic mini-pumps resulted in decreased antinociceptive potency and efficacy of spinal opioids [13]. Despite intensive research documenting the occurrence of antinociceptive tolerance, the mechanism underlying this phenomenon remains a subject of much debate. This review will give an overview of changes observed at the cellular as well as the systemic level following prolonged opiate exposure.

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**Signaling Changes as a Mechanism of Opiate Antinociceptive Tolerance**

*Homologous Desensitization and Down-Regulation*

Opiate drugs and endogenous opioid neuropeptides produce their physiological effects through the activation of three structurally distinct receptors that are members of the superfamily of G protein-coupled receptors (GPCR). The mu opioid receptor (MOR), the delta opioid receptor (DOR) and the kappa opioid receptor (KOR) are encoded by different genes and are well defined pharmacologically [14]. The acute activation of opioid receptors results in a decrease in neuronal excitability and neurotransmission by the activation of a class of inwardly rectifying potassium channels, and the inhibition of certain voltage-sensitive calcium channels (for review, see [15, 16]). In nociceptive transmission, for example, the activation of opioid receptors in the primary afferent C fibers results in hyperpolarization of these neurons, decreased firing, inhibited release of excitatory neurotransmitters including glutamate [17] and substance P [18, 19] from the central termini of these afferent fibers in the dorsal horn of the spinal cord. Opioid receptors are also expressed in post-synaptic, intrinsic neurons within the spinal cord [20–22]. Thus, activation of these receptors by spinal administration of opioid agonists may elicit antinociception by pre-synaptic modulation of the activity of primary afferent fibers, or by post-synaptic influence on other spinal neurons.

One proposed mechanism of antinociceptive tolerance after sustained opioid exposure is through agonist-induced receptor desensitization and downregulation of functional receptors present in target neurons [23, 24]. A decrease in functional, cell surface receptors as a result of prolonged agonist exposure is a characteristic common to many GPCR that have been studied to date and as such, receptor desensitization and downregulation as a cellular mechanism of drug tolerance is widely accepted [16, 24]. Like other members of the GPCR superfamily, prolonged agonist activation of opioid receptors results in receptor phosphorylation by G protein-coupled receptor kinases (GRKs), uncoupling of the receptors from G protein-mediated intracellular signaling, and recruitment of beta-arrestins [25–29]. Recruitment of beta-arrestin promotes the attachment of the G protein-coupled receptors to clathrin-coated pits that subsequently undergo endocytosis (i.e. receptor internalization) via a dynamin-dependent mechanism [26, 28, 30]. Upon receptor internalization the receptors may be recycled to the membrane, leading to resensitization, or targeted for degradation, leading to receptor downregulation [31, 32].
The role of beta-arrestins in opioid antinociceptive tolerance has been examined by antisense knockdown of beta-arrestin [33] and by studying transgenic mice that lack the gene that encodes beta-arrestin-2 [34–36]. Intrathecal administration of antisense for beta-arrestin was shown to decrease beta-arrestin mRNA by approximately 60% in vitro [33]. In vivo, spinal administration of these antisense oligonucleotides was shown to delay the development of tolerance to spinal morphine administration [33]. Administration of antisense for beta-arrestin was also reported to attenuate cold allodynia induced by nerve injury. The authors conclude that both morphine tolerance and neuropathic pain are mediated through receptor desensitization [33]. The relation between beta-arrestin and pro-nociceptive mechanisms within the spinal cord is unknown at this time. The beta-arrestin-2 null mutant mice showed increased sensitivity to the acute antinociceptive effects of morphine and did not develop antinociceptive tolerance [37]. The results from the beta-arrestin knockdown and knockout studies have led to the proposal that beta-arrestin-mediated desensitization, and presumably receptor endocytosis, of opioid receptors is induced by morphine in vivo and is suggested to contribute directly to the development of physiological tolerance to opiates [37]. However, these results are not easily reconciled with findings of an inverse relationship between receptor endocytosis and opioid tolerance, as well as reports showing that prolonged administration of opiates fails to lead to opioid receptor downregulation as discussed below.

Numerous studies have reported that distinct opioid agonists, all of which produce antinociceptive tolerance, differ in their ability to modulate the number of surface accessible opioid receptors [38–42]. Several studies report that morphine fails to induce GRK-mediated desensitization, and presumably receptor endocytosis, of opioid receptors is induced by morphine in vivo and is suggested to contribute directly to the development of physiological tolerance to opiates [37]. However, these results are not easily reconciled with findings of an inverse relationship between receptor endocytosis and opioid tolerance, as well as reports showing that prolonged administration of opiates fails to lead to opioid receptor downregulation as discussed below.

Sustained opioid treatment in certain cultured cell models consistently results in a significant increase in the activation of adenylyl cyclase (AC), known as AC superactivation, along with a corresponding increase in the basal level of cAMP [16, 52, 53]. It has been shown that AC superactivation and the corresponding cAMP upregulation is due to a variety of factors including the cell models in question and the agonist used [16, 54–59]. The increase in basal cAMP has been proposed to be a cellular mechanism of opioid tolerance by requiring a greater extent of adenylyl cyclase inhibition through the opioid receptor/Gi-coupled pathway [16]. Recent research shows that there is selectivity in the interactions of G-protein alpha subunits upon differential agonist activation of the opioid receptor [60]. Some have argued that chronic opioid-induced AC superactivation could be due to a switch from receptor activation of inhibitory G proteins (Gi) to enhanced receptor interaction with stimulatory proteins (Gs) upon chronic receptor activation [56, 57, 61]. Crain and Shen [61] proposed that the supersensitivity of mouse dorsal root ganglion neurons following chronic opioid treatment may be due to a shift of the opioid receptors’ coupling from a predominantly Gi-mediated pathway to a higher incidence of Gs activation by the receptors. Thus, a switch in the receptor-G protein activity could account for the development of cellular tolerance as indicated by AC superactivation.

There are nine different AC isoenzymes described to date (for review, see [62, 63]). All AC isoforms, except for type I, are stimulated by Gsα and forskolin, and AC activity can be further modulated by several different secondary factors such as Gsα subunits, Gβγ subunits, Ca2+-calmodulin, intracellular Ca2+, and phosphorylation by protein kinases A and C [62, 63]. Increased AC activity could result from enhanced receptor-Gs interactions and/or by directly enhancing the activity of the enzyme [56, 57]. In guinea pig ileum longitudinal muscle myenteric plexus preparations, chronic in vivo pretreatment with morphine results in a type of cAMP overshoot wherein a reversal from inhibition to stimulation of AC is observed.
This chronic, in vivo pretreatment with morphine also produced an increase of the phosphorylation of ACII that is dependent on protein kinase C and which can significantly increase their stimulatory responsiveness to Gsα and Gβγ [58]. Similarly, a CHO cell line stably transfected with the rat μ-opioid receptor exhibits AC superactivation that is unaffected by cycloheximide pretreatment, indicating that no up-regulation of AC or Gα occurs in this system [55]. Others have reported that increased basal cAMP levels after sustained exposure to δ opioid receptor agonists in transfected CHO cells that express the human δ opioid receptors was associated with the phosphorylation of AC type VI [64]. Further, it was demonstrated that Raf-1, a key protein kinase of the MAPK signal transduction cascade that directly phosphorylates and sensitized AC VI [65], plays a role in chronic delta opioid agonist mediated AC superactivation [66].

Using cAMP superactivation as a cellular marker of opioid tolerance, it was found to be inversely related to receptor endocytosis [48]. MOR mutations that facilitated endocytosis reduced the development of cAMP superactivation, whereas MOR mutations that inhibited endocytosis increased cAMP superactivation [48]. These data suggest that opioid tolerance is likely mediated through mechanisms other than receptor endocytosis that is facilitated by beta-arrestin. A shortcoming of AC superactivation as a mechanism of opioid tolerance is that the enhanced effects of cAMP should influence the efficacy of other receptor systems whose major function is to suppress cAMP production. To date, there is little debate or attention given to testing this hypothesis further by examining possible cross-tolerance to other Gα-coupled receptor function after sustained opioid exposure. Moreover, whether enhanced cAMP levels are physiologically relevant in peripheral or central nociceptive neurons of opioid tolerant animals remains to be validated.

**Protein Kinase Activation**

Opioid exposure has been reported to activate mitogen-activated protein kinases (MAPK) in cell lines expressing the mu opioid receptor [67, 68], as well as activating transcription factors such as cyclic AMP-response element DNA-binding protein (CREB) in cultured DRG neurons, NG108-15 cells, and in vivo [69–72]. Within cultured DRG cells, chronic application of morphine increased phosphorylation of MAPK including p38, ERK, and JNK as well as phosphorylation of the transcription factor CREB [70]. Increased expression of phosphorylation-activated CREB (pCREB), CGRP, and SP was observed based on immunolabeling analysis in the dorsal root ganglion of morphine tolerant rats [70, 73]. pCREB-like immunoreactivity (LI), CGRP-LI and SP-LI are colocalized to cultured dorsal root ganglion neurons that also express MOR. Furthermore, the MEK1 (mitogen-activated protein kinase/extracellular signal-regulated kinase) inhibitor, PD98059, which blocks the MAPK pathway, inhibited morphine-induced increased expression of CGRP-LI and SP-LI, as well as phosphorylation of ERK and CREB in cultured DRG neurons [70]. Other studies have shown that ERK/MAPK activated CREB in various cell types in vitro [74] and in different brain regions in vivo [75], and that both CGRP and preprotachykinin gene promoters contain CRE [76–79]. Thus, chronic morphine exposure appears to enhance the expression of nociceptive sensory neuropeptides such as CGRP and SP via ERK/MAPK pathway and is CREB dependent.

**Sustained Opioid-Induced Excitatory Neurotransmission**

Another proposed mechanism for antinociceptive tolerance is that sustained opiate administration induces neuroplasticity that enhances stimulus-evoked release of excitatory neurotransmitters including glutamate, CGRP, and SP from nociceptive primary afferent fibers within the spinal cord [80–83]. An upregulation of the excitatory neurotransmitters coupled with increased stimulus-evoked release of these pronociceptive neurotransmitters suggest that prolonged opiate exposure leads to sensitization of the nociceptive system. Supporting this hypothesis, opioid-induced abnormal pain has been demonstrated in several animal models after repeated spinal administration of opioids [84–86]. Moreover, rats that were made tolerant to either systemic or spinal morphine demonstrated hyper-reflexia and extreme sensitivity to handling upon the injection of either spinal or systemic naloxone [10]. Consistent with these observations, it has been interpreted that opioids given over time maintain their level of efficacy, but the concurrent development of hyperalgesia serves to counteract the antinociceptive effect of opioids, producing an impression of tolerance [87, 88]. Counter to this argument is that opioid-induced hyperalgesia is simply the result of an unmasking of a compensatory neuronal hyperactivity in response to morphine-induced inhibition of neuronal function [89]. This hyper-responsiveness, or sensitization, becomes evident either after the opioid is removed or occurs intermittently between injections such that opioid-induced hyperal-
against the tail flick test \[13\]. Concurrently, these animals rightward shift in the morphine dose-response curve in their antinociceptive effect within 6 days, and by a DAMGO or morphine, as demonstrated by a reduction in their antinociceptive effect within 6 days, and by a rightward shift in the morphine dose-response curve against the tail flick test \[13\]. Importantly, these behavioral signs of abnormal pain were present while DAMGO was still being infused into the intrathecal space \[13\]. In a related study, the continuous exposure of rats to morphine was assured by constant infusion or the s.c. implantation of a pair of pellets containing free-base morphine \[91\]. Within 7 days, the rats demonstrated reduced response thresholds to light tactile or noxious radiant heat stimuli, indicating the presence of tactile and thermal hypersensitivity \[91\]. As with the spinal DAMGO infusion, the continuous exposure to subcutaneous morphine also produced a significant rightward shift in the spinal or systemic morphine dose-response curves \[91\]. These studies demonstrate that abnormal pain is present during the continuous delivery of opioids by systemic or spinal delivery, and provides evidence that the sensory changes are not due to the development of states of ‘mini-withdrawals’. As pain may be thought of as a ‘physiological antagonist of antinociception (or analgesia, clinically), opioid-induced increased pain may manifest as ‘opioid tolerance’ \[13, 83, 90, 91\].

**Mechanisms Mediating Opioid-Induced Pain**

**NMDA Receptor**

Opioid-induced pain and antinociceptive tolerance may share some underlying mechanisms with the abnormal pain occurring after peripheral nerve injury \[85, 92–94\]. Both of these states are associated with greatly diminished antinociceptive effect of morphine and are sensitive to reversal by intrathecal NMDA antagonists, suggestive of spinal sensitization. It has long been appreciated that activation of the NMDA receptor by glutamate results in the sensitization of spinal neurons \[95\]. Moreover, NMDA receptor mediated central sensitization has been associated with enhanced nociception in chronic pain states \[85, 94, 96\]. This observation has been extended to include opioid-induced abnormal pain \[85, 97–99\]. The blockade and reversal of opioid tolerance by NMDA antagonists has been repeatedly noted, indicating the importance of the NMDA receptor function in this process \[86, 99–101\]. In these studies, MK801 did not produce antinociception alone, nor did it increase antinociceptive action of morphine in non-tolerant rats. The development of tolerance to spinal morphine was prevented by the co-infusion of the NMDA antagonists MK801 or dextromethorphan \[102\]. Hyperalgesia evoked by short-term administration of heroin or fentanyl also was blocked by NMDA antagonists, as well as hyperalgesia provoked by naloxone-precipitated opiate withdrawal \[87, 97, 98, 103–105\]. NMDA receptors are expressed on the central terminals of primary afferent fibers \[106, 107\] as well as in spinal cord neurons. Thus NMDA receptors may promote opioid induced pain and antinociceptive tolerance presynaptically by promoting neurotransmitter output \[107\] and/or postsynaptic potentiation of sensory transmission.

**Spinal Dynorphin**

Although dynorphin was originally identified as an endogenous κ-opioid agonist and may act as an endogenous antinociceptive agent under certain conditions \[108–110\], considerable evidence indicates that enhanced expression of spinal dynorphin is pronociceptive. States of chronic inflammation and peripheral nerve injury which are accompanied by manifestations of abnormal pain, including spontaneous pain, allodynia and hyperalgesia, are also associated with elevated spinal dynorphin content \[111–113\]. Pain behaviors associated with nerve injury were blocked by antiserum to dynorphin \[92, 114–117\]. Dynorphin-like immunoreactivity and prodynorphin mRNA levels were elevated in the spinal cord perfusate of polyarthritic rats \[118\]. A single spinal injection dynorphin has produced long-lasting tactile allodynia in rats and mice \[119, 120\].

Elevations in spinal dynorphin content are also seen in condition of opioid-induced pain states \[13, 121\]. Spinal infusion of DAMGO over 6–7 days produced tactile and thermal hypersensitivity while the opioid infusion was continuing \[13\]. This treatment also produced elevated dynorphin content in the lumbar cord as well as immunoreactivity for prodynorphin \[13\]. The spinal injection of antiserum to dynorphin blocked these behavioral signs of abnormal pain in the DAMGO-treated rats, but...
had no effect on sensory thresholds in normal, non-tolerant rats. More importantly, antiserum to dynorphin unmasked the antinociceptive action of the still-present DAMGO [13]. There is evidence that increased spinal dynorphin promotes the further release of excitatory transmitters from primary afferent neurons, thus provoking a positive feedback loop that amplifies further sensory input. Microdialysis studies have demonstrated localized, dose-dependent release of glutamate and aspartate elicited by exogenous dynorphin in the hippocampus and spinal cord [122–124], and stimulates the production of prostaglandin E2 in the spinal cord [123]. Dynorphin also enhances the release of substance P from trigeminal nuclear slices, and this effect was blocked by MK-801 but not by opioid antagonists [125]. Furthermore, capsaicin-evoked release of CGRP from spinal cord tissue was potentiated by dynorphin A(2-13), a non-opioid fragment [81]. Importantly, dorsal spinal cord tissue taken from rats exposed to morphine pellets for 7 days demonstrated enhanced capsaicin-evoked release of CGRP, which could be blocked in the presence of dynorphin antiserum in the perfusion medium [81].

The pronociceptive actions of elevated levels of endogenous spinal dynorphin as well as enhanced activity of the NMDA receptor may therefore represent two key components in the complex pathway that gives rise to sensitization of sensory neurotransmission at the spinal cord level. Disruptions made to this pathway at the receptors (NMDA, NK-1, CGRP), or enzymes that regulate receptors, channel function, and transcription factors (MAPK, PKC), or release of neurotransmitters and modulators (EAA, neuropeptides, prostaglandins, dynorphin, calcium channels) act to disrupt both the abnormal pain states and restore antinociceptive efficacy of opiates. The overwhelming similarities in the spinal mechanisms that promote opioid induced pain and antinociceptive tolerance, while provocative, do not proof the hypothesis that opioid tolerance is a manifest of enhanced pain. More recently, we began to examine a region of the brain stem, namely the rostral ventromedial medulla (RVM), which has been established as a critical origin of descending input from the brainstem to the spinal cord to regulate pain transmission. Our findings substantiate the hypothesis that sustained opioid exposure induces plasticity in the spinal/supraspinal nociceptive transmission pathway and underlies opioid antinociceptive tolerance.

**Descending Modulation of Pain Transmission**

**Abnormal Pain Is Promoted by Descending Facilitation from the RVM**

The RVM, which includes the nucleus raphe magnus and surrounding reticular neurons ventral to the nucleus gigantocellularis, has been identified as a critical region with respect to nociceptive processing and control [126–128]. Numerous studies have implicated the RVM and the surrounding tissue as a prominent source of descending modulation of nociception [129–136]. Focal brain stimulation of the RVM produced a biphasic modulatory effect on nociceptive activity, with low-intensity electrical stimulation producing facilitation whereas high-intensity electrical stimulation inhibited the tail flick reflex or activity of dorsal horn units [131, 137]. Furthermore, microinjection of the excitatory amino acid glutamate or of neureptensin into the RVM produced a similar biphasic effect, with low doses facilitating and high doses inhibiting the activity of spinal dorsal horn neurons [131, 137, 138]. Several studies have since demonstrated that nerve injury-induced hypersensitivity is dependent on descending facilitation arising from the RVM [132, 139]. The RVM is generally described as consisting of 3 classes of neurons, based on response characteristics to nociceptive inputs [126, 127]. The ‘off’-cells pause in their firing immediately before a withdrawal response to nociceptive stimuli occurs. The ‘on’-cells accelerate firing immediately before the nociceptive reflex occurs. The so-called ‘neutral’ cells show no electrophysiologic responses to nociception. Through an extensive series of experiments, it has been determined that activation of the off-cells produces an inhibition of nociceptive input and inhibition of nocifensive responses [126, 127, 140]. Conversely, the on-cells activate a descending facilitation of nociceptive processing through both local interactions within the RVM and descending systems projecting to the spinal cord [126, 127, 135, 136, 140]. It has been reported that spontaneous activity of on-cells increases along with facilitated pain behavior during naloxone-precipitated withdrawal [141, 142]. Thermal hyperalgesia induced by naloxone-precipitated withdrawal or prolonged delivery of a noxious thermal stimulus was blocked by administration of lidocaine into the RVM [135, 143]. We found that the tactile and thermal hypersensitivity induced by continuous exposure to morphine by subcutaneous implant of pellets or by osmotic minipump was reversibly blocked by the microinjection of lidocaine into the RVM [91].
The Dorsolateral Funiculus

RVM projection neurons extend to the spinal cord via the dorsolateral funiculus (DLF). Lesion of the DLF both prevents [139] and reverses [144] nerve injury-induced pain. DLF lesion also blocks opioid-induced pain and antinociceptive tolerance to spinal opioids [91]. Rats with morphine treatment and bilateral lesions of the DLF demonstrated dose-effect curves identical to those of non-tolerant rats, whereas those with sham DLF lesions and morphine pellets demonstrated a significant shift to the right of the morphine dose-response curves [91]. Normal nociceptive responses and the antinociceptive action of morphine in rats implanted with placebo pellets were not affected by DLF lesions, indicating that these changes were not due to a disruption of normal sensory processing [91]. These effects of DLF lesion are related to the changes induced in the spinal cord upon sustained opioid exposure. In fact, DLF lesion prevented the upregulation of spinal dynorphin [91] and prevented the enhanced release of CGRP from the primary afferent in spinal cord tissue slices [81]. It is important to note that, in these studies, manipulations that blocked abnormal pain did not enhance basal responses to nociception nor did they enhance the antinociceptive potencies of opioids given to non-tolerant animals. Rather, reversal of abnormal pain was specific for the tolerant state. Abolition of opioid-induced pain resulted in loss of antinociceptive tolerance, thus providing evidence that antinociceptive tolerance may reflect enhanced states of pain.

Role of CCK as an Endogenous Pronociceptive (or ‘Anti-Opioid’) Agent

It has been well established that CCK exists in heterogeneous distributions throughout the brain and spinal cord [145, 146]. Notably, the distributions of CCK and of CCK receptors in the CNS show significant overlap with the distributions of endogenous opioid peptides and of opioid receptors, suggesting the possibility of complementary roles in modulation of nociception [147, 148]. Importantly, immunoreactivity for CCK is seen in periaqueductal gray (PAG), raphe nuclei and the medullary reticular formation, and nerve terminals of CCK and enkephalin-containing neurons overlap in the PAG and RVM [145, 149]. CCK immunoreactivity is predominantly associated with fibers within the RVM [150], and CCK-containing projections from the RVM to the spinal cord have been observed [151]. Under normal conditions, CCK is not found in the DRG or terminals of primary afferents of non-primates, but is detected in the superficial laminae of the spinal cord [149, 152, 153]. Spinal CCK is derived from descending projections and interneurons [145].

The spinal and supraspinal administration of CCK has produced behavioral signs of hyperalgesia and enhanced activity of dorsal horn neurons consistent with a pronociceptive role [154–156]. Spinal or systemic CCK blocked antinociception mediated by endogenous opioids and exogenous morphine [157]. CCK antagonists elicited an enhancement of morphine-induced antinociception while producing no antinociceptive activity when given alone [153, 157–162]. Furthermore, the CCKB antagonist, L365,260, inactive alone, significantly enhanced the antinociceptive effect of systemic or spinally administered morphine in rats and mice [83, 163]. Antisense oligodeoxynucleotide ‘knock-down’ of the CCK2 receptor also enhances morphine antinociception [164]. Finally, antinociception mediated by endogenous opioids following blockade of enkephalinases was also enhanced by CCK antagonists [164–166]. Interestingly, CCK antagonists alone have not produced an antinociceptive effect, again indicating the lack of endogenous CCK tone in the normal condition [167].

Recently, it was found that CCK infused into the RVM blocked the antinociceptive effect of systemic morphine [168]. Although the circuitry is not fully understood, it appeared to do so by blocking the morphine-induced increase in firing of RVM off-cells [168]. Furthermore, the microinjection of CCK into the RVM of normal rats produced behavioral signs of pain demonstrated by reversible increased sensitivity to normally non-noxious mechanical stimuli and to noxious thermal stimulation [169, 170]. These results provided strong evidence that CCK is an important player in the RVM mediating descending facilitation of nociception.

There is considerable evidence that while CCK modulates the antinociceptive activity of opioids, the opioids in turn promote CCK release in the brain and the spinal cord, apparently keeping a harmonious balance between endogenous pronociceptive and antinociceptive systems [166, 167, 171–173]. Microdialysis techniques performed in vivo demonstrated that acute systemic and spinal morphine administration increased CSF levels of CCK in the spinal cord [172, 174]. Microdialysis studies also revealed a naloxone-reversible marked increase in extracellular CCK in the frontal cortex of conscious rats after acute systemic morphine administration [175]. Consistent with these observations after short-term opioid exposure, the development of antinociceptive tolerance to morphine is also associated with an upregulation of CCK within the brain and the spinal cord [171, 172, 176]. Prolonged ex-
Exposure to morphine also increases CCK expression within the brain and spinal cord, which in turn further attenuates the antinociceptive effect of morphine, thus resulting in antinociceptive tolerance [172, 177, 178]. Microdialysis performed in the spinal cord of morphine-tolerant rats indicated increases in K⁺-evoked release of CCK in vivo [179]. Moreover, sustained morphine administration correlated with persistent release of CCK in the frontal cortex [175]. Thus, sustained opioid administration increases CCK activity within the brain and spinal cord, which is known to block the antinociceptive effects of morphine and induce behavioral signs of hyperalgesia along with enhanced activity of dorsal horn neurons which is consistent with a pronociceptive role of CCK. Indeed, numerous studies have demonstrated that the co-administration of CCK antagonists with morphine prevents the development of antinociceptive tolerance [162, 180, 181]. Furthermore, behavioral signs of already established antinociceptive tolerance to morphine have been reversed by CCK antiserum or CCKB antagonists at doses that did not enhance morphine antinociception in naive rats [167, 182–184]. Thus, these studies indicate that CCK has a pivotal role in mediating antinociceptive tolerance to opioids. The mechanisms by which CCK acts as an ‘antiopioid’ are currently unknown. It was suggested that CCK counteracts the opioid-induced inhibition of depolarization-induced Ca²⁺ influx into primary afferent neurons by eliciting a mobilization of Ca²⁺ from intracellular stores, thus maintaining nociceptive neurotransmitter release [173]. More recent data suggest that CCK is also likely to act through the activation of pronociceptive systems arising from the RVM [185].

**Conclusion**

The development of tolerance to the analgesic action of opioids is well documented, and is generally considered to be an obstacle in the use of opioids for the treatment of chronic pain. Cellular adaptations such as receptor desensitization and endocytosis, adenylyl cyclase superactivation and increased cAMP levels, and activation of MAPK pathways have been proposed to explain tolerance development, possibly through changes in cytoplasmic signaling events and control of neural gene expression. Such adaptations likely lead to long-lasting changes in neural function. However, it has been difficult to link these cellular changes with systemic changes observed in response to prolonged opioid treatment. Many clinical and preclinical reports have shown that prolonged opioid administration produces paradoxical pain, requiring increased opioid dosages to induce analgesia and diminish the pain state being treated, and thus manifest behaviorally as antinociceptive tolerance. Recent evidence from our laboratories suggests that opioid-mediated paradoxical pain may be a result of neuroplastic changes at supraspinal sites that ultimately lead to the development of descending facilitation arising from the RVM. Growing evidence seems to indicate that increased facilitation arising from the RVM is likely to be mediated through increased CCK activity in this region. Descending facilitation increases expression of spinal dynorphin, which acts as an endogenous pronociceptive agent that promotes increased release of excitatory neurotransmitters from primary afferent neurons. Thus, an enhanced pain state is observed as a consequence of increased evoked release of excitatory neurotransmitters from primary afferent neurons. Based on these observations, we suggest that the combination of substances that block abnormal pain along with opioids would result in a therapeutic approach where opioid activity is maintained even over extended periods of time. Of note, the many substances shown to block opioid antinociceptive tolerance are blockers or inhibitors of endogenous substances which promote pain.
References


Opioid-Induced Pain


Ding XZ, Bayer BM: Increases of CCK mRNA and peptide in different brain areas following acute and chronic administration of morphine. Brain Res 1993;625:139–144.


