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Induction of pain facilitation by sustained opioid exposure: relationship to opioid antinociceptive tolerance

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Abstract

Opioid analgesics are frequently used for the long-term management of chronic pain states, including cancer pain. The prolonged use of opioids is associated with a requirement for increasing doses to manage pain at a consistent level, reflecting the phenomenon of analgesic tolerance. It is now becoming clearer that patients receiving long-term opioid therapy can develop unexpected abnormal pain. Such paradoxical opioid-induced pain, as well as tolerance to the antinociceptive actions of opioids, has been reliably measured in animals during the period of continuous opioid delivery. Several recent studies have demonstrated that such pain may be secondary to neuroplastic changes that result, in part, from an activation of descending pain facilitation mechanisms arising from the rostral ventromedial medulla (RVM). One mechanism which may mediate such pain facilitation is through the increased activity of CCK in the RVM. Secondary consequences from descending facilitation may be produced. For example, opioid–induced upregulation of spinal dynorphin levels seem to depend on intact descending pathways from the RVM reflecting spinal neuroplasticity secondary to changes at supraspinal levels. Increased expression of spinal dynorphin reflects a trophic action of sustained opioid exposure which promotes an increased pain state. Spinal dynorphin may promote pain, in part, by enhancing the evoked release of excitatory transmitters from primary afferents. In this regard, opioids also produce trophic actions by increasing CGRP expression in the dorsal root ganglia. Increased pain elicited by opioids is a critical factor in the behavioral manifestation of opioid tolerance as manipulations which block abnormal pain also block antinociceptive tolerance. Manipulations that have blocked enhanced pain and antinociceptive tolerance include reversible and permanent ablation of descending facilitation from the RVM. Thus, opioids elicit systems-level adaptations resulting in pain due to descending facilitation, upregulation of spinal dynorphin and enhanced release of excitatory transmitters from primary afferents. Adaptive changes produced by sustained opioid exposure including trophic effects to enhance pain

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transmitters suggest the need for careful evaluation of the consequences of long-term opioid administration to patients.

Keywords: Opioid; Pain; Antinociceptive tolerance; Rostral ventromedial medulla; CCK

Introduction

Although there have been several recent advances in the therapeutic management of painful conditions, the primary drugs of choice for the treatment of moderate to severe pain remain the mu-opioid receptor analgesics, represented by such drugs as morphine and fentanyl. Whereas these opioids enjoy well-deserved and accepted clinical efficacy, the use of opioid analgesics for the treatment of many chronic pain states is often offset by the development of tolerance, defined as a decrease in analgesic activity of a drug after a previous exposure to the same or a similar drug (Cox, 1990; Foley, 1993, 1995; Way et al., 1969). Opioid analgesic tolerance is well recognized experimentally and clinically, and can occur over a period of days to weeks (Foley, 1993, 1995; Way et al., 1969). Clinically, the need for increasing doses of opioids in cases of chronic pain is well documented and usually presented as a major obstacle to providing adequate pain relief over a long period of time (Cherney and Portenoy, 1999; Foley, 1993, 1995). In a review of the clinical experience of over 700 patients that received spinal morphine over an average of 124 days, it was found that analgesic tolerance to spinal opioid use developed to different degrees among patients, and appeared to be related to the type of pain and differences in pharmacokinetics among patients (Arner et al., 1988).

In animal studies, antinociceptive tolerance is best characterized by a rightward displacement of the analgesic dose-effect curve. Early studies had shown that the repeated daily systemic injections of morphine to mice or rats produced significant rightward shifts in the antinociceptive effect of morphine challenge in nociceptive assays (Fernandes et al., 1977a,b). Repeated systemic or intrathecal (i.th.) injections of morphine also produced a rightward shift in the dose-response curves for i.th. morphine in the hot-plate and tail-flick tests (Yaksh et al., 1977). Prolonged exposure to morphine pellets implanted subdermally likewise produced a significant shift to the right in the dose-effect curves for morphine given either i.th. or supraspinally (Roerig and Fujimoto, 1988; Roerig et al., 1984). Despite much intensive research documenting the occurrence of antinociceptive tolerance, however, the mechanisms which underlie this phenomenon remain largely unknown.

Many studies have appropriately focused on changes occurring at the cellular level in order to gain an appreciation of the mechanisms that may drive opioid tolerance (Bohn et al., 2000; Childers, 1991; Collin and Cesselin, 1991; Mayer et al., 1995; Sabbe and Yaksh, 1990) including possible alterations in coupling of G-proteins to receptors, or in activities of adenylate cyclase and protein kinases. While these changes are clearly important to observations made with sustained exposure of opioids in animals, changes in cellular mechanisms have not yet been directly related to opioid-induced changes at the systems level. Furthermore, mechanistic interpretation of preclinical studies of opioid antinociceptive tolerance is particularly difficult as 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 is summarized in Table 1. These substances include CGRP antagonists

Table 1
Compounds which block opioid tolerance block abnormal pain

Substrate/system	Endogenous mediator	Reference
NMDA antagonist	glutamate	(Menard et al., 1996b; Powell et al., 2000b)
AMPA antagonist	EAAs	(Kest et al., 1997)
CCK antagonist	CCK	(Dourish et al., 1988; Kellstein and Mayer, 1991; Watkins et al., 1984; Xu et al., 1992)
CGRP antagonist	CGRP	(Menard et al., 1995; Powell et al., 2000b)
Ca^{++} channel blocker	Ca^{++} channels	(Contreras et al., 1997)
Dynorphin antiserum	dynorphin	(Vanderah et al., 2000a)
NOS inhibitor	nitric oxide	(Aley and Levine, 1997; Powell et al., 1999)
PKC inhibitor	PKC	(Mao et al., 1995c; Trujillo and Akil, 1991)
PKA inhibitor	PKA	(Bernstein and Welch, 1997)
COX inhibitor	prostaglandins	(Powell et al., 1999)
Glutamate transporter activator	glutamate	(Nakagawa et al., 2001)
I_2 Imidazoline agonists	agmatine	(Boronat et al., 1998)
Orphanin FQ/nociceptin	nociceptin	(Lutfy et al., 2001)
Benzodiazepine antagonist	GABA	(Raghavendra and Kulkarni, 1999)

(Menard et al., 1996a; Powell et al., 2000a), NO synthase inhibitors (Powell et al., 1999), calcium channel blockers (Aley and Levine, 1997), cyclooxygenase inhibitors (Powell et al., 1999), protein kinase C inhibitors (Mao et al., 1995c), competitive and non-competitive antagonists of the N-methyl, D-aspartate (NMDA) receptor, AMPA antagonists (Kest et al., 1997), superoxide dismutase mimics (Salvemini and Porreca, unpublished observations), dynorphin antiserum (Vanderah et al., 2000a), and CCK antagonists (Dourish et al., 1988, Xu et al., 1992). Blockade of opioid tolerance by antagonists of NMDA receptors has been especially well studied (Lutfy et al., 1996; Manning et al., 1996; Mao et al., 1995b, 1996; Trujillo and Akil, 1991). A concept that has been recently gaining considerable experimental validation is the hypothesis 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 (Gardell et al., 2002; Vanderah et al., 2000a, 2001a,b). Here, we discuss the possible relationship of opioid-induced abnormal pain to antinociceptive tolerance.

Opioid-induced abnormal pain is a clinical concern

A number of clinical reports suggest that the sustained administration of opioids, intended to abolish pain, can unexpectedly produce abnormally heightened pain sensations. In a review of the clinical experiences of over 750 patients receiving epidural or spinal morphine over a mean period of 124 days, it was found that many patients developed hyperesthesias (increased sensitivity to sensory stimuli) and allodynia (pain elicited by normally innocuous sensory stimulation) (Arner et al., 1988). In an extreme case, one patient developed severe allodynia after receiving an i.th. infusion of 30 mg/ml of morphine (Arner et al., 1988). In another clinical report, a patient with an original pain complaint from advanced epidermoid carcinoma of the right lung and thoracic pain developed spontaneous pain, hyperesthesia and allodynia of the legs with a dermatomal distribution of pain corresponding from the lumbosacral region after receiving continuous subarachnoid infusion of 80 mg/day of morphine (De Conno et al., 1991).

Hyperesthesias were also induced by long-term i.th. morphine infusion in a cancer patient where the pain was originally controlled by 1 mg/day of i.th. morphine (Ali, 1986). Again, the abnormal pain manifested in a different way than the original pain complaint, and was described as a burning sensation of the whole leg, rather than an intermittent shooting type of pain originally described (Ali, 1986). More recently, a report was published where a spinal infusion of sufentanil in a patient with neuropathic pain secondary to arachnoiditis and laminectomy originally alleviated the pain but then evoked hyperesthesias in the lower extremities (Devulder, 1997). This abnormal pain state was described as being qualitatively different from the original complaint and included the back, abdomen and both legs. Cancer patients that received high doses of spinal morphine by bolus injections also reported paradoxical intense pain within one-half hour of the injections (Stillman et al., 1987). Prolonged hyperalgesia following short-term opioid exposure has been suggested as a plausible reason as to why clinical results of studies on pre-emptive analgesia have been disappointing (Eisenach, 2000).

Opioids produce abnormal pain in animal studies

Opioid-induced abnormal pain has been demonstrated in several animal models after either systemic or spinal administration. Large doses of i.th. morphine have been associated with paradoxical algesia and hyperesthesias (Woolf, 1981). Rats that received a large (50 μg) i.th. bolus injection of morphine demonstrated pain behavior that involved intermittent bouts of biting and scratching at the dermatomes corresponding to the injection site along with aggressive and nocifensive behaviors in response to light brushing of the flanks (Yaksh et al., 1986). Higher doses of i.th. morphine (90 to 150 μg) also provoked periodic bouts of spontaneous agitation and nocifensive responses to light touch, and these effects of morphine were not sensitive to naloxone (Yaksh and Harty, 1988). The systemic injection of heroin produced antinociception in the tail flick test and the antinociceptive effect of a subsequent dose given immediately after the effect of the initial dose was terminated produced a significantly lower effect (Larcher et al., 1998). Additionally, the injection of naloxone after a single dose of heroin unmasked a latent hyperalgesia, indicated by shortened tail flick response times (Larcher et al., 1998). In a separate study, the s.c. injection of heroin produced antinociception that was followed by decreased thresholds to evoke paw-pressure induced vocalization (Laulin et al., 1998). The same group also demonstrated that a single injection of morphine produced an initial antinociception followed by thermal hyperalgesia (Celerier et al., 1999). Furthermore, the administration of naloxone during the antinociceptive phase of morphine or fentanyl unmasked an NMDA-mediated hyperalgesic effect (Celerier et al., 1999). Most recently, it was found that repeated injections of fentanyl at 15 minute intervals produced a significant hyperalgesia lasting up to 5 days after the fentanyl injections (Celerier et al., 2000).

Similarly, several studies have demonstrated that sustained or repeated spinal administration of opioids has resulted in abnormal pain states to include thermal hyperalgesia and tactile hypersensitivity (Mao et al., 1995b; Trujillo and Akil, 1991; Woolf, 1981; Yaksh and Harty, 1988). Moreover, rats that were made tolerant to either systemic or spinal morphine demonstrated hyperreflexia and extreme sensitivity to handling upon the injection of either spinal or systemic naloxone (Yaksh et al., 1977). The development of thermal hyperalgesia in response to either repeated injections or constant infusion of morphine has been confirmed (Mao et al., 1994, 1995b; Trujillo and Akil, 1991). 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 (Colpaert, 1996; Laulin et al., 1999). In contrast, it has been argued 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 (Gutstein, 1996a). This hyperresponsiveness, or sensitization, becomes evident either after the opioid is removed or occurs intermittently between injections such that opioid-induced hyperalgesia might be interpreted as a result of repeated episodes of opioid withdrawal (“miniwithdrawals”) (Gutstein, 1996a,b).

In order to address such concerns, we have recently demonstrated that *continuous* exposure to opioids, either by constant infusion or subcutaneous pellet implantation, produced behavioral signs of exaggerated pain (Vanderah et al., 2000a, 2001a, 2001b). Critically, the enhanced, abnormal pain occurred while the opioid was continuously present in the system (Vanderah et al., 2000a, 2001b). For example, the continuous spinal infusion of [D-Ala^2,N-Me-Phe4,Gly-ol^5]enkephalin (DAMGO) delivered through an osmotic minipump to rats produced antinociceptive tolerance to 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 (Vanderah et al., 2000a). Concurrently, these animals expressed tactile and thermal hyperesthesias, indicated by significant reductions in paw withdrawal responses to light tactile or noxious radiant heat applied to the hindpaws (Vanderah et al., 2000a). Importantly, these behavioral signs of abnormal pain were present while DAMGO was still being infused into the intrathecal space (Vanderah et al., 2000a). 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 (Vanderah et al., 2001b). Within 7 days, the rats demonstrated reduced response thresholds to light tactile or noxious radiant heat stimuli, indicating the presence of tactile and thermal hyperesthesias (Vanderah et al., 2001b). As with the spinal DAMGO infusion, the continuous exposure to s.c. morphine also produced a significant rightward shift in the i.th. or s.c. morphine dose-response curves (Vanderah et al., 2001b). These studies demonstrated that abnormal pain was present during the continuous delivery of opioids by systemic or spinal delivery, and provide evidence that the sensory changes were 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” (Vanderah et al., 2000a, 2001a, 2001b).

Mechanisms mediating opioid-induced pain

It has been suggested that opioid-induced pain and antinociceptive tolerance may share some underlying mechanisms with the abnormal pain occurring after peripheral nerve injury (Bian et al., 1999; Mao et al., 1995a,b; Wegert et al., 1997). Both of these states are associated with greatly diminished antinociceptive effect of morphine and are sensitive to reversal by 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 (Baranauskas and Nistri, 1998; Ma and Woolf, 1995; Wilcox, 1991). Moreover, NMDA receptor mediated central sensitization has been associated with enhanced nociception in chronic pain states (Haley and Wilcox, 1992; Mao et al., 1995b; Wegert et al., 1997). This observation has been extended to include opioid-induced abnormal pain (Celerier et al., 2000; Larcher et al., 1998; Laulin et al., 1998; Mao et al., 1994, 1995b). The blockade and reversal of opioid tolerance by NMDA antagonists has been repeatedly noted, (Mao et al., 1994; Trujillo and

Akil, 1991, Tiseo et al., 1994; Tiseo and Inturrisi, 1993) indicating the importance of the NMDA receptor in this process. For example, repeated daily injections of i.th. morphine to rats produced tolerance to the antinociceptive effect of morphine along with thermal hyperalgesia of the hindpaws, and both of these effects were prevented by concurrent injections of MK801 (Mao et al., 1994). In another study, the systemic coadministration of morphine with MK801 to rats prevented, in a dose-dependent manner, the development of tolerance to the antinociceptive effect of morphine (Trujillo and Akil, 1991). 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 (Manning et al., 1996). In addition, dextromethorphan or MK-801 reversed the behavioral manifestations of morphine-induced pain and antinociceptive tolerance in rats (Elliott et al., 1995; Gardell et al., 2001; Vanderah et al., 2000b). 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 (Celerier et al., 1999, 2001; Celerier et al., 2000; Larcher et al., 1998; Laulin et al., 1998, 1999).

The presence of presynaptic NMDA receptors on central terminals of primary afferent fibers has been demonstrated (Liu et al., 1994, 1997). Since these sites also contain mu-opioid receptors, an anatomical link between these two systems exists, where NMDA receptors may promote enhanced neurotransmitter output and opioid receptor inhibit the same (Liu et al., 1997). However, such observations have not been designed to address the blockade of opioid tolerance by mechanisms involving multiple and independent sites. Although primary afferent neurons release excitatory amino acids when stimulated, these nerve fibers do not tend to discharge spontaneously, (Price, 1988). Supraspinal pathways do not appear to be involved in modulating lumbar NMDA receptor activity since spinal cord transection does not abolish the ability of i.th. MK801 to prevent morphine tolerance and the i.th. injection of MK801 at thoracic, rather than lumbar, sites did not prevent tolerance to morphine administered at the lumbar level (Gutstein et al., 1992; Mao et al., 1995b). In addition, opioid-induced pain is mediated by different mechanisms from those necessary to produce antinociception, since opioid-induced pain is readily blocked by NMDA antagonists whereas NMDA antagonists themselves are not antinociceptive (Celerier et al., 1999; Laulin et al., 1999).

Recent evidence suggests that enhanced neurotransmitter release subsequent to opioid administration may be promoted, in part, by activation of descending facilitation from supraspinal sites and consequent changes in the spinal cord. Convergent evidence suggests that prolonged exposure to opioids induces neuroplastic changes resulting in enhanced ability of CCK to excite spinopetal facilitatory pathways arising from the rostroventromedial medulla (RVM) (Gardell et al., 2002; Vanderah et al., 2000a, 2001a, 2001b). This pronociceptive mechanism enhances morphine-induced pain and tolerance, and also leads to an upregulation in spinal dynorphin content. These pathologically elevated levels of dynorphin further promote the release of excitatory neurotransmitters (e.g., CGRP and perhaps excitatory amino acids) from the primary afferent fibers. Enhanced excitatory transmitter release following sustained opioid exposure represents a physiological mechanism by which opioids can promote enhanced pain which may manifest as antinociceptive tolerance. Thus, morphine-induced elevated spinal dynorphin may further promote nociceptive input into the spinal cord, and contribute to a feed-forward cycle that serves to maintain this abnormally sensitized pain state, manifesting behaviorally in decreased antinociceptive actions of opioids (i.e., antinociceptive tolerance) (Gardell et al., 2002; Vanderah et al., 2000a, 2001a, 2001b).

Role of CCK as an endogenous pronociceptive (or "anti-opioid") agent

It has been well established that CCK exists in heterogenous distributions throughout the brain and spinal cord (Baber et al., 1989; Savasta et al., 1988). 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 (Ghilardi et al., 1992; Stengaard-Pedersen and Larsson, 1981). 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 (Baber et al., 1989; Hokfelt et al., 1988). Moreover, CCK-containing projections from the RVM to the spinal cord have been identified (Mantyh and Hunt, 1984). 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 (Hokfelt et al., 1988; Stanfa et al., 1994; Verge et al., 1993). Spinal CCK is derived from descending projections and interneurons (Baber et al., 1989).

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 (Hong and Takemori, 1989; Jeftinija et al., 1981; Pittaway et al., 1987). Spinal or systemic CCK blocked antinociception mediated by endogenous opioids and exogenous morphine (Faris et al., 1983). CCK antagonists elicited an enhancement of morphine-induced antinociception while producing no antinociceptive activity when given alone (Dourish et al., 1990; Faris et al., 1983; Hughes et al., 1991; Stanfa et al., 1992, 1994; Suh and Tseng, 1990; Watkins et al., 1985a,b). Furthermore, the CCK_B antagonist, L365,260, inactive alone, significantly enhanced the antinociceptive effect of systemic or i.th. morphine in rats and mice (Ossipov et al., 1994; Vanderah et al., 1996a). Antisense oligodeoxynucleotide "knock-down" of the CCK_B receptor also enhances morphine antinociception (Vanderah et al., 1994). Finally, antinociception mediated by endogenous opioids following blockade of enkephalinases was also enhanced by CCK antagonists (Maldonado et al., 1993; Noble et al., 1993; Valverde et al., 1994; Vanderah et al., 1994). Interestingly, CCK antagonists alone have not produced an antinociceptive effect, again indicating the lack of endogenous CCK tone in the normal condition (Wiesenfeld-Hallin and Xu, 1996).

There is considerable evidence that while CCK modulates the antinociceptive activity of opioids, the opioids in turn promote CCK release, apparently keeping a harmonious balance between endogenous pronociceptive and antinociceptive systems (Noble et al., 1993; Stanfa et al., 1994; Wiesenfeld-Hallin and Xu, 1996; Zhou et al., 1992,1993). Microdialysis techniques performed in vivo demonstrated that systemic and spinal morphine increased CSF levels of CCK (de Araujo Lucas et al., 1998). Systemic morphine resulted in an 89% increase in CCK levels in spinal cord perfusate (Zhou et al., 1993). Microdialysis studies also revealed a naloxone-reversible marked increase in extracellular CCK in the frontal cortex of conscious rats after systemic morphine (Becker et al., 1999). Consistent with these observations after short-term opioid exposure, the development of antinociceptive tolerance to morphine is also associated with an upregulation of CCK (Ding and Bayer, 1993; Zhou et al., 1992). Prolonged exposure to morphine has resulted in an accelerated increase in CCK expression which in turn further attenuated the antinociceptive effect of morphine, thus resulting in antinociceptive tolerance (Zhou et al., 1993). For example, after 1, 3 and 6 days of exposure to morphine, whole brain levels of proCCK mRNA increased by 52%, 62% and 97%, respectively (Zhou et al., 1992). It was also shown that a single s.c. injection of morphine produced 2- to 3-fold increases in CCK mRNA content in the hypothalamus and spinal cord (Ding and Bayer, 1993). The development of tolerance to morphine was

accompanied by elevated CCK content in the amygdala (Pu et al., 1994). Increased release of CCK has also been seen in cases of sustained morphine administration. Microdialysis performed in spinal cord of morphine tolerant rats indicated increases in K^+-evoked release of CCK in vivo (Lucas et al., 1999). Finally, sustained morphine administration correlated with persistent release of CCK in the frontal cortex (Becker et al., 2000).

In contrast, numerous studies have demonstrated that the co-administration of CCK antagonists with morphine prevented the development of antinociceptive tolerance (Dourish et al., 1990; Kellstein and Mayer, 1991; Xu et al., 1992). Furthermore, behavioral signs of already established antinociceptive tolerance to morphine have been reversed by CCK antiserum or CCK_B antagonists at doses that did not potentiate morphine in naive rats (Ding et al., 1986; Hoffmann and Wiesenfeld-Hallin, 1994; Singh et al., 1996; Wiesenfeld-Hallin and Xu, 1996). 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 Ca^{++} influx into primary afferent neurons by eliciting a mobilization of Ca^{++} from intracellular stores, thus maintaining nociceptive neurotransmitter release (Stanfa et al., 1994). More recent data suggest that CCK is also likely to act through the activation of pronociceptive systems arising from the RVM.

Abnormal pain is promoted by descending facilitation from the RVM

Increased pain as a contributing mechanism of antinociceptive opioid tolerance to opioids is a consequence, in part, of descending facilitation arising from the medullary modulatory sites (Vanderah et al., 2001a, 2001b). 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 (Fields and Basbaum, 1999; Fields et al., 1983; Fields and Heinricher, 1985). This region is generally described as consisting of 3 classes of neurons, based on response characteristics to nociceptive inputs (Fields and Basbaum, 1999; Fields et al., 1983; Fields and Heinricher, 1985). 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 (Fields and Basbaum, 1999; Fields et al., 1983; Fields and Heinricher, 1985; Heinricher et al., 1992). 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 (Fields et al., 1983, 1991; Fields and Heinricher, 1985; Heinricher et al., 1992; Heinricher and Roychowdhury, 1997).

Numerous studies have implicated the RVM and the surrounding tissue as a prominent source of descending facilitation (Calejesan et al., 2000; Fields, 1992; Heinricher and Roychowdhury, 1997; Kaplan and Fields, 1991; McNally, 1999; Urban et al., 1999a, 1999b; Zhuo and Gebhart, 1992, 1997). Focal brain stimulation of the RVM produced intensity dependent inhibition or facilitation of the tail flick reflex or activity of dorsal horn units (Zhuo and Gebhart, 1990, 1992). Furthermore, microinjection of the excitatory amino acid glutamate or of neurotensin into the RVM also produced signs of facilitation (Urban and Gebhart, 1997; Zhuo and Gebhart, 1990, 1992). Thermal hyperalgesia secondary to

naloxone-precipitated withdrawal or prolonged delivery of a noxious thermal stimulus has been blocked by RVM lidocaine (Kaplan and Fields, 1991; Morgan and Fields, 1994). It has been reported that spontaneous activity of on-cells increases along with facilitated nocisponsive behavior during naloxone-precipitated withdrawal (Bederson et al., 1990; Kim et al., 1990). Moreover, these actions were blocked by microinjection of lidocaine into the RVM (Kaplan and Fields, 1991). Most recently, it was found that CCK infused into the RVM blocked the antinociceptive effect of systemic morphine (Heinricher et al., 2001). Although the circuitry is not fully understood, it appeared to do so by blocking the morphine-induced increase in firing of RVM off-cells (Heinricher et al., 2001). 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 hyperalgesia (Kovelowski et al., 2000; Xie et al., 2002). It has been shown that on-cells are the only RVM neurons directly inhibited by mu-opioids (Heinricher et al., 2001). Based on this observation, mu-opioid expressing neurons of the RVM were selectively lesioned by the microinjection into the RVM of saporin conjugated to dermorphin (Burgess et al., 2002a,b). This method has been demonstrated to produce a selective reduction in neurons expressing mRNA for the mu-opioid receptor in this region (Burgess et al., 2002a,b). Ablation of RVM cells expressing mu opioid receptors by the dermorphin-saporin conjugate resulted in a loss of the thermal and tactile hyperesthesias induced by microinjection of CCK into the RVM (Burgess et al., 2002b). These results provided strong evidence that CCK is an important player in the RVM mediating descending facilitation of nociception.

Enhanced pain mediated through descending facilitation from the RVM has been shown to apparently underlie opioid-induced pain in several studies. The continuous exposure to morphine by s.c. pellet implantation or by osmotic minipump has been shown to produce tactile and thermal hyperesthesias that was reversibly blocked by the microinjection of lidocaine into the RVM (Vanderah et al., 2001b). Such abnormal pain developed over a period of days and did not reflect the acute activity of the opioid (Vanderah et al., 2001b). Similarly, tactile and thermal hyperesthesias in animals receiving morphine pellets were blocked by the CCK_B antagonist L365,260 administered into the RVM (Xie et al., 2002). Moreover, physical disruptions of the dorsolateral funiculus (DLF), which include the spinopetal projections from the RVM, also blocked tactile and thermal hyperesthesias resulting from sustained opioid delivery by s.c. pellets or osmotic minipump (Vanderah et al., 2001b).

Descending facilitation promotes upregulation of spinal dynorphin and enhanced primary afferent activity

The mechanisms through which activation of tonic descending facilitation may promote opioid-induced paradoxic pain is unclear, but an important factor may be the elevation in the expression of spinal dynorphin (Vanderah et al., 2000a, 2001a, 2001b). Although dynorphin was originally identified as an endogenous opioid κ-opioid agonist and may act as an endogenous antinociceptive agent under certain conditions (Akil et al., 1984; Goldstein et al., 1979; Ossipov et al., 1996), 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 (Draisci et al., 1991; Dubner and Ruda, 1992; Kajander et al., 1990). Pain behaviors associated with nerve injury were blocked by antiserum to dynorphin (Bian et al., 1999; Malan et al., 2000; Wagner and

Deleo, 1996; Wagner et al., 1993; Wang et al., 2001). Dynorphin-like immunoreactivity and prodynorphin mRNA levels were elevated in the spinal cord perfusate of polyarthritic rats (Pohl et al., 1997). A single spinal injection dynorphin has produced long-lasting tactile allodynia in rats and mice (Laughlin et al., 1997; Vanderah et al., 1996b).

Elevations in spinal dynorphin content are also seen in rats that have been constantly exposed to systemic or spinal morphine or spinal DAMGO (Gardell et al., 2002; Vanderah et al., 2000a, 2001b). Spinal infusion of DAMGO over 6-7 days produced tactile and thermal hyperesthesias while the opioid infusion was continuing (Vanderah et al., 2000a). This treatment also produced elevated dynorphin content in the lumbar cord as well as immunoreactivity for prodynorphin (Vanderah et al., 2000a). The spinal injection of antiserum to dynorphin blocked these behavioral signs of abnormal pain in the DAMGO-treated rats, but did not elicit any changes in non-tolerant rats. More importantly, antiserum to dynorphin unmasked the antinociceptive action of the still-present DAMGO (Vanderah et al., 2000a). Furthermore, lesions of the DLF, which have been shown to block opioid-induced pain, also prevented the upregulation of spinal dynorphin in rats with morphine pellets (Gardell et al., 2002). Although the precise mechanisms through which increased spinal dynorphin expression promotes pain remains to be elucidated, 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 (Faden, 1992; Koetzner et al., 2003; Skilling et al., 1992). Dynorphin has been shown to enhance release of substance P from trigeminal nuclear slices, and this effect was blocked by MK-801 but not by opioid antagonists (Arcaya et al., 1999). Furthermore, capsaicin-evoked release of CGRP from spinal cord tissue was potentiated by dynorphin $A_{(2\text{-}13)}$, a non-opioid fragment (Claude et al., 1999; Gardell et al., 2002). Importantly, dorsal spinal cord tissue taken from rats exposed to morphine pellets for 7 days demonstrated enhanced capsaicin-evoked release of CGRP (Gardell et al., 2002). The enhanced release of CGRP was prevented in rats with lesions of the DLF, which also blocks abnormal pain and upregulation of spinal dynorphin, as well as by the addition of antiserum to dynorphin to the perfusate (Gardell et al., 2002). These data present convincing evidence of interactions linking persistent opioid exposure, descending facilitation, spinal dynorphin upregulation and opioid-induced abnormal pain.

Manipulations that block enhanced pain also block antinociceptive tolerance

The manipulations employed to block opioid-induced pain were also demonstrated to block the behavioral manifestation of opioid antinociceptive tolerance (Vanderah et al., 2001a). Rats that were implanted with subdermal morphine pellets demonstrated a significant shift to the right of the antinociceptive dose-response curves for morphine given either systemically or spinally (Vanderah et al., 2001b; Xie et al., 2002). These dose-response curves were shifted to the left when lidocaine was administered into the RVM, demonstrating a normalization of the antinociceptive effect of systemic or spinal morphine (Vanderah et al., 2001b). The A_{50} values were not significantly different from those of non-tolerant rats (Vanderah et al., 2001b). In addition, the ability of lidocaine microinjected into the RVM to restore the antinociceptive potency of i.th. morphine was reversible and consistent with the duration of action of lidocaine (Vanderah et al., 2001b). Similar results were obtained when the CCK_B antagonist L365,260 was given into the RVM. The antinociceptive dose-response curve for spinal

morphine was returned to the same degree of potency of that found in non-tolerant rats when L365,260 was given into the RVM (Xie et al., 2002). Neither of these treatments elicited any changes in dose-effect curves for morphine in non-tolerant animals (Vanderah et al., 2001b; Xie et al., 2002). Conversely, the microinjection of CCK itself into the RVM of naive rats produced a significant shift to the right of the antinociceptive dose-response curve for spinal morphine (Xie et al., 2002). Furthermore, bilateral DLF lesions made prior to the implantation of the morphine pellets prevented the development and expression of morphine antinociceptive tolerance (Vanderah et al., 2001b). 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 (Vanderah et al., 2001b). Normal nocifensive responses and the antinociceptive action of morphine in rats implanted with placebo pellets was not affected by DLF lesions, indicating that these changes were not due to a disruption of normal sensory processing (Vanderah et al., 2001b). Finally, it was also shown that spinal infusion of DAMGO over 7 days produced upregulation of spinal dynorphin and abnormal pain (Vanderah et al., 2000a). These effects were accompanied by antinociceptive tolerance to spinal morphine, demonstrated by a significant shift to the right of the morphine dose-response curve when compared to that of naive rats (Vanderah et al., 2000a). The i.th. injection of antiserum to dynorphin resulted in a restoration of the dose-effect curve for spinal morphine to that of naive rats (Vanderah et al., 2000a). Antiserum to dynorphin did not alter the potency of morphine in naive rats. 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, the reversals of abnormal pain were specific for the tolerant state. Moreover, abolition of opioid-induced pain resulted in loss of antinociceptive tolerance, thus providing evidence that what is measured as antinociceptive tolerance may reflect enhanced states of pain.

Summary

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. Although cellular mechanisms have been proposed to explain tolerance development, it has not been possible to clearly interpret these effects in terms of mechanisms which elicit antinociception. Many clinical and preclinical reports have shown that prolonged opioid administration produces paradoxical pain, which would require increased opioid dosages and would be manifested behaviorally as tolerance. Recent evidence from our laboratories suggest 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 the increased activity of CCK in this region, which promotes descending facilitation. Such descending facilitation promotes expression of spinal dynorphin, which acts as an endogenous pronociceptive agent, thus resulting in an enhanced pain state as a consequence of enhancement of evoked release of excitatory neurotransmitters from primary afferent neurons, notably CGRP. CCK is recognized as an endogenous ‘‘anti-opioid’’ peptide that promotes nociception but may be better described as a ‘‘pronociceptive’’ agent. Therefore, an opioid agonist with CCK antagonist properties should also block increased nociceptive input and prevent the

development of antinociceptive tolerance. Based on these observations, it is possible to 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 (Table 1).

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