Spinal NGF restores opioid sensitivity in neuropathic rats: Possible role of NGF as a regulator of CCK-induced anti-opioid effects

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The breadth of peripheral effects produced by nerve growth factor (NGF) in nociceptive processing has been well documented. However, less is known about the functional significance of central NGF in nociceptive transmission. The effect of NGF on the nervous system is dependent on the developmental stage. During the prenatal developmental period, NGF is critical for survival of nociceptors; in the postnatal period it regulates the expression of nociceptor phenotype, and in the adult it contributes to pain following an inflammatory insult. The implications for central NGF in the expression and regulation of spinal neuropeptides that are involved in pain mechanisms are reviewed. Knowledge has been gained by studies using peripheral nerve injury models that cause a deprivation of central NGF. These models also give rise to the development of pain syndromes, which encompass spontaneous pain, hyperalgesia and allodynia, routinely referred to as neuropathic pain. These models provide an approach for examining the contribution of central NGF to nociceptive transmission. Chronic pain emanating from a nerve injury is typically refractory to traditional analgesics such as opioids. Recent evidence suggests that supplementation of spinal NGF restores morphine-induced antinociception in an animal model of neuropathic pain. This effect appears to be mediated by alterations in spinal levels of cholecystokinin. The authors hypothesize that NGF is critical in maintaining neurochemical homeostasis in the spinal cord of nociceptive neurons, and that supplementation may be beneficial in restoring and/or maintaining opioid analgesia in chronic pain conditions resulting from traumatic nerve injury.

Key Words: Allodynia; Cholecystokinin; Nerve growth factor; Opiate; Pain

Le NGF spinal rétablit la sensibilité aux opioïdes chez les rats neuropathiques : rôle possible du NGF comme régulateur des effets anti-opioïdes induits par la cholécystokinine

RÉSUMÉ : L’étendue des effets périphériques produits par le facteur de croissance nerveux (NGF) dans le processus nociceptif est bien documentée. Cependant, la signification fonctionnelle du NGF central dans la transmission nociceptive est moins connue. L’effet du NGF sur le système nerveux dépend du stade de développement de ce système. Pendant la période de développement prénatale, le NGF est essentiel à la survie des nocicepteurs ; dans la période postnatale, il régule l’expression du phénotype des nocicepteurs, et chez l’adulte, il contribue à la douleur résultant d’une atteinte inflammatoire.

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Implications of NGF central dans l’expression et dans la régulation des
neuropeptides spinaux qui sont impliqués dans les mécanismes de la
douleur sont passées en revue. Les connaissances se sont aussi
améliorées en procédant à des études utilisant des modèles de lésions
nerveuses périphériques qui entraînent une carence de NGF central. Ces
modèles entraînent aussi le développement de syndromes douloureux,
qui englobent la douleur spontanée, l’hyperalgie et l’allodynie,
communément appelés douleur neuropathique. Ces modèles fournissent
une approche pour étudier la contribution du NGF central à la
transmission nociceptive. Une douleur chronique provenant d’une
lésion nerveuse est typiquement réfractaire aux analgésiques classiques
comme les opioïdes. Des données récentes laissent à penser que
l’administration d’un supplément de NGF spinal rétablit
l’antinoception induite par la morphine dans un modèle animal de
douleur neuropathique. Cet effet semble être médié par des
modifications survenant dans les niveaux de cholecystokinin sp nale.
Les auteurs émettent l’hypothèse que le NGF est essentiel au maintien de
l’homéostase neurochimique des neurones nociceptifs dans la moelle
épinière et que l’administration d’un supplément de NGF pourrait
permettre de rétablir et/ou de maintenir une analgésie opioïde dans les
cas de douleur chronique résultant d’une atteinte nerveuse d’origine
traumatique.

Injury to the peripheral or central nervous system (CNS)
gives rise to the development of pain syndromes that usu-
ally include spontaneous pain, hyperalgesia and allodynia
(1,2), routinely referred to as neuropathic pain. Following
peripheral nerve injury in humans, neuropathic pain devel-
ops, including mechanical allodynia, defined as perception
of pain in response to a normally innocuous tactile stimulus
(3). Several animal models of neuropathic pain have been de-
veloped to investigate the mechanisms and pathologies that
precipitate chronic pain (4-6). The development of neu-
ropathic pain has been attributed to an increase in excitability, a
decrease in inhibition and structural reorganization of neu-
rons within the dorsal spinal cord (7) (Table 1). One of the
morphological changes that occurs following damage to a
peripheral nerve is the sprouting of myelinated cutaneous
A-beta fibres into lamina II of the dorsal spinal cord (8-12).
This creates close proximity between fibres that normally
mediate nonpainful mechanical information and primary af-
ferents that are involved in nociceptive transmission. In fact,
one study reported that the A fibres that sprout within the su-
perficial lamina make synaptic connections (11). It has been
hypothesized that this morphological change underlies the
expression of mechanical allodynia. One might speculate
that the activation of large afferent fibres could cause the
depolarization of small diameter afferent nerve terminals, and
thus induce the release of transmitters and neuropeptides involved in
nociceptive transmission. Therefore, innocuous mechanical stimuli could give rise to a painful response via
the new synaptic connections made between large and small
diameter afferent terminals within the spinal dorsal horn. It is not known what triggers the morphological
change; however, there is a correlation between the reduction
in the retrograde supply of peripherally produced trophic fac-
tors (including nerve growth factor [NGF]) and nerve
injury-induced effects. Certainly, NGF is reduced in sensory
neurons following axotomy (13). We review evidence that
NGF can reverse some of the adverse effects produced by pe-
}
gion (DRG) cells, although other studies using both in situ hybridization (28,29) and immunocytochemical (31) techniques have found very few DRG cells expressing CCK. Others have claimed that CCK is not normally present in normal DRG cells in the rat (32,33). In the rat dorsal horn of the spinal cord, numerous interneurons and descending fibres contain CCK (29,34,35).

CCK differs from most other neuropeptides that modulate nociceptive transmission in that it appears to act indirectly by interaction with the opioid system. The hypothesis that CCK may be an anti-opioid peptide is derived from studies demonstrating that exogenous application of CCK attenuates the analgesic effect of morphine and beta-endorphin (36,37). This observation has since been confirmed by many reports that used both behavioural and electrophysiological nociceptive techniques. Under normal conditions, CCK causes a marked inhibition of the antinociceptive effects of morphine and selective mu opioid receptor agonists (38,39), and attenuates morphine-induced inhibition of C fibre-evoked discharges of dorsal horn nociceptive neurons (37). Endogenous CCK tonically inhibits opioid-induced antinociception, and selective CCK antagonists potentiate morphine-induced antinociception (23,24,40-45). Furthermore, an antisense oligonucleotide directed against the CCK-B receptor mRNA was shown to enhance morphine-induced antinociception (46), suggesting that CCK produces a tonic inhibition of morphine-induced analgesic effects through an action at CCK-B receptors. The results of a recent clinical study underscore the contribution of CCK and its anti-opioid effects in chronic pain states because the CCK receptor antagonist proglumide increased the analgesic effect of morphine in some patients with chronic benign pain (47).

Many studies have demonstrated that opioids enhance the release of CCK through activation of opioid receptors on CCK-containing neurons (Figure 1). Recently, morphine was shown to evoke the release of CCK from cortical regions via activation of a delta-opioid receptor (48). High concentrations of morphine or a delta-opioid receptor agonist, (D-Ser8)-leucine enkephalin-Thr, were shown to enhance a calcium-dependent release of CCK from the rat substantia nigra (49), and [D-Ala2]deltorphin augmented CCK release from dorsal horn lumbar spinal cord slices (50). This excitatory action of opioids via activation of the delta-opioid receptor subtype has also been implicated in the development of opioid tolerance.

CCK does not elicit hyperalgesic effects, nor do CCK antagonists produce antinoceception, indicating that endogenous CCK has no tonic inhibition on endogenous opioids (51). The mechanism by which CCK produces its anti-opioid effect remains elusive, although many hypotheses have been formulated. Within the CNS, the distribution of CCK parallels that of the endogenous opioids within the pain processing areas (52-55), providing anatomical evidence that a functional relationship may exist between these two transmitter systems. One of the possible mechanisms of CCK’s anti-opioid activity was proposed to be consequential to CCK producing an attenuation of the binding affinity of mu opioid receptors for its ligands (56). It has also been proposed that CCK’s anti-opioid effects involve interactions between intracellular signalling cascades following activation of both CCK and mu opioid receptors (57). Although anatomical studies have yet to verify the coexistence of CCK-B and mu opioid receptors on the same nerve terminal, CCK-B receptor binding sites are present on capsaicin-sensitive small diameter primary afferent neurons (58). Moreover, CCK reverses the effects of mu opioid agonists in a whole cell patch clamp recording of acutely dissociated DRG neurons (59). Together, these studies strongly suggest the colocalization of CCK-B and opioid receptors, and the occurrence of CCK-opioid interactions within the same primary afferent neuron.

It has been established that CCK receptor signalling occurs predominantly via the phospholipase C-1,4,5-inositol triphosphate/1,2-diacylglycerol (IP3/DAG)-protein kinase C (PKC) pathway (27). CCK receptors activate pertussis toxin-sensitive G proteins that are coupled to membrane-bound phospholipase C, which in turn causes an increase in IP3, and the subsequent release of intracellular calcium. DAG activates PKC, resulting in its translocation to the membrane, where it can phosphorylate various proteins. It has been shown that stimulation of PKC can result in the phosphorylation and desensitization of opioid receptors (60-62). It is highly possible that this signalling cascade may underlie CCK’s anti-opioid effects because it has recently been demonstrated that local pretreatment with a PKC inhibitor, calphostin C, abolished the inhibitory effects of CCK on opioid-induced antinociception (63).

**CCK INCREASES FOLLOWING NERVE INJURY**

It was hypothesized that one of the possible reasons for the relative refractory analgesic efficacy of opioids in neuropathic pain may be an increase in CCK in the spinal cord. Thus, the lack of morphine effectiveness in alleviating neuropathic nociception may partially result from an increase in

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**Figure 1** Schematic representation of the interaction between cholecystokinin (CCK) and the opioid system at the level of the spinal cord. Chronic administration of morphine has been shown to increase endogenous CCK via activation of the delta opioid receptor (OR). In turn, CCK decreases the levels of endogenous enkephalins and activates CCK-B receptors to elicit an anti-opioid effect, resulting in the attenuation of morphine-induced antinociception. Blockade of CCK-B receptors with selective antagonists has been shown to attenuate the development of tolerance to opioid-induced antinociception.
NGF IN NOCICEPTION

NGF has an established role in the survival, differentiation, development and maintenance of phenotype for small diameter, primary afferent neurons involved in nociceptive transmission (75). Nociceptive neurons are generally thought to express the NGF-selective trkA receptor. In the absence of NGF, C and A-delta fibres are absent in sensory ganglia, and their corresponding nerve endings are missing both in the periphery and spinal cord (76,77). Overexpression of NGF produces the opposite effect, resulting in the hypertrophy of small diameter, primary afferent neurons innervating the skin (77-79), a condition that produces thermal hyperalgesia in the affected animal (80). The scope of NGF’s action in establishing and maintaining nociceptors for normal pre- and postnatal development is well documented (81).

The contribution of NGF to inflammatory hyperalgesia was suggested to be mediated by its activation of high affinity trkA receptors on mast cells and primary sensory neurons (82). Peripherally produced NGF normally maintains the sensitivity of nociceptive sensory neurons, but in some inflammatory states, an increase in NGF occurs in the skin (83). The augmentation of NGF appears to be partly responsible for the hyperalgesia and allodynia that typically accompany such an insult. Thus, neutralization of NGF during inflammation with the use of either antibodies directed at NGF (84,85) or trkA-immunoglobulin G immunoadhesin molecules for NGF (86) attenuates hyperalgesia. Moreover, NGF is sufficient to induce hyperalgesia because administration either locally or systemically induces a decrease in thermal nociceptive thresholds (85,87). Similarly, it was demonstrated that NGF was necessary for the mechanical hyperalgesia that ensues following an inflammatory insult (88). The peripheral and central mechanisms implicated in NGF-induced hyperalgesia have been extensively reviewed (81).

NGF plays a role in the dynamic control of neuropeptide levels in adult sensory neurons (89-91) and, therefore, may contribute to inflammatory hyperalgesia by altering the release of peptides from sensory neurons. The role of peripheral NGF in nociception is demonstrated in studies showing that anti-NGF or trkA fusion molecules attenuate Freund’s adjuvant-induced increase in preprotachykinin A mRNA (92), substance P and calcitonin gene-related peptide (CGRP) (86,91).

ALTERATIONS IN NGF FOLLOWING NERVE INJURY

Although NGF is not necessary for neuronal survival in adults, it does appear to influence neuronal growth following denervation induced by peripheral nerve damage. In sensory systems, NGF is specifically taken up along peripheral and central processes of sensory neurons and retrogradely transported to the cell body (93). The depletion of NGF in the spinal dorsal horn following sciatic nerve axotomy (10) or chronic constriction injury (12) is thought to precipitate collateral sprouting of large diameter afferent fibres of laminae III to V into lamina II of the spinal cord. In support of this hypothesis, it was discovered that supplementation of spinal NGF by continuous intrathecal infusion suppressed the sprouting of these axon terminals into neighbouring denervated lamina (94). Sciatic nerve transection was shown to decrease the density of NGF binding sites on DRG neurons, and intrathecal NGF infusion partially reversed this reduction but did not influence NGF binding to neurons with intact axons (95).

Studies have demonstrated that disruption of NGF transport is correlated with changes in neuropeptide levels in the spinal cord and DRG that occur following peripheral nerve axotomy or constriction injury (95,96). Moreover, continuous infusion of NGF to the proximal stump of a transected sciatic nerve mitigates some of the morphological, biochemical and electrophysiological alterations in axotomized DRG...
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TABLE 2
Percentage maximum possible effect (MPE) produced by intrathecal morphine (20 µg) obtained on day 14 following sciatic nerve constriction of nerve-injured animals

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>% MPE of intrathecal morphine (20 µg) in neuropathic rats (day 16 after injury)</th>
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<tbody>
<tr>
<td></td>
<td>Chronic spinal IgG</td>
</tr>
<tr>
<td>Thermal plantar test</td>
<td>12.5±2.80</td>
</tr>
<tr>
<td>Cold water response frequency</td>
<td>20.6±1.59</td>
</tr>
<tr>
<td>Cold water response duration</td>
<td>21.9±4.80</td>
</tr>
<tr>
<td>50% Von Frey threshold</td>
<td>26.1±3.86</td>
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Values are means ± SEM (n = five to nine per group). Statistical analysis using a paired t test revealed that mechanical response thresholds, and withdrawal latencies to either cold or heat following intrathecal morphine in nerve growth factor (NGF)-treated rats were significantly increased compared with premorphine values. *P=0.05; **P=0.01. IgG Immunoglobulin G.

perikarya (97-102). Several studies have provided evidence that NGF is involved in neural, anatomical and molecular plasticity of primary afferents (103-106). Although the occurrence of collateral sprouting and the decrease in spinal NGF are accepted, the clinical relevance of these events elicited in animal models of neuropathic pain is unclear. However, a functional correlate is suggested in that NGF supplementation attenuates injury-induced allodynia and hyperalgesia (107,108). There is also evidence that NGF may be neuroprotective to DRG neurons against drug- and diabetes-induced neuropathies (109). Beneficial effects of NGF supplementation have also been reported in alleviating neuropathic pain, in that infusion of NGF directly on the sciatic nerve prevents the development of thermal hyperalgesia and partially blocks mechanical allodynia in a sciatic nerve constriction model (110,111). However, local administration of anti-NGF decreased the severity of autotomy and blocked collateral sprouting, suggesting that neutralization of peripheral NGF may also be advantageous in blocking neuropathic pain (111).

NGF REVERSES CCK-INDUCED OPIOID INSENSITIVITY

As discussed above, many studies have demonstrated a decrease in NGF in the sciatic nerve and central terminals in animal models of neuropathic pain (112). However, Verge and colleagues (105) demonstrated that delayed chronic intrathecal administration of NGF counteracted sciatic nerve constriction-induced changes in neuropeptide content, including the injury-induced increase in CCK. The phenotypic changes displayed within sensory neurons of the dorsal horn may also be advantageous in blocking neuropathic pain (111). CCK levels within the hypothalamus (115). This may explain the observation in mice overexpressing NGF that there is an enhanced efficacy of morphine following induction of thermal hyperalgesia.

We have confirmed the observation that intrathecal morphine is ineffective in increasing mechanical response thresholds, latencies to radiant heat or sensitivity to cold water in rats exhibiting nerve injury-induced allodynia and hyperalgesia (18-22). Nevertheless, delayed intrathecal NGF infusion was beneficial in restoring morphine-induced anti-hyperalgesic and antiallodynic effects indicative of neuropathic pain (unpublished data). In the study by Cahill and Codere (unpublished data), delayed chronic intrathecal infusion of NGF could not alleviate decreased nociceptive thresholds in neuropathic rats, it only influenced morphine sensitivity (Table 2). While the mechanism by which NGF may regulate morphine antinociception in this model remains elusive, we have implicated the potential relevance of CCK as an important inhibitory modulator of opioid antinociception in neuropathic pain states.

To validate further the hypothesis that endogenous spinal NGF is important in maintaining opioid-induced antinociception, we attempted to mimic the decreased effectiveness of opioid antinociception that occurs in neuropathic pain models by neutralizing endogenous levels of NGF with chronic intrathecal infusion of antibodies directed against purified 2.5S NGF. A previous study (116) demonstrated that changes in density and distribution of calcitonin gene-related peptide could be manipulated by changes in endogenous levels of NGF. Thus, chronic intrathecal anti-NGF treatment resulted in altered expression of CGRP in the dorsal horn of rat spinal cord (117). We recently discovered (unpublished data) that treating rats with intrathecal anti-NGF had no significant effect on morphine-induced antinociception compared with that seen in control rats infused with immunoglobulin G (IgG). However, whereas morphine-induced antinociception was potentiated by pretreatment with an intrathecal CCK-B receptor antagonist in anti-NGF-treated rats, there was no significant effect in IgG-treated controls (Table 3). These results draw a positive correlation between a decrease in NGF and CCK receptor antagonism restoring opioid antinociception. It is tempting to suggest that endogenous NGF appears to be important in maintaining the neuropeptide homeostasis,

TABLE 3
Percentage change in morphine-induced antinociception by cholecystokinin (CCK)-B receptor antagonist

<table>
<thead>
<tr>
<th>Immunoglobulin G</th>
<th>Antinerve growth factor</th>
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<tr>
<td>8.3±0.72</td>
<td>84.8±10.9*</td>
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Percentage change in morphine (5 µg) -induced antinociception following pretreatment of intrathecal vehicle or CCK receptor antagonist (LY225910, 10 nmol) in rats chronically infused for seven days with intrathecal immunoglobulin G or antinerve growth factor (NGF) via osmotic minipumps. Statistical analysis using a paired t test revealed that the percentage increase in antinociception elicited by intrathecal morphine following LY225910 in anti-NGF treated rats was significantly increased compared with that in IgG-treated rats. *P=0.05,

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including CCK, that is required for maintaining opioid effectiveness (Figure 2).

**CONCLUSIONS**

Many authors have attempted to understand the mechanisms involved in the development and maintenance of chronic pain. The ultimate goal of these authors is to facilitate the development of new therapies for optimal pain treatment. Pain is generated by a number of processes that are qualitatively different, requiring multiple modalities for treatment. Accordingly, opioids are often very effective for the management of pain with an inflammatory origin, whereas opioids have low efficacy or no effect on alleviating pain resulting from nerve injuries. The upregulation of CCK that ensues following nerve injury depresses the ability of endogenous opioids to modulate nociceptive transmission, resulting in the appearance of neuropathic pain syndromes and the reduction of the effectiveness of exogenously administered opioids.

NGF may play a role in the central plasticity and sensitization that occur following traumatic nerve injury. The low amount of central NGF, due to the attenuation of its transport to the CNS, forms part of the adaptive response that may inadvertently lead to the development of chronic pain. Changes in NGF may be related to the clinical phenomenon of opioid insensitivity that occurs in chronic pain of neuropathic origin. Nerve injury leads to complex changes in neuropeptide expression within primary afferents that correlate with changes in their phenotype; these events, particularly increases in CCK and its receptor, are thought to elicit neuropathic pain. It must be kept in mind that there are considerable species and strain differences in the reaction to neuronal damage (68). Moreover, variations in peptide levels are evident in the various animal models implemented for the induction of neuropathic pain-like characteristics within a species strain. Thus, clinically useful drugs for treating neuropathic pain may need to be tailored to specific patient populations where the pathology is well defined and symptoms have been properly charted.

Consequently, it has been proposed that NGF may, at the appropriate time, dose, site and mode of administration, provide prophylaxis and treatment in conditions that lead to chronic pain (117). Although intrathecal NGF does not alter neuropathic nociceptive behaviours, it does restore antiallodynic and antihyperalgesic effects of morphine. We propose that by normalizing the spinal levels of CCK, intrathecal administration of NGF may ameliorate the chronic pain associated with traumatic nerve injury by re-establishing the effectiveness of conventional opioid therapies for pain management.

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