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Intrathecal nerve growth factor restores opioid effectiveness in an animal model of neuropathic pain

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Abstract

It is without dispute that the treatment of neuropathic pain is an area of largely unmet medical need. Available analgesics, such as morphine, either have minimal effects in neuropathic pain patients, or are not always well tolerated due to concurrent adverse effects. The chronicity of neuropathic pain is thought to be related to many neurochemical changes in the dorsal root ganglia (DRG) and spinal cord, including a reduction in the retrograde transport of nerve growth factor (NGF). In this study, we have determined the ability of chronic intrathecal (i.t.) infusion of NGF to reverse neuropathic pain symptoms and to restore morphine's effectiveness in an animal model of neuropathic pain. Seven days after sciatic nerve constriction injury, NGF was administered to the spinal cord by continuous infusion (125 ng/μl/h) via osmotic pumps attached to chronically implanted i.t. catheters. Spinal infusion of NGF did not affect the expression of tactile allodynia or thermal (hot) hyperalgesia in neuropathic rats, although it significantly increased cold water responses frequency at day 14. Following infusion of vehicle, i.t. morphine (20 μg) was ineffective in altering somatosensory thresholds in neuropathic rats. In contrast, morphine substantially attenuated the neuropathy-induced warm and cold hyperalgesia, as well as tactile allodynia, in neuropathic rats chronically infused with i.t. NGF. In addition, we demonstrate that i.t. morphine-induced antinociception was augmented by a cholecystokinin (CCK) antagonist in animals chronically infused with i.t. antibodies directed against NGF. We 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.
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Keywords: Morphine; Neuropathic pain; Nerve growth factor; Spinal cord; Allodynia; Hyperalgesia

1. Introduction

Neuropathic pain is a chronic debilitating condition that is characterized by its lancinating or continuous burning-type pain, and is typically associated with the occurrence of allodynia (pain resulting from normally innocuous stimuli) and/or hyperalgesia (increased sensitivity and exaggerated responses to painful stimuli). Injury to the peripheral and central nervous systems can give rise to the development of neuropathic pain, which is considered to be inherently resistant to opioids (Arnér and Meyerson, 1993; Max et al., 1988). With the development of animal models of neuropathic pain, several studies have supported clinical observations that spinal administration of opioids, such as morphine, has a reduced therapeutic benefit in alleviating neuropathic pain (Mao, 1999; Xu et al., 1993, 1994).

It has been well established that nerve growth factor (NGF) has a role in the development and maintenance of neurons in the peripheral (Barde, 1989) and central (Shelton and Reichardt, 1986) nervous systems. In adult rats, NGF is synthesized in peripheral tissues and retrogradely transported to the cell bodies of sensory neurons (dorsal root ganglia, DRG) (Goedert et al., 1981). Once in the DRG, NGF exhibits a trophic effect on the regulation of substance P and calcitonin gene related peptide

(CGRP) in unmyelinated sensory neurons (Lindsay and Harmar, 1989). Disruption of NGF transport due to a nerve constriction injury or nerve transection has been correlated with altered neuropeptide expression in the spinal cord and DRG neurons (Csillik et al., 1985; Verge et al., 1992). For example, sciatic nerve injury was shown to decrease the levels of substance P and CGRP, as well as causing increases in vasoactive intestinal peptide, galanin, neuropeptide Y and cholecystokinin (CCK) (reviewed by Wiesenfeld-Hallin and Xu, 1996). However, supplementation of NGF via continuous infusion to the proximal stump of a transected sciatic nerve was shown to mitigate some of the morphological, biochemical and electrophysiological alterations in DRG neuronal perikarya (Fitzgerald et al., 1985; Otto et al., 1987; Rich et al., 1987; Verge et al., 1989). Moreover, naïve animals injected with antiserum against NGF exhibited similar changes in sensory neuropeptide levels as traumatic nerve injury (Shadiack et al., 2001). There are numerous reports that have demonstrated peripheral administration of NGF produces pain responses. However, there is also evidence that NGF, administered directly to DRG or spinal cord, may either reduce neuropathic pain or re-set the chemical imbalance created by the nerve injury. In keeping with this hypothesis, infusion of NGF directly on the sciatic nerve prevents the development of thermal hyperalgesia, and partially blocks mechanical allodynia induced by sciatic nerve constriction (Ren et al., 1995; Ro et al., 1999), as well as preventing the behavioural and biochemical manifestations of diabetic neuropathy (Apfel et al., 1994).

In this study, we assessed whether spinal administration of NGF, by continuous i.t. infusion, attenuates the expression of tactile allodynia and hot and cold hyperalgesia in rats with a unilateral nerve constriction injury. The effectiveness of i.t. morphine to alleviate tactile allodynia, as well as cold and hot hyperalgesia, associated with sciatic nerve constriction injury following NGF treatment was also determined. Finally, we assessed the modulatory effects of a CCK receptor antagonist on morphine-induced antinociception in rats chronically treated with antibodies to NGF. This study was used to examine the role of endogenous NGF on morphine sensitivity in neuropathic pain, and to provide further evidence for a relationship between NGF and CCK anti-opioid effects.

2. Methods

2.1. Animals

Experiments were performed on male Long Evans hooded rats (300–350 g; Charles River, Que., Canada) housed in groups of three per cage. Rats were maintained on a 12/12 h light/dark cycle and were allowed free

access to food and water. Experiments were carried out according to a protocol approved by the animal care committee at McGill University, and in accordance with guidelines of the NIH guide for care and use of laboratory animals. The rats were sacrificed immediately following the 14 day experimentation protocol, and separate groups of animals were used for the three experimental protocols described below.

2.2. Sciatic nerve constriction

Sciatic nerve injury was accomplished by the method previously described by Fisher et al. (1998). Briefly, rats were anesthetised with halothane (2.5%) and the sciatic nerve of the left hind limb was exposed following blunt dissection. A polyethylene (PE-90) cuff (2 mm in length) was wrapped around the entire sciatic nerve. Care was taken to ensure that the nerve was not pinched by the cuff, and that it was not too tight so as to occlude the perineural blood flow. The muscle was stitched, and the incision was closed with wound clips. Animals received topical antibiotic and subcutaneous saline (5 ml). For sham-operated animals, the surgical procedure was identical except the sciatic nerve was not manipulated.

2.3. Intrathecal cannulation

Both naïve rats (for anti-NGF infusion), or 7 day neuropathic rats (for β-NGF infusion), were implanted with chronic i.t. catheters, according to the method previously described by Cahill et al. (1995). Briefly, rats were anesthetised with sodium pentobarbital (60 mg/kg). A small opening was made at the cisterna magna and a catheter (PE 10 tubing attached to PE 60 tubing for attachment to an osmotic pump) was inserted into the subarachnoid space and caudally directed 8 cm to the lumbar enlargement of the spinal cord. After anchoring the catheter, an osmotic mini-pump (model 2001, Alzet, Coopertino, CA) was attached to it, and the pump was implanted subcutaneously. Only animals exhibiting no motor deficits were used for behavioural testing.

2.4. Drugs and drug delivery

In the first series of experiments, osmotic pumps attached to i.t. catheters were filled with either 125 ng/µl rat recombinant β-NGF (Sigma, St Louis, MO) in a solution of artificial cerebral spinal fluid (ACSF) containing 1 mg/ml rat serum albumin (Sigma, St Louis, MO), or vehicle. The osmotic pumps were model 2001 Alzet pumps (Alza Corp., Cupertino, CA), which pumped at a rate of 1 µl/h for 7 days, producing an i.t. infusion dose of 125 ng/h. The same 125 ng/µl concentration of NGF was previously shown to be effective in restoring neuropeptide levels (Verge et al., 1995), and preventing A fibre sprouting (Bennett et al., 1996) in neuropathic

rats. In a separate experiment, naïve rats received either antibodies to NGF (anti-NGF IgG, 1 µg/µl) or control IgG (1 µg/µl) (Sigma, St Louis, MO), via model 2001 osmotic pumps attached to i.t. catheters. We chose a 1 µg/h infusion dose of anti-NGF, since this dose administered i.t. to naïve rats produces similar changes in sensory neuropeptide levels, as is seen following traumatic nerve injury (Shadiack et al., 2001). IgG was used as a vehicle to determine if there are any non-specific effects of antibody administration.

The effect of acute administration of 3 µg NGF (in a 30 µl volume of ACSF) was also determined in this study to compare with that of chronic infusion. This dose was equivalent to the cumulative daily dose of NGF given by osmotic pumps, and it served as a control for possible behavioural effects of i.t. NGF administration. For morphine antinociception studies, morphine sulfate (Sabex, Mississauga, ON) was prepared in sterile saline and administered at a dose (20 µg/30 µl) that produced maximum analgesia in thermal nociceptive tests in normal animals. LY 225910, a CCK antagonist, (10 nmol/30 µl, Tocris, Ellisville, MO) was prepared in 10% dimethylsulfoxide in saline and administered i.t. to the rats. NGF, morphine and LY 225910, or combinations of these agents, were all administered via lumbar puncture between vertebrae L5 and L6 while the rats were under brief halothane anesthesia.

2.5. Behavioural testing

At various time points rats were tested for mechanical, thermal and cold sensitivity using the following tests.

2.5.1. Mechanical sensitivity

Mechanical response thresholds were quantified by measuring the hind paw withdrawal response to von Frey filament stimulation according to the method described by Chaplan et al. (1994). In brief, animals were placed in a Plexiglas® box ($21 \times 16 \times 27$ cm³) with a wire grid bottom through which the von Frey filaments (Stoelting, Wood Dale, IL) were applied to the plantar surface of the injured hind limb. Filaments were applied in either ascending or descending strength as necessary to determine the filament closest to the threshold of response. The minimum stimulus intensity was 0.25 g and the maximum was 15 g. Based on the response pattern and the force of the final filament, the 50% response threshold (g) was calculated. The resulting pattern of positive and negative responses was tabulated using the convention, X, withdrawal; O, no withdrawal, and the 50% response threshold was interpolated using the formula:

50% g threshold $= (10^{[x_f+k\delta]})/10000$

where x_f = value (in log units) of the final von Frey hair used; k = tabular value (see Chaplan et al., 1994) for

pattern of positive/negative responses; and δ = mean difference (in log units) between stimuli (here 0.224).

2.5.2. Cold sensitivity

Rats were placed on a metal surface in a 1 cm deep, 1 °C water bath for a 75 s period. A response was recorded when the rat lifted its hind paw completely out of the water when not ambulating. The frequency of responses, and the total duration of time the rat kept it's hind paw elevated, within the 75 s period was recorded. Rats received a single exposure to the cold water stimulus at each time point.

2.5.3. Thermal sensitivity

Withdrawal latencies from noxious heat were assessed using the plantar test as previously described by Hargreaves et al. (1988). Rats were placed in Plexiglas® boxes positioned on a glass surface. Animals were allowed to habituate for 15 min before testing. Paw withdrawal latency in response to radiant heat was measured using the plantar test apparatus (Stoelting, Wood Dale, IL). The heat source was positioned under the plantar surface of the affected hind paw and activated at a setting of 25. The digital timer connected to the heat source automatically recorded the response latency for paw withdrawal to the nearest 0.1 s. The intensity of the light beam was chosen to give baseline latencies of 10–12 s in sham rats. A cut off time of 20 s was imposed to prevent tissue damage. Rats were only tested once at each time point.

2.6. Experiment 1

Rats were initially divided into two groups, those that received sciatic nerve constriction or sham surgeries. Behavioural testing was performed prior to sciatic nerve injury, and on days 4, 7, 11 and 14 after nerve injury. On day 7, after behavioural testing, all rats underwent surgery to implant chronic i.t. catheters and osmotic pumps, which were primed so that they immediately started pumping NGF (125 ng/h) or vehicle (1 µl/h). Thus, each of the original two groups was subdivided into two more groups that received either i.t. NGF or vehicle infusion, creating four groups in total: sham-vehicle, sham-NGF, neuropathic-vehicle and neuropathic-NGF. All rats were tested with von Frey hair stimulation to evaluate mechanical response thresholds, a cold water test to determine cold sensitivity, and a radiant heat stimulus (plantar test) to assess withdrawal latencies to noxious heat.

2.7. Experiment 2

(A) On day 14 following sciatic nerve constriction, and following 7 days of NGF or vehicle infusion, separate groups of rats were tested in the various behavioural

paradigms prior to and following i.t. morphine. Morphine (20 µg/30 µl) was administered a minimum of 3 h following the first (pre) testing paradigm. Rats were again tested 30 min following morphine administration.

(B) The effects of *acute* i.t. morphine (20 µg/30 µl), NGF (3 µg/30 µl), or morphine + NGF (30 µl total volume injected for the combination) were also assessed on day 14 after nerve injury in neuropathic rats that had no prior pharmacological treatment. Morphine was again administered a minimum of 3 h following the first (pre) testing paradigm, and rats were tested again 30 min following morphine and/or NGF administration.

2.8. Experiment 3

Following determination of baseline values on the plantar test, rats were divided into two groups that received either chronic infusion of anti-NGF (1 µg/h) or IgG (1 µl/h) (both from Sigma, St Louis, MO) for 7 days. All rats underwent implantation of chronic i.t. catheters and osmotic pumps as above. IgG was used as a control for any occurrence of antibody-induced non-specific effects. Seven days after onset of i.t. infusion, thermal nociceptive latencies were evaluated prior to and following acute i.t. administration of the following pharmacological agents: CCK antagonist, LY 225910 (10 nmol/30 µl) + morphine (5 µg/30 µl), or the vehicle for LY 225910 (30 µl, 10% dimethylsulfoxide in saline) + morphine. As before, drug administration was performed 3 h after the pre-test, and post-drug thermal latencies were determined 30 min after i.t. drug administration.

2.9. Statistical analysis

All data are expressed as means ± S.E.M. Statistical analyses used included parametric tests for continuous data and non-parametric tests for discrete measurements performed using GB-Stat (Dynamic Microsystems, Silver Spring, MD). Statistical analysis on withdrawal latencies to radiant heat and cold water duration were performed using parametric two way analysis of variance followed by the Dunnett's test and student Newman–Keuls test for post hoc comparisons on all time course studies. Statistical analysis on mechanical thresholds and cold water frequency of responses were performed with Friedman ANOVA for repeated non-parametric data followed by Wilcoxon signed-rank test for post hoc comparisons on all time course experiments. Further analysis was performed with a Mann–Whitney *U*-test for independent groups to compare vehicle- and NGF-treated rats for mechanical and cold water frequency measures. Paired *t*-tests (thermal latencies and cold water duration) or Wilcoxon signed-rank tests (von Frey thresholds and cold water frequencies) were also used to evaluate the effectiveness of morphine following chronic or acute administration of i.t. NGF (i.e. pre- versus post-

morphine). Finally, a one way ANOVA followed by Newman–Keuls post hoc test was performed for the plantar test in Experiment 3.

3. Results

3.1. Experiment 1: to determine the effects of continuous delivery of i.t. NGF on nociceptive thresholds in neuropathic and control rats

Fig. 1 illustrates the time course of alterations in sensitivity to mechanical stimulation, radiant heat and cold water, in both sham and nerve constriction groups. Rats with unilateral nerve constriction exhibited significant increases in mechanical, heat and cold sensitivity ipsilateral to the injury as reflected by decreased mechanical thresholds (A) and thermal latencies (B), or increased cold water responses (C and D), at all time points following nerve injury (solid symbols) when compared to baseline values within each testing paradigm. Results of mechanical thresholds indicate a significant main effect of time for the vehicle-treated ($\chi^2(5) = 15.2$, $P < 0.01$) and NGF-treated ($\chi^2(5) = 17.7$, $P < 0.05$) in nerve constriction rats (Fig. 1A). Results of cold water frequency assay indicate a significant main effect of time for the vehicle-treated ($\chi^2(5) = 8.59$, $P < 0.05$) and NGF-treated ($\chi^2(5) = 9.45$, $P < 0.05$) in nerve constriction rats (Fig. 1C). Post hoc comparisons (Wilcoxon signed-rank test) on mechanical thresholds and cold water frequency of responses revealed that compared to baseline, vehicle-treated and NGF-treated rats had significantly lower mechanical thresholds, and an increase in frequency of cold responses, on all test days following sciatic nerve injury, demonstrating the development of tactile allodynia and cold hyperalgesia (Fig. 1A,C). Analysis of cold water duration of responses (Fig. 1D) also demonstrated an effect of time in vehicle- ($F(4,65) = 4.19$, $P < 0.01$) and NGF-treated ($F(4,65) = 3.22$, $P < 0.01$) nerve constricted groups. Analysis of plantar test data revealed a significant decrease in thermal latencies over time for vehicle- ($F(4,65) = 4.56$, $P < 0.01$) and NGF-treated nerve constricted ($F(4,65) = 3.99$, $P < 0.05$) groups. Post hoc comparisons (Dunnett's test) revealed that both nerve constriction groups had significantly lower latencies in the plantar test, and higher cold water duration scores on all test days, compared to baseline, with the exception of day 4 for both groups for cold water duration. In contrast, the mechanical and cold water responses measures, as well as thermal responses, in sham-operated rats, remained unchanged throughout the testing period (hollow symbols). The shaded box indicates the timing of continuous i.t. administration of NGF or vehicle for both neuropathic and sham groups.

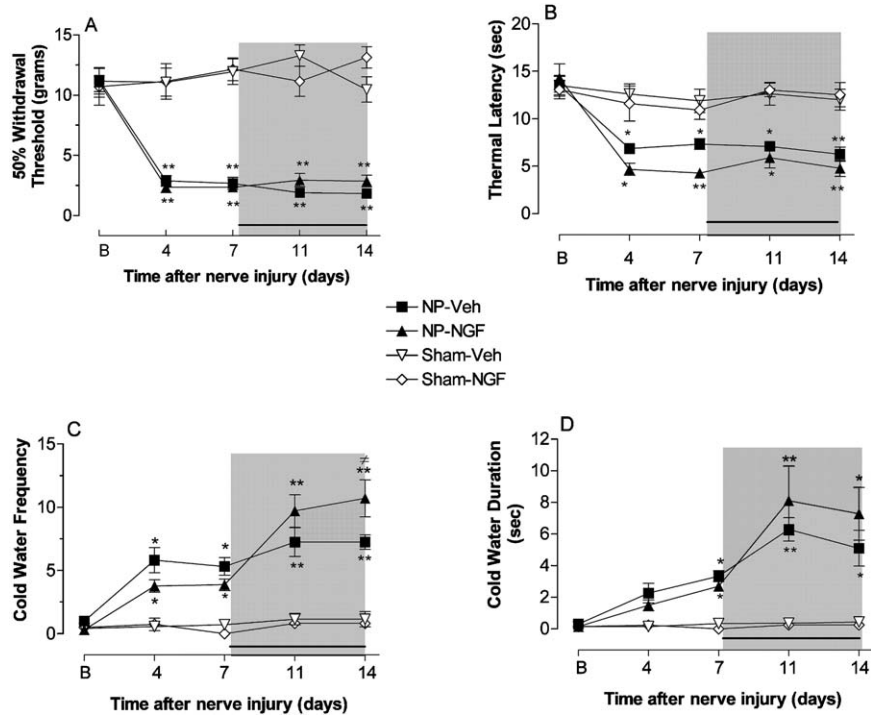It has been well documented that peripheral adminis-

Fig. 1. Effect of chronic administration of i.t. NGF on nociceptive thresholds in sham and nerve-injured rats. Fifty percent von Frey response thresholds (A), withdrawal latencies from radiant heat (B), cold water response frequencies (C) and cold water duration (D) obtained for the hind limb ipsilateral to sciatic nerve constriction of both sham (hollow symbols) and nerve injured animals (solid symbols). The time course of chronic i.t. vehicle (veh) or NGF (125 ng/µl/h) infusion is indicted by the shaded area and the line bar. Values represent means ± S.E.M. for n = 5–9 per group. B refers to baseline scores obtained prior surgery and NP refers to neuropathic injury. Significant differences between baseline values and post nerve injury were evaluated by Wilcoxon signed-rank test for mechanical thresholds and cold water response frequencies and the Dunnett's for withdrawal latencies to radiant heat and cold water response duration. Post hoc analysis revealed a significant decrease from baseline values at all time points following nerve injury for mechanical response threshold and radiant heat latencies and significant increases in cold water frequency and duration, with the exception of day 4 for the cold water duration ($*P < 0.05$, $**P < 0.01$). In addition, a Mann–Whitney U-test revealed a significant difference of NGF compared to vehicle treatment in neuropathic rats on day 14 for cold water frequencies ($\neq P < 0.05$).

tration of NGF induces tactile allodynia and thermal hyperalgesia, therefore, the comparison of response thresholds and latencies between vehicle and NGF-treated groups was important to establish. Further analysis was performed to determine whether i.t. NGF infusion altered response thresholds compared to vehicle-treatment in the sham and nerve constriction groups. Results indicate that i.t. infusion with NGF (125 ng/h) had no significant effect on tactile or heat sensitivity in any of the testing paradigms, when compared to vehicle treatment, for either sham or neuropathic rats. However, compared to vehicle, NGF-treated neuropathic rats had a significantly higher cold water frequency response on day 14 (Fig. 1C) ($P < 0.05$, Mann–Whitney U), although a similar increase was not exhibited for cold water duration in neuropathic rats, or for either cold water response measure in sham-injured rats. In general, i.t. NGF appeared to be well tolerated, as there were no significant differences in grooming behaviour, weight changes or any obvious manifestation of stress.

3.2. Experiment 2A: to determine the effects of continuous administration of NGF on morphine-induced antinociception in neuropathic rats

On day 14 post-nerve injury, the nociceptive responses in each behavioural testing paradigm were evaluated prior to and post i.t. morphine administration. Fig. 2 illustrates the response thresholds to mechanical stimulation (Fig. 2A), withdrawal latencies to noxious heat stimulation (Fig. 2B), and cold water responses (Fig. 2C,D), prior to and 30 min following morphine administration. Statistical analyses demonstrate no significant differences between pre- and post-morphine response values for mechanical thresholds or cold water testing in vehicle- or NGF-treated sham-operated rats. In contrast, i.t. morphine significantly increased thermal thresholds in both vehicle and NGF-treated sham operated rats ($P < 0.05$).

When comparing the responses to pre- versus post-morphine injection in neuropathic rats, morphine was
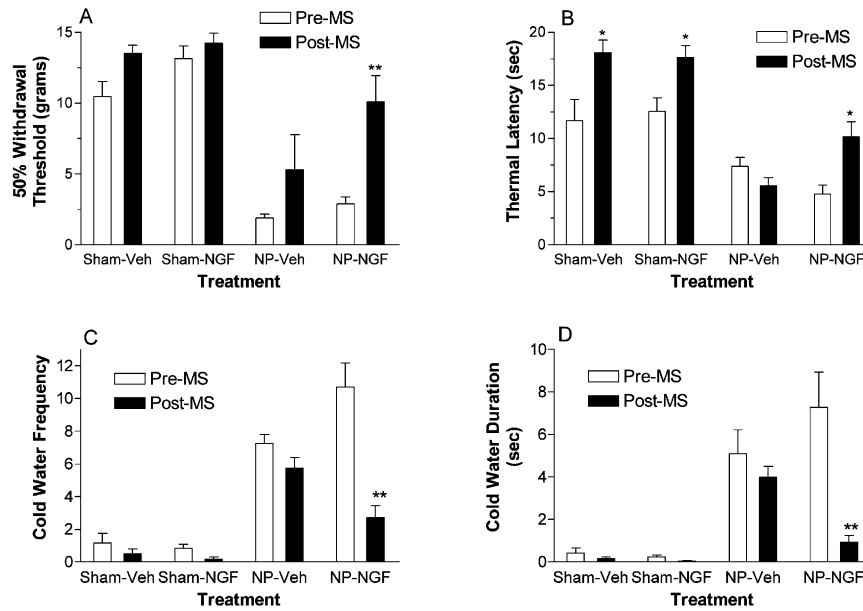
Fig. 2. Anti-allodynic and antinociceptive effects of morphine in NGF- and vehicle-treated rats. Fifty percent von Frey response thresholds (A), withdrawal latencies from radiant heat (B), cold water response frequency (C) and cold water response duration (D) obtained on day 14 following sciatic nerve constriction of both sham and nerve injured animals prior to (open bars) and 30 min following (shaded bars) i.t. morphine (20 µg). NP refers to neuropathic injury and MS abbreviates morphine sulfate. Values represent means ± S.E.M. for n = 5–9 per group. Statistical analysis using a paired t-test and Wilcoxon signed-rank tests revealed that i.t. morphine increased thermal and mechanical thresholds, as well as cold water responses, in NGF-treated neuropathic rats compared to pre-morphine values ($*P < 0.05$, $**P < 0.01$), whereas a similar effect was not observed in vehicle-treated neuropathic rats. Morphine also significantly increased thermal withdrawal latencies in sham-treated rats ($*P < 0.05$).

found to have no significant effect on mechanical, thermal or cold water responses in the vehicle-treated groups, indicating the lack of effectiveness of i.t. morphine to attenuate nerve injury-induced tactile allodynia or thermal hyperalgesia. However, in NGF-treated neuropathic rats, i.t. morphine produced a significant increase in mechanical and thermal thresholds demonstrating the ability of morphine to reverse tactile allodynia and thermal hyperalgesia following chronic NGF treatment. In addition, in NGF-treated neuropathic rats, i.t. morphine produced a significant decrease in both cold water response frequency and cold water response duration, when compared to pre-morphine values, ($P < 0.05$).

3.3. Experiment 2B: to determine the effects of acute administration of NGF on morphine-induced antinociception in neuropathic rats

At this point, it was important to establish whether *acute* administration of NGF, morphine, or the combination of acute NGF and morphine, had any effect on response thresholds in a neuropathic control group. Neither acute i.t. morphine, NGF, nor the combination, had any effect on the thresholds of responses to mechanical, radiant heat or on the frequency or duration of responses to the cold water stimulus in 14 day neuro-

pathic rats (Fig. 3). Thus, there was a lack of effect of acute i.t. NGF or morphine on mechanical allodynia or thermal and cold hyperalgesia in neuropathic rats.

3.4. Experiment 3: to determine whether neutralizing NGF alters morphine-induced antinociception

In the final series of experiments, rats were chronically infused with either anti-NGF or IgG for 7 days via chronically implanted i.t. catheters, to determine whether suppression of NGF altered thermal thresholds. Also, after the 7 days of anti-NGF or IgG infusion, thermal thresholds were evaluated in all rats prior to and following an i.t. injection of either: (1) morphine (5 µg) + the CCK antagonist LY225910 (10 nmol/30 µl), or (2) morphine + vehicle (10% dimethylsulfoxide in saline) for the CCK antagonist. No differences in thermal withdrawal latencies between baseline and following chronic anti-NGF or IgG infusion were evident (data not shown). Statistical analyses were performed to compare the antinociceptive effects produced by i.t. morphine (5 µg) when combined with either vehicle or LY225910 within each immunoglobulin group. There was no significant difference in the thermal antinociception produced by morphine when comparing vehicle to LY225910 in rats chronically treated with IgG. However, LY225910 significantly increased morphine-induced thermal antinoc-
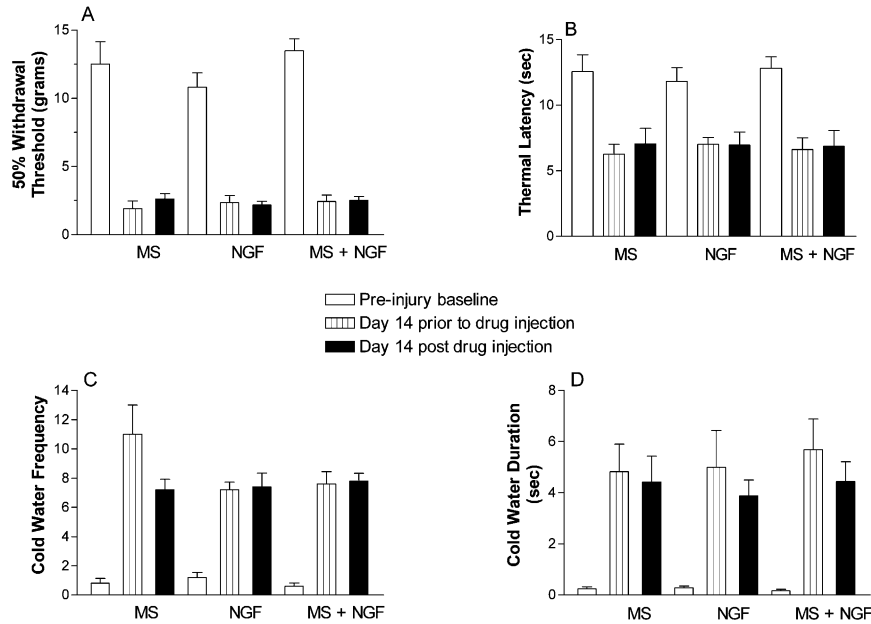
Fig. 3.   Effect of acute administration of NGF and morphine on nociceptive thresholds in neuropathic rats. Fifty percent von Frey response thresholds (A), thermal latencies (B), cold water response frequency (C) and cold water response duration (D) obtained on day 14 following sciatic nerve constriction of nerve-injured animals prior to and 30 min following *acute* i.t. administration of either morphine (20 μg), NGF (3 μg) or morphine (20 μg) + NGF (3 μg) are presented. Values represent means ± S.E.M. for $n = 6$ per group. Statistical comparisons using a paired *t*-test and Wilcoxon signed-rank tests compared response thresholds pre- versus post-drug administration. No significant differences were demonstrated in any of the testing paradigms.

iception compared to vehicle in rats chronically treated with anti-NGF (reflected by increases in % MPE) (Fig. 4).

4. Discussion

The intent of this study was to determine whether exogenous spinal supplementation of i.t. NGF would reverse neuropathic pain symptoms, or alter opioid analgesic effectiveness, in an animal model of neuropathic pain. In the present study, we found that continuous spinal administration of a relatively low dose of NGF did not alter thermal (hot) or mechanical nociceptive thresholds in either sham or neuropathic rats. While cold water response frequency was increased after i.t. NGF infusion in neuropathic rats, a significant effect was only observed on day 14, and was not observed for cold water response duration. This increase was also observed at a time period when cold water responses are generally increased for nerve injured rats, so it is difficult to attribute that effect to NGF infusion alone.

Although there is evidence that peripheral NGF treatment can reduce neuropathic pain in some clinical states (McArthur et al., 2000), it has been consistently shown that peripheral or systemic administration of NGF elicits mechanical and thermal hyperalgesia (Lewin and Mend-
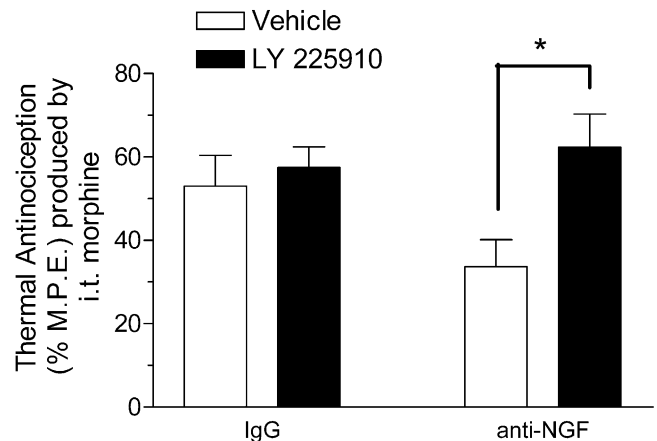

Fig. 4.   Effect of CCK antagonist on morphine-induced antinociception in rats treated with anti-NGF. Percent maximum possible effect (% MPE) of i.t. morphine (5 μg) with or without i.t. CCK receptor antagonist (LY225910, 10 nmol) in rats chronically infused with i.t. IgG or anti-NGF. Statistical analysis using a one-way ANOVA revealed that in anti-NGF-treated rats the MPE of i.t. morphine + LY225910 was significantly higher compared to i.t. morphine + vehicle (*$P < 0.05$).

ell, 1993). In contrast, reports on the effects of spinal administration of NGF on nociception have not been consistent. Thus, i.t. NGF was found to produce thermal hyperalgesia (Malcangio et al., 2000), and acute i.t. administration of a novel NGF receptor antagonist, ALE-0540 dose-dependently reduced mechanical allodynia in a rodent model of neuropathic pain (Owolabi et al., 1999). Conversely, chronic i.t. anti-NGF infusion had minimal effect on mechanical thresholds in a model of neuropathic pain induced by spinal nerve ligation (Deng et al., 2000). In the present study, we did not observe the NGF-induced thermal hyperalgesia as demonstrated by Malcangio et al. (2000). This discrepancy may have occurred because Malcangio et al. (2000) used a dose that was four times higher than the dose we used. However, the dose and delivery method of NGF chosen in the current study did augment one of our measures of cold water responses.

In rats exhibiting nerve injury-induced allodynia and hyperalgesia, i.t. morphine, at the dose (20 μg) chosen in this study, was found to be ineffective in increasing mechanical response thresholds, latencies to radiant heat or sensitivity to cold water, a finding previously reported by many others (Xu and Wiesenfeld-Hallin, 1991; Mao et al., 1995; Xu et al., 1993, 1994). Here we demonstrate for the first time that i.t. NGF infusion restored morphine-induced anti-hyperalgesic and anti-allodynic effects in neuropathic rats. While the mechanism by which NGF may regulate morphine antinociception in this model remains elusive, we propose that NGF supplementation may reduce elevated levels of CCK, an important inhibitory modulator of opioid analgesia, that accompany nerve injury, to pre-injury levels. Hence, changes in CCK levels have been shown to occur following peripheral nerve injury, such that a dramatic up-regulation of CCK-like material and CCK mRNA in the rat DRG has been reported after sciatic nerve transection (Hökfelt et al., 1994; Verge et al., 1993). Indeed, i.t. co-administration of a CCK antagonist and morphine has been found to produce an anti-allodynic action in nerve-injured rats, while i.t. morphine alone was without effect (Nichols et al., 1995). Furthermore, in the flexor-reflex model in spinal rats, systemic morphine exhibited a reduced antinociceptive potency in axotomized compared to normal rats, and administration of a CCK-B antagonist was found to potentiate the effect of morphine in this model (Xu et al., 1994).

Several studies have provided evidence that NGF is involved in neural anatomical and molecular plasticity of primary afferents, and in the regulation of peptides known to be involved in nociceptive pathways (Ma et al., 1995; Verge et al., 1995). Sciatic nerve transection was shown to decrease the density of NGF binding sites on DRG neurons, but i.t. NGF infusion could partially reverse the effects of axotomy on binding sites without altering NGF binding on intact neuronal axons (Verge et al., 1992). Additionally, Verge et al. (1995) demonstrated that chronic i.t. administration of NGF counteracted sciatic nerve injury-induced changes in neuropeptide content, including the induced increase in CCK. Others have proposed that nerve injury-induced increases in CCK may partially mediate the lack of morphine effectiveness, and the supplementation of NGF may help to restore CCK levels to pre-injury levels; thus restoring morphine antinociception.

In the current study, we have also tried to mimic nerve injury-induced increases in CCK levels by neutralizing endogenous levels of NGF with chronic i.t. infusion of antibodies directed against purified 2.5S NGF. Previous studies have demonstrated that anti-NGF treatment produced altered neuropeptide expression (Christensen and Hulsebosch, 1997; Shadiack et al., 2001). We showed here that morphine-induced antinociception was potentiated by i.t. administration of a CCK-B receptor antagonist in anti-NGF rats, but that the same potentiation was not observed in IgG-treated rats. These results draw a positive correlation between the morphine enhancing effects of NGF supplementation in neuropathic rats, and CCK receptor antagonism in anti-NGF-treated rats. Thus, these finding support the hypothesis that the level of spinal NGF may influence morphine sensitivity by affecting CCK, although further studies will be necessary to evaluate the levels of CCK in anti-NGF-treated rats.

In summary, NGF may play a role in the development of chronic pain that results from nerve injury, where changes in NGF expression at the early stages of nerve injury or disease form part of the adaptive response which may inadvertently lead to the development of chronic pain. Conversely, it has been proposed that NGF may at appropriate time, dose, site and mode of administration provide prophylaxis and treatment in conditions that lead to chronic pain (Anand, 1995). In this study, i.t. NGF had minimal effect on neuropathic nociceptive behaviours, however, it also restored the anti-allodynic and anti-hyperalgesic effects of i.t. morphine. Further studies are necessary to evaluate the beneficial effects of spinal administration of NGF for adjuvant treatment of chronic pain.

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