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Attenuation of hyperalgesia in a rat model of neuropathic pain after intrathecal pre- or post-treatment with a neurokinin-1 antagonist

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Abstract

Although many studies have demonstrated a role for substance P in pain, there have been conflicting reports implicating the involvement of substance P in neuropathic pain models. In this study, the non-peptide neurokinin-1 (NK-1) receptor antagonist, L-732,138 was chronically administered by intrathecal (i.t.) injection to rats with mono-neuropathy produced by sciatic nerve constriction. Rats exhibited tactile allodynia and cold hyperalgesia over a 16-day testing period. L-732,138 (5–200 nmol) administered i.t. prior to and for 3 consecutive days post-surgery attenuated the mechanical allodynia and cold hyperalgesia on days 4 and 8 post-surgery. The effects of i.t. L-732,138 were also determined in rats with established nerve injury-induced neuropathy. The NK-1 receptor antagonist was injected for 4 consecutive days starting on day 8 post-sciatic nerve injury. Administration of L-732,138 (5–200 nmol) i.t. produced both anti-allodynic and anti-hyperalgesic effects on day 12, but the effect was not permanent, as nociceptive thresholds were similar to controls by day 16. These results demonstrate that substance P is involved both in the *induction* and the *maintenance* of neuropathic pain and provides justification for the development and administration of substance P antagonists for the management of clinical neuropathic pain. © 2002 International Association for the Study of Pain. Published by Elsevier Science B.V. All rights reserved.

Keywords: Substance P; Neuropathy; Allodynia; Nociception; Analgesia; Sciatic nerve constriction

1. Introduction

Neuropathic pain resulting from nerve injury is a persistent condition characterized by spontaneous pain, allodynia (nociceptive responses to normally innocuous stimuli), and hyperalgesia (increased sensitivity and exaggerated responses to nociceptive stimuli). Although patients sometimes find pain relief with conventional analgesics such as opioids, neuropathic pain is typically poorly managed (Arnér and Meyerson, 1988; Max et al., 1988; Kupers et al., 1991). Moreover, the use of current therapies such as tricyclic anti-depressants is not always well tolerated or adequately effective (McQuay et al., 1996). The development of animal neuropathic pain models has greatly advanced our understanding of the pathophysiology and mechanisms of neuropathic pain states (Bennett and Xie, 1988; Kim and Chung, 1992; Seltzer et al., 1990). One

valid model indicative of neuropathic pain is produced by chronic constriction of the rat sciatic nerve with polyethylene tubing (cuff) (Mosconi and Kruger, 1996). This model induces behavioral characteristics analogous to the model described by Bennett and Xie (1988), including thermal hyperalgesia as well as tactile allodynia, however, the behavioral manifestations produced by the cuff are reportedly less variable (Mosconi and Kruger, 1996).

Injury to a peripheral nerve produces altered peptide expression within the spinal cord and dorsal root ganglia (DRG). Following sciatic nerve injury, many neurochemical changes occur. Accordingly, these changes may include a decrease in the levels of substance P and calcitonin gene related peptide (CGRP), as well as increases in vasoactive intestinal peptide, galanin, neuropeptide Y, and cholecystokinin (CCK) over various times after nerve injury (reviewed by Wiesenfeld-Hallin and Xu, 1996). It has been suggested that alterations in peptide expression may represent adaptive responses that promote survival and recovery of damaged neurons (Hököfelt et al., 1994). However, these changes also may be responsible for

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central sensitization and the precipitation of chronic pain states (reviewed by Coderre and Katz, 1997).

Substance P is the preferred tachykinin at neurokinin-1 (NK-1) receptors and is released into the dorsal horn following noxious stimulation (Duggan et al., 1987; Brodin et al., 1987; McCarron and Goldstein, 1991). Substance P produces thermal hyperalgesia following exogenous application by intrathecal (i.t.) administration, which is blocked by NK-1 receptor antagonists (Yashpal et al., 1993). Moreover, electrophysiological recordings of spinal neurons have demonstrated that iontophoretic application of substance P elicits firing of nociceptive specific neurons (De Koninck and Henry, 1991; Radhakrishnan and Henry, 1991). Reportedly, substance P is not involved in the genesis of acute pain and may only be involved in conditions of persistent pain (Henry, 1993). This has been confirmed by knockout studies whereby pain thresholds are unaffected, but responses to intense noxious stimulation are significantly attenuated, and there is a substantial reduction in hyperalgesia elicited in models of persistent pain (De Felipe et al., 1998; Cao et al., 1998). Furthermore, hyperalgesia was absent in rats treated with toxin-conjugated substance P that results in the selective destruction of lamina I neurons expressing NK-1 receptors (Mantyh et al., 1997; Benoliel et al., 1999).

Although substance P has been reported to be down-regulated in animal models of neuropathic pain (Garrison et al., 1993; Munglani et al., 1995), the number of NK-1 receptors is up-regulated (Aanonsen et al., 1992; Goff et al., 1998), and it has been proposed that these receptors are sensitized. Furthermore, substance P expression is initially enhanced in DRG cells following sciatic nerve injury (Marchand et al., 1994). Evidence that NK-1 receptors may play a vital role in nerve injury-induced pain behaviors has been provided by experiments demonstrating that i.t. CP-96,345 blocks spinal sensitization and attenuates the development of flexor reflex hypersensitivity following axotomy of the sciatic nerve (Luo and Wiesenfeld-Hallin, 1995). Moreover, i.t. substance P caused spinal release of excitatory amino acids in rats with partial nerve ligation (Skilling et al., 1992). Recent studies reported the effectiveness of novel NK-1 receptor antagonists in producing dose-dependent mechanical anti-hyperalgesic or anti-allodynic effects in a model of neuropathic pain in the guinea pig (Campbell et al., 1998; Gonzalez et al., 2000) and rat (Coudoré-Civiale et al., 1998; Gonzalez et al., 2000). Additionally, nerve injury-induced mechanical hyperalgesia was significantly depressed in NK-1 receptor knockout mice compared to wild-type controls (Mansikka et al., 2000).

While these latter reports support a role for NK-1 receptors in behavioral manifestations presumed to reflect neuropathic pain following nerve injury, no study has systematically examined the contribution of NK-1 receptors in the development and maintenance of neuropathic pain behaviors. Since substance P is an integral component of

central sensitization and may be an important mediator of the plastic changes that occur in the dorsal spinal cord after nerve injury, substance P is likely to contribute to the development and/or maintenance of neuropathic pain. In this study, we have demonstrated that chronic administration of an NK-1 receptor antagonist during either the induction or maintenance of neuropathic pain, attenuates cold hyperalgesia and tactile allodynia. Parts of this manuscript have been previously published in abstract form (Cahill and Coderre, 1998).

2. Methods

2.1. Animals

Experiments were performed on male Long Evans hooded rats (300–350 g; Charles River, Quebec, 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 the Clinical Research Institute of Montreal and in accordance with guidelines from the Canadian Council on Animal Care and International Association for the Study of Pain Committee for Research and Ethical Issues.

2.2. Surgical procedures

2.2.1. Sciatic nerve constriction

Sciatic nerve injury was accomplished by the method previously described by Fisher et al. (1998). Briefly, rats were anaesthetized with halothane (2.5%) and the lateral left thigh was shaved and swabbed with 70% ethanol. A skin incision was made in the cleaned area; the sciatic nerve was exposed following blunt dissection and gently freed from adhering tissue. 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 separated muscle was stitched and the incision was closed with wound clips. Animals received topical antibiotic and subcutaneous saline (5 ml). For sham-operated control animals, identical surgical procedures were employed with the exception of dissection and cuffing of the sciatic nerve.

2.3. Behavioral testing

2.3.1. Mechanical allodynia testing

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 × 16 × 27 cm³) with a wire grid bottom through which the von Frey filaments (Stoelting) were applied to the plantar surface of the injured hind paw. Fila-

ments 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:

$$50\% \text{ g threshold} = (10^{[x_f + k\delta]})/10,000$$

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).

2.3.2. Cold water testing

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 latency to the first response, frequency of responses, and the total duration of time the rat kept its hind paw elevated within the 75 s period was recorded.

2.3.3. Paw posture rating

Rats were placed in a Plexiglas® box (30 × 30 × 30 cm³) and habituated for 5 min prior to evaluating the posture of the hind paw ipsilateral to the constriction injury over a 180 s period. The index of nociceptive behavior was assessed based on that described previously by Attal et al. (1990). This was accomplished by scoring the behavior of the animal in terms of a weighted pain intensity scale: 0, flat posture and pressed normally on the floor with equal weight distribution between hind paws; 1, curved posture with favoring of hind paw such that it is resting lightly on the floor; 2, elevation of hind paw; 3, licking of hind paw. A pain score was calculated by using a weighted scoring method as previously described for the formalin test (Coderre et al., 1993). Thus the numerical ratings were calculated by multiplying the amount of time the rats spent in each category by the weighted factor indicated above. This was expressed by the following formula: (time spent in category 0 × 0 + time spent in category 1 × 1 + time spent in category 2 × 2 + time spent in category 3 × 3)/180 s.

2.4. Experimental protocol

Rats were divided into two groups, those that underwent sciatic nerve constriction and those that received only sham surgeries. Administration of L-732,138 (NK-1 antagonist; RBI, Natick, MA, USA) i.t. was accomplished by acute lumbar puncture between L4 and L5, while rats were briefly anesthetized with halothane. The choice of

NK-1 receptor antagonist used in this study was based on the fact that L-732,138 was commercially available and could be delivered in aqueous solution of neutral pH, rather than other commercially available products that require dissolving in dimethylsulfoxide. Moreover, it has the advantage of not antagonizing calcium channels, providing less side effect potential (personal communication, Merck Pharmaceuticals). Analgesic effects were determined by administering various doses of L-732,138 (5–200 nmol) according to two injection schedules: (1) induction phase, 15 min prior to sciatic nerve constriction and then every 24 h for 3 days or (2) maintenance phase, drug was administered on days 8–11 post-surgery. Behavioral testing was performed 2 days prior to sciatic nerve injury and on days 4, 8, 12, and 16 after nerve injury. All rats were tested with von Frey hair stimulation for evaluating mechanical response thresholds and cold sensitivity using a 1°C cold water bath.

An additional group was included to assess the effects of the NK-1 receptor antagonist (200 nmol) following a single injection via lumbar puncture. Rats were tested prior to and at 15 and 30 min after drug administration. Separate groups of rats were used for mechanical and cold water testing as the time course prevented the ability to test both behavioral parameters in the same animals.

2.5. Statistical analysis

All data are expressed as means ± SEM. Statistical analysis on cold water duration and response latencies were performed using repeated measures one way analysis of variance (ANOVA) followed by the Dunnett's test and Fisher's LSD (least squares difference) test for post hoc comparisons on all time course studies. Statistical analysis on mechanical thresholds and cold water frequency of responses were performed using the non-parametric Friedman's repeated measures ANOVA on ranks followed by Wilcoxon signed-rank test for post hoc comparisons on all time course experiments. Statistical analysis for the pain scoring assessed for paw posture was performed using the non-parametric *t*-test followed by the Mann Whitney test for post hoc comparisons.

3. Results

3.1. Induction

Fig. 1 illustrates the time course of alterations in sensitivity to mechanical stimulation in the chronic constriction injury (CCI) rats when the treatment was given during the induction of the neuropathy. Results indicate a significant main effect of time for the vehicle-treated ($\chi^2(7) = 25.2$, $P < 0.01$) and L-732,138-treated ($\chi^2(7) = 7.73$, $P < 0.05$) CCI rats. Post hoc comparisons (Wilcoxon signed-rank test) revealed that compared to baseline, vehicle-treated rats had significantly lower mechanical thresholds on all

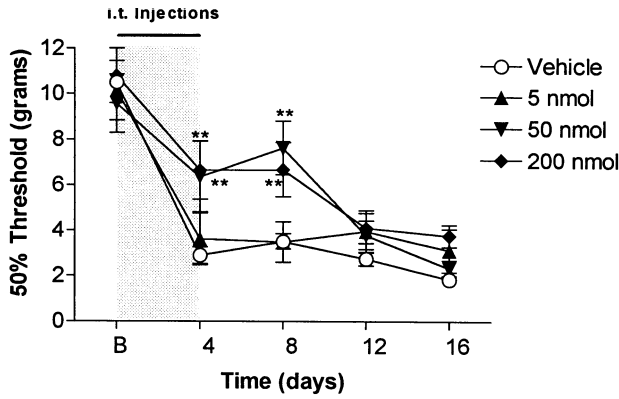


Fig. 1. Dose- and time-related anti-allodynic effects of i.t. NK-1 receptor antagonist L-732,138 for mechanical response thresholds assessed by von Frey hairs. The shaded area represents the time course of drug administration (*induction*). All data are expressed as means \pm SEM for $n = 6-8$ per group. Post hoc analysis for mechanical thresholds (Wilcoxon signed-rank sum) indicate significant difference from vehicle-treated groups on appropriate days (** $P < 0.01$).

test days following sciatic nerve injury (Fig. 1) demonstrating the development of tactile allodynia. However, L-732,138-treated animals exhibited significantly lower response thresholds only on day 12 compared to baseline responses, suggesting that the NK-1 antagonist delayed the development of tactile allodynia up to at least day 8 post-surgery, 4 days after the treatment had ended.

Cold hyperalgesic effects produced by sciatic nerve constriction following four consecutive daily injections of i.t. L-732,138 were assessed. Rats with unilateral nerve constriction exhibited significant increases in cold sensitivity ipsilateral to the injury as reflected by the increased response frequencies (Fig. 2A), and duration of response (Fig. 2B), at all time points following nerve injury when compared to baseline values. Fig. 2 also illustrates the effects of L-732,138 utilizing two testing parameters of the cold water test. Analysis of the cold water response frequencies (Fig. 2A) recorded for the ipsilateral hind paw of nerve-injured rats indicated a significant effect of time ($\chi^2(7) = 11.40$, $P < 0.05$) and treatment ($\chi^2(7) = 7.94$, $P < 0.05$). Subsequent post hoc analysis revealed significant anti-hyperalgesic effects with the 50 and 200 nmol doses on days 4, 8, and 12 post-surgery. Significant differences in cold water duration (Fig. 3B) were also demonstrated, where ANOVA analysis revealed an effect of time ($F(4, 70) = 4.21$, $P < 0.01$) and treatment ($F(4, 70) = 3.21$, $P < 0.05$). Post hoc comparisons revealed significant differences between vehicle and drug treatment (50 and 200 nmol) on individual days up to day 12, 8 days after treatment ended, suggesting that early treatment with the NK-1 receptor antagonist attenuates the development of cold hyperalgesia. Paw posture ratings were also significantly improved on days 4, 8, and 12 in the L-732,138 treatment groups (50 and 200 nmol) compared to control (data not shown).

In contrast, mechanical and cold sensitivity in sham-operated rats remained unchanged throughout the testing period (data not shown). L-732,138 i.t. appeared to be well tolerated as there were no significant differences in grooming behavior, weight changes or any obvious manifestation of stress, and the drug had no analgesic effects in sham-operated rats. Importantly, i.t. administration of L-732,138 during either the induction phase or the maintenance phase had no effect on either mechanical or cold thermal thresholds in sham-surgery rats (data not shown).

3.2. Maintenance

Fig. 3 illustrates the time course of alterations in sensitivity to mechanical stimulation in the CCI rats prior to and following i.t. administration of L-732,138 when the treatment was given after the neuropathy was established. Mechanical response thresholds were determined in the

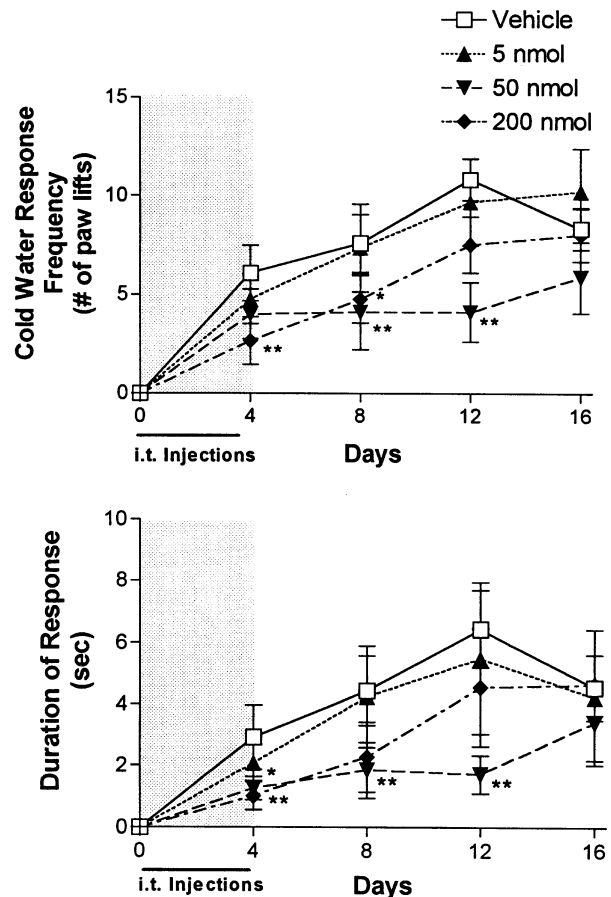


Fig. 2. Dose- and time-related anti-hyperalgesic effects of i.t. NK-1 receptor antagonist L-732,138 in the cold water test. The shaded area represents the time course of drug administration (*induction*). All data are expressed as means \pm SEM for $n = 6-8$ per group. Post hoc analysis for frequency of responses (Wilcoxon signed-rank sum) and duration of response (Fisher LSD) indicate significant difference from vehicle-treated groups on appropriate days (* $P < 0.05$, ** $P < 0.01$).

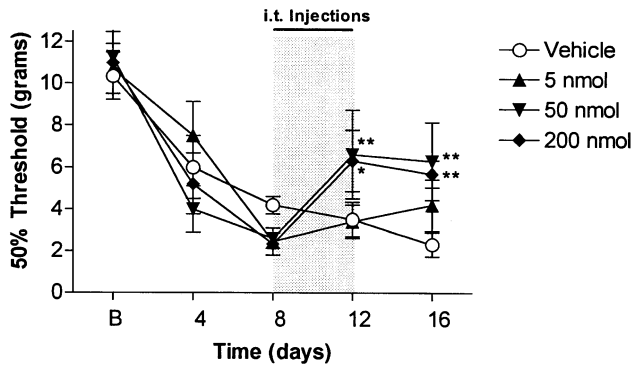


Fig. 3. Dose- and time-related anti-allodynic effects of i.t. NK-1 receptor antagonist L-732,138 for mechanical response thresholds assessed by von Frey hairs. The shaded area represents the time course of drug administration (maintenance). All data are expressed as means ± SEM for n = 6–8 per group. Post hoc analysis for mechanical thresholds (Wilcoxon signed rank sum) indicate significant difference from vehicle-treated groups on appropriate days (*P < 0.05, **P < 0.01).

absence of any pharmacological treatment on days 4 and 8 following nerve injury. Mechanical thresholds were significantly lower than baseline on days 4 and 8 post-CCI for all groups. Following testing on day 8, i.t. L-732,138 was

administered every 24 h for the next 4 consecutive days. Post-treatment testing was performed on days 12 and 16 following nerve injury. Results indicate a significant main effect of time for the vehicle-treated ($\chi^2(7) = 24.3, P < 0.01$) and L-732,138-treated ($\chi^2(7) = 8.21, P < 0.05$) CCI rats. Post hoc comparisons (Wilcoxon signed-rank test) revealed that compared to baseline, vehicle-treated rats had significantly lower mechanical thresholds on days 12 and 16 following sciatic nerve injury (Fig. 3). In contrast, L-732,138-treated animals (50 and 200 nmol doses) did not exhibit significantly lower response thresholds on days 12 and 16 compared to baseline responses suggesting that the NK-1 antagonist attenuated the occurrence of tactile allodynia after commencement of treatment.

Cold hyperalgesia was assessed in rats receiving i.t. L-732,138 for 4 consecutive days after the neuropathy was established. Rats with unilateral nerve constriction exhibited significant increases in cold sensitivity ipsilateral to the injury as reflected by the increased latency to first response (Fig. 4A) and the duration of response (Fig. 4B) on days 4 and 8 following nerve injury when compared to baseline values within each testing paradigm. Fig. 4 also illustrates the effects of L-732,138 utilizing two testing parameters of the cold water test. Analysis of the cold water response latencies (Fig. 4A) recorded for the ipsilateral hind paw of nerve-injured rats indicated a significant effect of time ($F(4, 70) = 4.05, P < 0.05$) and treatment ($F(4, 70) = 3.83, P < 0.05$). Subsequent post hoc analysis revealed significant effects with the 50 and 200 nmol doses on day 12 and a significant effect with the 200 nmol dose on day 16 post-surgery when compared to vehicle. Significant differ-

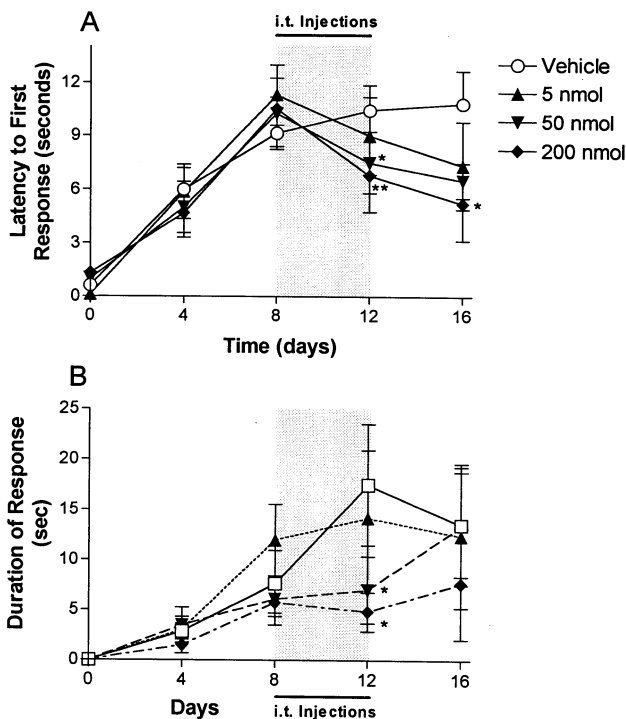


Fig. 4. Dose- and time-related anti-hyperalgesic effects of i.t. NK-1 receptor antagonist L-732,138 for the cold water test. The shaded area represents the time course of drug administration (maintenance). All data are expressed as means ± SEM for n = 6–8 per group. Post hoc analysis for latency to first response and duration of response (Fisher LSD) indicate significant difference from vehicle-treated groups on appropriate days (*P < 0.05, **P < 0.01).

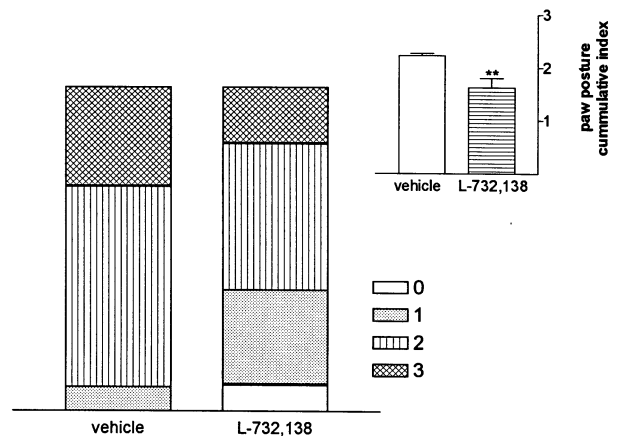


Fig. 5. Index of spontaneous nociceptive behavior evaluated over a period of 15 min on day 16 post-sciatic nerve constriction injury for both vehicle and L-732,138 (200 nmol). The large bar graph represents the ratio of the amount of time spent in each of the four categories over a 180 s testing period. The insert bar graph demonstrates the relative paw posture rating based on a cumulative index scoring method (see Section 2 for description). Values represent means ± SEM for n = 6–8 per group. Post hoc analysis for pain ratings (Mann Whitney) indicate significant difference from vehicle-treated groups (*P < 0.05).

ences in cold water duration (Fig. 5B) were also demonstrated, where ANOVA analysis revealed an effect of time ($F(4, 70) = 3.94, P < 0.05$) and treatment ($F(4, 70) = 3.33, P < 0.05$). Post hoc comparisons revealed significant differences between vehicle and drug treatment (50 and 200 nmol) on day 12. These results suggest that late treatment with the NK-1 receptor antagonist can attenuate cold hyperalgesia once it has been fully established. Finally, paw posture ratings were also significantly improved on day 12 in the L-732,138 treatment groups (50 and 200 nmol) compared to control (data not shown).

3.3. Acute administration

The effects of acute administration of L-732,138 were also determined on days 4, 8, 12, and 16 post-sciatic nerve constriction. L-732,138 i.t. (200 nmol) had no effect on either mechanical response thresholds or cold water response parameters (data not shown). However, acute administration of L-732,138 significantly improved neuropathic paw posture rating. On post-operative day 16 following sciatic nerve constriction, all rats exhibited abnormal ipsilateral hind paw posture. The index of nociceptive behavior for rats treated with either i.t. L-732,138 (200 nmol) or vehicle is illustrated in Fig. 5. Results indicate that there is a significant effect of treatment which was revealed by post hoc comparisons (Mann Whitney) indicating that the drug-treated group showed significantly less nociception compared to rats that were injected with vehicle. There was no change in paw posture in sham rats (data not shown).

4. Discussion

In this study, we demonstrate that NK-1 receptors are involved in the development of tactile allodynia and cold hyperalgesia induced by sciatic nerve constriction. However, antagonism of these receptors during the induction phase does not prevent the neuropathic pain from developing, rather it delays its onset. In addition, prolonged but not acute administration of an NK-1 receptor antagonist alleviates nociceptive behaviors associated with neuropathic injury after the neuropathy is established. These results demonstrate that NK-1 receptors are functionally important for the development and maintenance of mechanical allodynia and cold hyperalgesia associated with nerve injury. The lack of effect of L-732,138 in the cold water test in sham animals suggests that substance P and the NK-1 receptor are not involved in acute nociceptive mechanisms; as previously suggested by others (Fleetwood-Walker et al., 1990; Henry, 1993).

Results from this study demonstrate that not only are NK-1 receptors involved in stimuli-induced hyperalgesia and allodynia but they are also important in one of the most common features associated with clinical neuropathic pain; spontaneous pain. Thus, the spontaneous behavior

exhibited by the rats injected with an NK-1 receptor antagonist was attenuated compared to those rats injected with vehicle. It could be argued that although the behavior is classified as spontaneous, the floor of the testing apparatus may be considered pain-inducing and reflect mechanical allodynia. However, the assessment of the nociceptive index is based on the fact that the rat's behavior is observed without intervention from the experimenter in contrast to the other tests used as previously validated by Attal et al. (1990).

Our results are also in agreement with other reports implicating NK-1 receptors in neuropathic pain behaviors. Thus, systemic administration of the NK-1 receptor antagonist GR205171 blocked the receptive field expansion of dorsal horns and attenuated mechanical allodynia induced by CCI (Cumberbatch et al., 1998). Moreover, Coudoré-Civiale et al. (1998) demonstrated that i.t. administration of the NK-1 receptor antagonist SR-48,968 reduces mechanically induced vocalizations in both CCI and diabetic rats. Similarly, other studies demonstrated anti-allodynic and anti-hyperalgesic activity of NK-1 receptor antagonists in an animal model of neuropathic pain (Campbell et al., 1998; Gonzalez et al., 2000). In these latter studies, the drug administration was employed after the onset of the neuropathy, whereas in our study the NK-1 antagonist was administered either before or after onset to compare its effects on both induction and maintenance of neuropathic pain; both of which were differentially affected by the drug treatment.

Our results are, however, inconsistent with those of Yamamoto and Yaksh (1992) who concluded that the NK-1 antagonist CP-96,345 did not influence heat hyperalgesia induced by CCI. This inconsistency may reflect their assessment of heat, rather than mechanical allodynia, or cold hyperalgesia, assessed in the present study. However, it should be noted that the NK-1 antagonist was able to significantly elevate withdrawal latencies in this latter study, an observation that was overlooked since the ipsilateral–contralateral differences scores were similar for drug-treated and control rats. Our results also do not support the results of Malcangio and Tomlinson (1998) who reported that the NK-1 antagonist RP 67580 was not effective at reversing mechanical hyperalgesia in rats with diabetic neuropathy. These differences may reflect alternate mechanisms that underlie the pathophysiology in CCI compared to diabetic neuropathy.

The functional importance of NK-1 receptors in neuropathic pain has been previously suggested by the use of toxins conjugated to substance P (Benoliel et al., 1999; Nichols et al., 1999). In these latter studies, mechanical allodynia following sciatic nerve constriction injury was attenuated in rats with cytotoxic injury to spinal cord NK-1 receptor expressing neurons. However, the contribution of other neurotransmitter systems that were co-expressed in these cells to the incidence of neuropathic pain behaviors cannot be disregarded. Additionally, studies in knockout

animals have not confirmed or disproved the contribution of NK-1 receptors in neuropathic pain since they have reported conflicting evidence for the role of tachykinins. Thus, mechanical hyperalgesia was shown to be significantly attenuated in NK-1 receptor knockout mice compared to wild-type controls (Mansikka et al., 2000), whereas Cao et al. (1998) failed to observe a difference in the mechanical allodynia that ensued following nerve injury in substance P/neurokinin A knockout compared to wild-type litter mates. Taken together, controversy still remains as to the physiological importance of substance P and its receptor in neuropathic pain and more careful analysis of the existing literature may be required to explain difference in experimental outcomes, such as genetic variability, compensatory developmental changes, and differences between animal neuropathic models.

Substance P has been demonstrated to facilitate transmission in the spinal cord dorsal horn (Henry, 1979; Wiesenfeld-Hallin, 1986). It has been proposed that substance P is the main excitatory sensory neuropeptide of primary afferent C-fibers and contributes to central sensitization in the spinal cord dorsal horn. Substance P is found in small diameter primary afferents and is released into the dorsal horn of the spinal cord following noxious stimulation (Duggan and Hendry, 1986). A role for substance P in neuropathic pain is implicated by studies demonstrating alterations in substance P-like immunoreactivity following nerve injury. CCI has been found to produce decreases in substance P-like immunoreactivity in the ipsilateral spinal cord dorsal horn (Cameron et al., 1991; Garrison et al., 1993; Xu et al., 1996) and DRG (Munglani et al., 1995) for up to 60 days following the nerve injury, although this effect is dependent on the type of ligatures used (Xu et al., 1996). Nerve injury can also lead to increases in substance P mRNA in the ipsilateral spinal cord dorsal horn (Delander et al., 1997), as well as increases in the level of pre-protachykinin A mRNA, a precursor of substance P, in the DRG (Marchand et al., 1994; Noguchi et al., 1994). This latter increase may reflect alterations in neuronal phenotypes, since the increase in pre-protachykinin A mRNA is dependent on the initiation of expression in large diameter A-fibers in the DRG which normally do not express substance P (Marchand et al., 1994; Noguchi et al., 1994). The reported reductions in SP-like immunoreactivity and increases in substance P and pre-protachykinin A mRNA in DRG and spinal cord following nerve injury suggest that there may be an increase in substance P utilization and turnover associated with neuropathic pain. A nerve-injury enhanced release of substance P is suggested by the finding that K^+ -evoked release of substance P is augmented in spinal cord slices taken from rats with diabetic neuropathy (Kamei et al., 1990). It has also been hypothesized that there is an increased spinal sensitivity to substance P after nerve injury, which permits even low levels of substance P to drive the nociceptive system

(Kuwahara et al., 1987; Skilling et al., 1992). This conclusion is supported by the observation that NK-1 receptors are increased following nerve injury (Aanonsen et al., 1992). Furthermore, Cameron et al. (1997) have reported a significant correlation between thermal allodynia and maximal changes in substance P and NK-1 receptor expression.

We propose that NK-1 receptor antagonists may at appropriate time, dose, site, and mode of administration prove beneficial for pain relief in conditions that lead to chronic pain and that spinal administration of NK-1 receptor antagonists may ameliorate the chronic pain associated with traumatic nerve injury.

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