

Antiallodynic Effects of Systemic and Intrathecal Morphine in the Spared Nerve Injury Model of Neuropathic Pain in Rats

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Background: The efficacy of opioids for neuropathic pain remains controversial. The effects of morphine on pain behavior were investigated in two animal models of neuropathic pain: the spared nerve injury (SNI) model and the spinal nerve ligation (SNL) model.

Methods: Nerve injuries were created in rats either by tight ligation and section of the left tibial and common peroneal nerves (SNI) or by unilateral ligation of L5 and L6 spinal nerves (SNL). Paw withdrawal threshold to mechanical stimuli was measured using the up-down method in the hairy and glabrous skin territories of the sural nerve for SNI rats or in the mid-plantar paw of SNL rats.

Results: Before SNI, the median paw withdrawal thresholds in hairy and glabrous skin were similar (26 g [25%, 75% quartiles: 26, 26 g]). The paw withdrawal threshold decreased after SNI in both hairy and glabrous skin ($P < 0.001$). Thirty days after the SNI, the threshold in hairy skin (0.3 g) was significantly lower than in glabrous skin (1.9 g; $P < 0.001$). In blinded experiments, both subcutaneous and intrathecal morphine (0.1–10 μ g) dose-dependently attenuated mechanical allodynia induced by SNI measured in the hairy skin, an effect that was naloxone reversible. The ED_{50} for the intrathecal morphine was 0.52 μ g (95% confidence interval, 0.31–0.90 μ g). Morphine (1 μ g intrathecal) attenuated SNI-induced mechanical allodynia in glabrous skin with potency similar to that in hairy skin. In SNL rats, morphine (30 μ g intrathecal) almost completely reversed the SNL-induced mechanical allodynia.

Conclusions: (1) SNI-induced mechanical allodynia is characterized by a lower paw withdrawal threshold in hairy versus glabrous skin; (2) systemic and intrathecal morphine reverse SNI-induced mechanical allodynia in a dose-dependent fashion; and (3) intrathecal morphine also reverses SNL-induced mechanical allodynia. These results suggest that intrathecal opioids are likely to be effective in the treatment of neuropathic pain.

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NEUROPATHIC pain is a devastating consequence of injury or diseases of the peripheral or central nervous system.^{1,2} It is associated with severe, chronic sensory disturbances characterized by spontaneous pain, increased responsiveness to painful stimuli (hyperalgesia), and pain perceived in response to normally innocuous stimuli (allodynia). The mechanisms underlying neuropathic pain are not fully understood. Neuropathic pain is particularly difficult to treat, and conflicting data exist regarding the effectiveness of opioids.^{3–13} For example, opioids were reported to be ineffective in a group of patients with neuropathic pain,³ whereas other observations suggest that opioids are effective in attenuating neuropathic pain.^{4,6,13–15} Intrathecal administration of opioids has become a popular method of pain control in patients with pain of malignant origin, but intrathecal opioids have been less widely used in patients with chronic neuropathic pain, partly because of conflicting reports of its efficacy in neuropathic pain.

In an attempt to clarify the mechanisms of pain and associated processes after nerve injury, animal models of chronic neuropathic pain have been developed over the past two decades.^{16–20} However, conflicting results regarding the efficacy of opioids on neuropathic pain in different neuropathic animal models have been reported. For example, in the chronic constriction injury model, systemic or intrathecal administration of opioids effectively attenuated neuropathic pain behaviors.^{21–29} However, in the spinal nerve ligation (SNL) model, administration of morphine systemically or intracerebroventricularly attenuated pain behaviors, but intrathecal administration of morphine had less effect on mechanical allodynia.^{30,31} In contrast, intrathecal morphine dose-dependently reversed mechanical allodynia in a rat model of central pain, whereas systemic morphine had little effect on this measure.³² These results indicate the efficacy of opioids in neuropathic pain is variable and seems to depend on several factors (e.g., the kind of nerve injury and the route of drug administration). However, no clear pattern has emerged.

Recently, a new neuropathic pain model, termed the *spared nerve injury (SNI) model*, was established in rats.³³ The SNI model involves tightly ligating and cutting two of the three terminal branches of the sciatic nerve (tibial and common peroneal nerves), leaving the remaining sural nerve intact. After the SNI, rats develop mechanical allodynia in the lateral side of the hind paw ipsilateral to nerve injury (sural nerve territory).

Mechanical allodynia represents an important clinical

sign of neuropathic pain that is difficult to treat with currently available therapies, and intrathecal opioids offer promise because of the low doses used and the decreased incidence of side effects. Therefore, we investigated the effects of systemic and intrathecal morphine on mechanical allodynia after SNI in the current study. Because a recent observation showed that intrathecal morphine resulted in significant antiallodynic effect in an SNL model,³⁴ which was in contrast to previous reports,^{30,31} we also examined the effects of intrathecal morphine on the allodynia in animals after SNL. We postulated that the discrepancies between our observation of an antiallodynic effect of morphine in the neuropathic pain models and the lack of effect reported in earlier studies^{30,31} could result from methodologic differences in catheterization technique or the site of testing. Hence, we examined whether the effects of morphine varies with skin type (hairy *vs.* glabrous). In addition, we tested whether the effect of morphine varies with the duration of spinal catheterization.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing 200–250 g were used. Rats were placed in plastic cages with sawdust bedding and were housed in a climate-controlled room under a 14 h–10 h light–dark cycle. The Johns Hopkins University Animal Care and Use Committee (Baltimore, Maryland) approved the experimental protocol. Experiments adhered to the guidelines for animal experimentation of the International Association for the Study of Pain.³⁵

Surgical Procedures for Producing the Neuropathic Pain Models

The SNI Model. The SNI surgery was performed on the left hind limb as previously reported.³³ Briefly, under isoflurane anesthesia (3% for induction, 2% for maintenance), the sciatic nerve and its three terminal branches (sural, common peroneal, and tibial) were exposed at the mid-thigh. Just distal to the trifurcation of the sciatic nerve, the common peroneal and tibial nerves were tightly ligated with 6-0 silk sutures and transected distal to the ligation. Approximately 2–3 mm of the distal nerve stump was then excised. Care was taken to avoid damage to the sural nerve. Muscle layers were closed using 4-0 chromic gut, and the skin incision was closed with wound staples.

The SNL Model. The left L5 and L6 spinal nerves were tightly ligated as previously reported.¹⁷ Briefly, under isoflurane anesthesia (3% for induction, 2% for maintenance), an incision was made above the lumbar spine, and the transverse process of vertebra L6 was exposed. After this process was carefully removed, the L5 and L6 spinal nerves were exposed and tightly ligated using 6-0 silk sutures. Muscle layers were closed using 4-0 chromic gut, and the skin incision was closed with wound staples.

Intrathecal Catheterization

The method for intrathecal catheterization is modified from that previously reported.³⁶ Briefly, 30 days after the SNI or spinal nerve injury, the rats were anesthetized with isoflurane. A 5-mm longitudinal incision was made through the skin a few millimeters lateral to the midline and 10–15 mm caudal to a line between the two anterior iliac spines. A guide cannula (20 gauge, 0.9×38 mm) was inserted through the incision and between the L5 and L6 vertebra into the intrathecal space. The correct intrathecal localization was confirmed by a tail flick or a paw retraction. A PE-10 tube (length, 28 cm) was inserted through the guide cannula until 3 cm extended beyond the tip of the guide cannula. The guide cannula was then carefully removed, avoiding displacement of the catheter. The catheter was tied in a loose knot and sutured on the back under the skin. The external end of the tube was passed subcutaneously and secured to the back of the neck. The external end of the tube was closed by fire flare. After all the other behavioral experiments were completed, 10 μ l lidocaine (2%) was administered to confirm whether the catheter was in the correct position. Lidocaine induces a transient paralysis of the hind paws when injected into the lumbar enlargement; if paralysis did not occur within 5 min, data from the rat were excluded from the study.

Behavioral Tests

Behavioral testing was performed by experimenters blinded to drugs being administered. Rats were placed under clear plastic boxes above a wire mesh floor, which allowed full access to the paws. Behavioral acclimation was allowed for at least 30 min. Mechanical paw withdrawal thresholds (PWTs) were measured with the up–down testing paradigm.^{37,38} von Frey hairs in log increments of force (0.42, 0.74, 1.2, 2.1, 3.5, 6.0, 9.3, and 15.8 g) were applied for a duration of 7 s to the lateral side of the hind paw (glabrous or hairy skin) in SNI rats or to the mid-plantar paw in SNL rats. To prevent the von Frey hair from sliding from the lateral hairy skin, the tip of the von Frey hair was targeted just below the lateral border of the metatarsal bone. The 2.1-g stimulus was applied first. Whenever a withdrawal response to a given probe occurred, the next smaller von Frey probe was applied. Whenever a negative response occurred, the next higher von Frey probe was applied. The test continued until (1) the responses of four more stimuli after the first change in response had been obtained or (2) the upper/lower end of the von Frey hair was reached. The 50% PWT values were derived according to the method described by Chaplan.³⁷ If the animal showed no response to any of the von Frey hairs, a value of 26 g, corresponding to the next log increment in potential von Frey hairs, was assigned as the threshold.³⁹

Drugs and Drug Administration

Morphine sulfate was purchased from Abbott Laboratories (North Chicago, IL). All drugs for intrathecal injection were preservative-free. Under gentle restraint, intrathecal drug injections were performed using a 50- μ l Hamilton syringe. The saline or morphine injection (10 μ l) was followed by a 12- μ l saline flush (dead space of 28-cm PE-10 tube). All pharmacologic experiments using intrathecal injection were performed 1 day after catheterization except when stated otherwise. Systemic morphine, naloxone, or saline was administered by subcutaneous or intraperitoneal injection in a volume of 250 μ l.

A cumulative drug-dosing regimen was used in the experiment investigating the antiallodynic effects of systemic morphine (1, 3, and 10 mg/kg subcutaneous) in the SNI model. The interval for drug injection was 40 min. Behavioral responses to mechanical stimuli were tested 30 min after the injection of each drug dose. To maintain blinding, the control animals received three injections of normal saline.

Statistical Analysis

Paw withdrawal thresholds are presented as median (25%, 75% quartiles). Response to drug therapy as measured by PWT was calculated using the percent maximum possible effect (%MPE) using the following equation: $\%MPE = ([\text{Measured Value}] - [\text{Pretreatment Value}]) \times 100 / (26 - [\text{Pretreatment Value}])$, wherein 26 is the ceiling of measurement of PWT. %MPE is presented as mean \pm SD. A similar analysis of PWT has been used by others.^{30,31} Sigmoidal nonlinear regression curve fitting for dose-response data and estimation of ED_{50} was performed using GraphPad Prism 3 software (GraphPad Software, San Diego, CA). The average %MPE during the peak effect of morphine (30 and 45 min after intrathecal injection) was used in the calculation of ED_{50} of morphine. ED_{50} is presented as mean (95% confidence interval [CI]). Parametric and nonparametric analyses were used where appropriate. Wilcoxon matched pairs, Friedman test with Dunn *post hoc* test, two-way repeated-measures analysis of variance, and *t* tests were used. All statistical analyses were performed using the statistical software package, STATISTICA 6, StatSoft, Inc. (Tulsa, OK). $P < 0.05$ was considered significant.

Results

Establishment of Neuropathic Pain

Before the SNI, the median PWT (26 g [25%, 75% quartiles: 26, 26 g]) measured in the hairy skin of sural nerve territory was not significantly different from that (26 [26, 26] g) in glabrous skin ($P > 0.05$, Wilcoxon matched pairs, $n = 16$). The PWT significantly decreased after SNI both in hairy skin ($P < 0.001$, Friedman test with Dunn *post hoc* test) and glabrous skin ($P < 0.001$, Friedman test with Dunn *post hoc* test) (data not

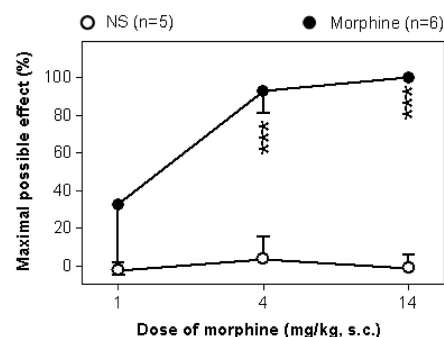


Fig. 1. Effect of subcutaneous morphine administration on the mechanical allodynia measured on hairy skin in the spared nerve injury model. Data are presented as percent maximum possible effect \pm SD. *** $P < 0.001$ versus normal saline (NS). s.c. = subcutaneous.

shown). The PWT in hairy skin (0.33 [0.21–1.91] g) 30 days after SNI was significantly lower than that in glabrous skin (1.85 [0.59–3.5] g) ($P < 0.001$, Wilcoxon matched pairs, $n = 16$).

Similarly, the PWT measured in the mid-plantar paw ipsilateral to L5 and L6 SNL dropped from 26 (26, 26) g before the injury to 2.7 (1.47, 3.51) g 30 days after the injury ($P < 0.001$, Wilcoxon matched pairs, $n = 10$), indicating rats with SNL also developed mechanical allodynia.

Rats with PWTs greater than 4.0 g after SNI ($n = 3$) or SNL ($n = 2$) were excluded from further experimentation.

Antiallodynic Effect of Systemic Morphine in SNI Model

Eleven rats with mechanical allodynia 30 days after SNI were randomly assigned to two groups. One group was given morphine using the cumulative dose regimen described in the Materials and Methods section, and the other was given repeated injection of normal saline as vehicle. Compared with normal saline, systemic morphine dose-dependently reversed mechanical allodynia (group main effect: $F_{1,9} = 85.60$, $P < 0.001$; dose main effect: $F_{2,18} = 15.33$, $P < 0.001$; fig. 1). The ED_{50} of subcutaneous morphine is 1.2 g (95% CI, 0.6–2.3 g).

Antiallodynic Effect of Intrathecal Morphine in SNI Model

Thirty-two rats with mechanical allodynia measured in hairy skin were randomly assigned to six groups. After baseline measurement of the PWT, normal saline or morphine (0.1, 0.3, 0.6, 1.0, or 10 μ g) was administered intrathecally, and then the PWT was measured every 15 min. Compared with the normal saline group, morphine dose-dependently reversed the mechanical allodynia induced by SNI (group main effect: $F_{5,25} = 50.71$, $P < 0.001$; time main effect: $F_{7,175} = 19.33$, $P < 0.001$; fig. 2A). The ED_{50} of intrathecal morphine is 0.52 μ g (95% CI, 0.31–0.90 μ g; fig. 2B).

To investigate whether the antiallodynic effects of in-

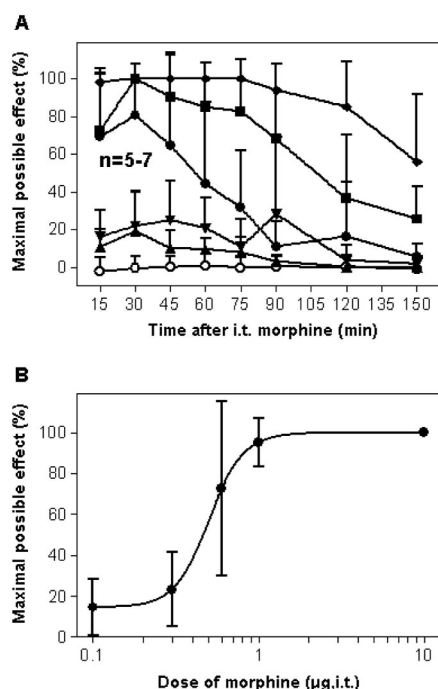


Fig. 2. Effect of intrathecal (i.t.) morphine administration on the mechanical allodynia measured on hairy skin in the spared nerve injury model. (A) Time course of analgesia is plotted for different drug doses: open circles = normal saline; triangles = 0.1 µg morphine; inverted triangles = 0.3 µg morphine; filled circles = 0.6 µg morphine; squares = 1.0 µg morphine; diamonds = 10 µg morphine. Repeated two-way analysis of variance showed that intrathecal morphine produced a dose-dependent and significant reversal of mechanical allodynia compared with normal saline. (B) Dose-response curve: $ED_{50} = 0.52$ µg (95% confidence interval, 0.31–0.90 µg).

trathecal morphine in the SNI model is mediated by opioid receptors, 13 rats with mechanical allodynia measured in hairy skin were randomly assigned to two groups. After baseline measurement of the PWT, morphine (1.0 µg) was administered intrathecal, and then the PWT was measured every 15 min. Immediately after measurement of the PWT at 30 min post morphine administration, one group was given naloxone (5 mg/kg, intraperitoneal) and the other given normal saline as vehicle in a blinded manner. The measurement of PWT was continued as indicated in figure 3. Compared with normal saline, naloxone significantly reversed the antiallodynic effects of intrathecal morphine (group main effect: $F_{1,11} = 15.89$; $P < 0.001$; time main effect: $F_{5,55} = 9.14$; $P < 0.001$; fig. 3).

To investigate whether the duration of intrathecal catheterization has an influence on the antiallodynic effects of intrathecal morphine, 14 rats with obvious mechanical allodynia in hairy skin were randomly assigned to two groups 7 days after the intrathecal catheterization. After baseline measurement of PWT, normal saline or morphine (1 µg) was administered intrathecal, and then PWT was measured every 15 min. Compared with normal saline, morphine reversed the me-

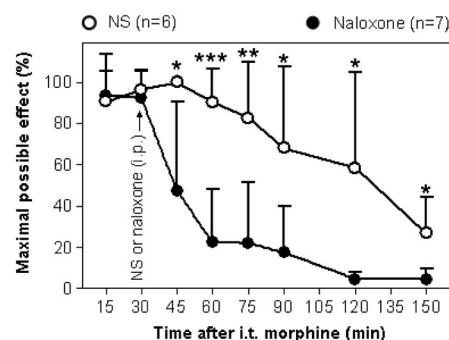


Fig. 3. Reversal of antiallodynic effects of intrathecal (i.t.) morphine by naloxone in the spared nerve injury model. Naloxone (5 mg/kg) was administered intraperitoneally 30 min after intrathecal morphine (1 µg) administration. Compared with normal saline (NS), naloxone significantly reversed the antiallodynic effects of intrathecal morphine. Behavioral tests were performed in the sural nerve-innervated hairy skin of the hind paw (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. normal saline).

chanical allodynia (group main effect: $F_{1,12} = 113.45$, $P < 0.001$; time main effect: $F_{7,84} = 4.85$, $P < 0.001$; fig. 4). These results are similar to those obtained 1 day after the catheterization (group main effect: $F_{1,11} = 0.11$, $P = 0.75$; fig. 2A), suggesting that the duration of intrathecal catheterization has no influence on the antiallodynic effect of intrathecal morphine.

To investigate whether morphine has different antiallodynic effects in glabrous skin compared with hairy skin, 14 rats with mechanical allodynia were randomly assigned to two groups. After baseline measurement of PWT in glabrous skin, normal saline or morphine (1 µg) was administered intrathecal, and then PWT was measured every 15 min. Compared with normal saline, morphine reversed the mechanical allodynia (group main effect: $F_{1,12} = 160.70$, $P < 0.001$; time main effect: $F_{7,84} = 6.23$, $P < 0.001$; fig. 5). These results are similar to those in hairy skin (group main effect: $F_{1,11} = 0.02$, $P = 0.88$; fig. 2A). These data suggest the antiallodynic efficacy of morphine is not different in glabrous versus hairy skin.

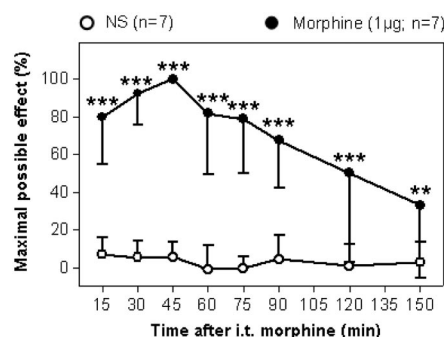


Fig. 4. Effect of intrathecal (i.t.) morphine administration on the mechanical allodynia after spared nerve injury measured on hairy skin 7 days after intrathecal catheterization. Repeated two-way analysis of variance showed intrathecal morphine produced significant reversal of mechanical allodynia compared with normal saline (NS) (** $P < 0.01$, *** $P < 0.001$ vs. NS).

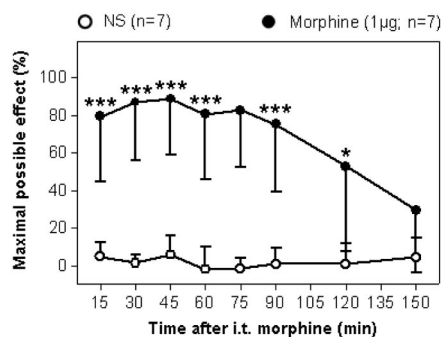


Fig. 5. Effect of intrathecal (i.t.) morphine administration on the mechanical allodynia after spared nerve injury measured on glabrous skin 1 day after intrathecal catheterization. Repeated two-way analysis of variance showed intrathecal morphine produced significant reversal of mechanical allodynia compared with normal saline (NS) (* $P < 0.05$, *** $P < 0.001$ vs. NS).

Antiallodynic Effect of Intrathecal Morphine in SNL Model

Ten rats with mechanical allodynia induced by SNL were randomly assigned to two groups. After baseline measurement of PWT, normal saline or morphine (30 μ g) was administered intrathecally, and then PWT was measured every 15 min. Compared with normal saline, morphine almost completely reversed mechanical allodynia during the entire period (150 min) of measurement (group main effect: $F_{1,8} = 708.00$, $P < 0.001$; time main effect: $F_{7,56} = 0.74$, $P = 0.64$; fig. 6), indicating that morphine is also antiallodynic in SNL model.

Discussion

Our study showed that mechanical allodynia develops in the sural nerve territory, ipsilateral to nerve injury, in rats. This observation is consistent with that reported by Decosterd and Woolf.³³ Although there was no difference in the PWT between hairy and glabrous skin in the sural nerve territory of naive rats, we found the mechanical allodynia in hairy skin was characterized by a lower withdrawal threshold than that in glabrous skin after the

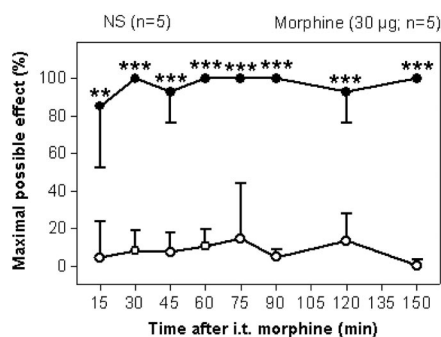


Fig. 6. Effect of intrathecal (i.t.) morphine administration on the mechanical allodynia in spinal nerve ligation model. Repeated two-way analysis of variance showed intrathecal morphine produced significant reversal of mechanical allodynia compared with normal saline (NS) (** $P < 0.01$, *** $P < 0.001$ vs. NS).

SNI. In contrast, Decosterd and Woolf³³ reported that before the nerve injury, the PWT to mechanical stimuli in the dorsal hairy skin was considerably higher than that in the glabrous skin in the plantar surface within the sural nerve territories. In addition, the mechanical allodynia induced by SNI in the hairy skin was less than that in the glabrous skin. The reason for the differences between our observations and those of Decosterd and Woolf³³ is unclear. Differences in test sites and methods used to measure PWTs may partly explain the discrepancies. For example, our hairy-skin test site was approached from the bottom and was just dorsal to the glabrous/hairy skin border placing it near the center of the sural nerve territory. Decosterd and Woolf³³ used a dorsal approach for testing, and therefore, their hairy skin site was widely farther from the hairy/glabrous border. In the current study, because the mechanical threshold was lower in hairy skin, pharmacologic studies with morphine examined the effects of the drug on SNI-induced mechanical allodynia in hairy skin.

Our study indicates that systemic morphine produces antiallodynic effects in SNI rats, which is consistent with previous reports.⁴⁰ Furthermore, we found that intrathecal morphine produced a dose-dependent antiallodynic effect in SNI rats. Reversibility of the antiallodynic effect of intrathecal morphine by naloxone indicates that the antiallodynic effect of morphine is mediated by opioid receptors. The ED₅₀ of intrathecal morphine in our study is 0.52 μ g (95% CI, 0.31–0.90 μ g). It is well known that intrathecal morphine produces antinociception in rats,⁴¹ but the minimum effective dose depends on the particular test used. The general minimum effective dose range of intrathecal morphine modifying nociception in most tests is 0.3–1 μ g,⁴² which is similar to the minimum effective dose of intrathecal morphine producing an antiallodynic effect in the current study. In addition, Penning *et al.*⁴³ reported that the ED₅₀ of intrathecal morphine in the paw pressure test is 1.1 (0.8–1.4) μ g, which is similar to that obtained in the current experiment. Furthermore, Zahn *et al.*⁴² reported that the effective dose range of intrathecal morphine to reverse mechanical allodynia in a rat model of postoperative pain was 0.5–5.0 μ g, which is consistent with our observations. Our results indicate that intrathecal morphine can produce complete reversal of mechanical allodynia in SNI rats in the dose range that is antinociceptive in naive rats.

There are conflicts in the clinical literature regarding opioid efficacy in patients with neuropathic pain.^{7,44} Some reports have shown that opioids are effective in attenuating neuropathic pain,^{7,44–46} whereas others have shown little to no efficacy of opioids on neuropathic pain.³ Recent controlled clinical trials have shown that oral opioids are effective in the treatment of postherpetic neuralgia.^{13,15}

Results from neuropathic pain animal models are also controversial. Opioids have been reported to be effec-

tive, mildly or moderately effective, or ineffective on neuropathic pain behaviors, depending on the route of drug administration.^{30,31,34,47} Intrathecal administration of opioids may be a promising method to treat neuropathic pain because of the decreased drug dose and decreased side effects. The SNL model has been widely used to investigate the effect of intrathecal morphine on neuropathic pain. Bian *et al.*³⁰ reported that intrathecal morphine failed to alleviate mechanical allodynia even at doses up to 100 μg in the SNL model, which is inconsistent with the current results showing that intrathecal morphine produced an antiallodynic effect in SNI rats in the dose range of morphine that produces antinociception in naive rats. It is noteworthy that Bian *et al.*³⁰ measured mechanical allodynia in the glabrous skin of the plantar hind paw in the SNL model, whereas we tested mechanical allodynia in the hairy skin of the lateral hind paw in the SNI model. It is reasonable to ask whether the skin type might cause a difference in the effectiveness of morphine in alleviating mechanical allodynia. However, our additional observations that intrathecal morphine (1 μg) produced similar antiallodynic effects in the hairy skin and glabrous skin in SNI rats do not support this hypothesis. Also, our intrathecal injections were given only 1 day after catheterization, whereas in previous reports, the interval between catheterization and intrathecal injection was 3–7 days.^{30,31,34} However, the interval between catheterization and intrathecal injection cannot account for the different antiallodynic effect of morphine because our results show that intrathecal morphine (1 μg) either 1 day or 7 days after catheterization produced a similar antiallodynic effect in SNI rats.

The discrepant results on the antiallodynic effect of intrathecal morphine in the SNL model reported by Bian *et al.*³⁰ and in the SNI model presented in the current study motivated us to recheck the efficacy of intrathecal morphine on mechanical allodynia in the SNL model. To our surprise, we found that even 30 μg intrathecal morphine, which was the lowest dose used by Bian *et al.*,³⁰ produced almost complete reversal of mechanical allodynia in our SNL rats. Beneficial effects of intrathecal morphine at high doses (30 μg or larger) should be interpreted with caution because these doses might induce side effects such as catalepsy³¹ that might potentially influence the mechanical allodynic behavior. However, Lee *et al.*³¹ reported that catalepsy has no relation to PWT. Our results are also consistent with a recent report that intrathecal morphine produced marked antiallodynic effects in SNL rats.³⁴ The estimated ED₅₀ in the above report is approximately 1.09 μg (95% CI, 0.725–1.625 μg), which is not significantly different from that in SNI rats in the current study. Furthermore, studies in mice also indicate that intrathecal morphine is effective in attenuating mechanical allodynia in the SNL model.⁴⁷ Even more intriguing, in an electrophysiologic study, it

has been shown that intrathecal morphine had an enhanced potency on the C fiber-evoked and noxious natural stimuli-evoked neuronal response of spinal nerve-ligated rats.⁴⁸ Our results are consistent with clinical observations that intrathecal opioids are effective in treating neuropathic pain.^{49–51}

The reasons for the varied responsiveness of neuropathic pain to opioids are unclear. Several hypotheses on this issue have been developed and are discussed below.

Type of Nerve Injury

It has been reported that the efficacy of morphine on thermal hyperalgesia induced by a chronic constriction injury is more prominent than that induced by a partial sciatic nerve injury,⁵² suggesting that the type of nerve injury plays a role in the responsiveness of neuropathic pain to opioids. The different reorganization in the central nervous system induced by different nerve injuries might contribute to the variation in the responsiveness of neuropathic pain to opioids. For example, chronic constriction injury increases the number of μ -opioid receptors, and tight ligation of the sciatic nerve decreases the number of μ -opioid receptors,^{53,54} which might lead to different morphine sensitivities.

Routes of Drug Administration

In a model of central pain, intrathecal administration of morphine effectively attenuated pain behavior, but the effect of systemically administered morphine was reduced.³² In contrast, Bian *et al.*³⁰ reported that systemic or intracerebroventricular morphine but not intrathecal morphine produced an antiallodynic effect in an SNL model.

Potency of Opioid Agonists

Nichols *et al.*⁵⁵ reported that intrathecal morphine did not alter allodynia at doses up to 100 μg , but the higher efficacy μ -opioid receptor-selective agonist [D-al₁, (2)N-MePhe, (4)Gly-ol(5)] enkephalin (DAMGO) produced a full dose-related antiallodynic effect when given intrathecally to nerve-injured rats. Przewlocka *et al.*⁵⁶ also reported that spinal injection of highly selective endogenous ligands, endomorphins, seem to be effective to relieve neuropathic pain behaviors. These data suggest that the potency of opioid receptor agonists might be another determining factor on the opioid action in neuropathic pain. However, no clear pattern has emerged. Some methodologic differences, such as lack of blinding of investigators, may also explain the discrepant results between studies. Undoubtedly, further studies are necessary to understand better the effects of opioids in neuropathic pain.

In summary, our study indicates that (1) SNI-induced mechanical allodynia is characterized by a lower PWT in hairy *versus* glabrous skin; (2) systemic and intrathecal morphine dose-dependently reverse the SNI-induced me-

chanical allodynia by an opioid receptor-mediated effect; and (3) intrathecal morphine is also effective in reversing the mechanical allodynia induced by SNL. Our results indicate that neuropathic pain is not resistant to opioids and suggest that morphine, especially intrathecal morphine, is likely to be effective in the treatment of neuropathic pain.

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