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Effects of Intrathecally Administered Nociceptin, an Opioid Receptor-like$_1$ Receptor Agonist, and N-methyl-D-aspartate Receptor Antagonists on the Thermal Hyperalgesia Induced by Partial Sciatic Nerve Injury in the Rat

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Background: **Nociceptin is a 17-amino acid peptide and acts as a potent endogenous agonist of the opioid receptor-like$_1$ receptor. Nociceptin is reported to depress glutamatergic transmission and to block the spinal facilitation that is thought to be mediated by the N-methyl-D-aspartate (NMDA) receptor. In the present study, the authors investigated the effect of intrathecally administered nociceptin and NMDA antagonists on the level of thermal hyperalgesia after partial sciatic nerve injury in the rat.**

Methods: **Partial sciatic nerve injury was created by tight ligation of one third to one half of the right sciatic nerve. The level of thermal hyperalgesia was evaluated by the difference score, which was calculated by subtracting the paw withdrawal latency against thermal nociceptive stimulation in the uninjured paw from that in the injured paw. Drugs were administered intrathecally 7 or 11 days after the nerve injury, and the level of thermal hyperalgesia was measured 5, 15, 30, 60, and 90 min after the drug injection.**

Results: **Intrathecal injection of nociceptin, but not of NMDA antagonists, attenuated the level of thermal hyperalgesia in a dose-dependent manner at a dose of 0.17–17 nM (post-drug difference score: saline-treated rats, −4.9 ± 2.2 s; 17 nM nociceptin–treated rats, −1.3 ± 0.9 s).**

Conclusions: **Intrathecal injection of nociceptin attenuated the level of thermal hyperalgesia induced by partial sciatic nerve injury, and NMDA receptor–dependent spinal facilitation does not play an important role in maintaining thermal hyperalgesia in rats with partial sciatic nerve injury. (Key words: Nerve(s), injury: neuropathic pain. Receptors: N-methyl-D-aspartate. Receptors, opioid receptor-like$_1$, receptor agonist: nociceptin. Pain, thermal hyperalgesia.)**

RECENTLY nociceptin was identified as a potent endogenous agonist of the opioid receptor-like$_1$ (ORL_1) receptor.[1,2] The ORL_1 receptor is a G protein–coupled receptor[3,4] and mediates inhibition of adenylyl cyclase.[4] Nociceptin is a 17-amino acid peptide (Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH) and is similar in sequence to dynorphin A.

Nociceptin has been reported to depress glutamatergic transmission.[5,6] Repetitive input from C-fibers can evoke a powerful and spinally mediated facilitation (wind-up) of the dorsal horn wide-dynamic-range neurons.[7] The wind-up phenomenon was reported to be mediated by activation of N-methyl-D-aspartate (NMDA) receptor, which is one of the receptors for excitatory amino acids, such as glutamate.[8] Intrathecally administered nociceptin inhibited this wind-up of dorsal horn neurons, but not the baseline C-fiber–evoked response.[9] We recently found that intrathecal injection of nociceptin attenuated the level of thermal hyperalgesia induced by paw carrageenan injection,[10] which was reported to be maintained by NMDA receptor–dependent spinal facilitation.[11] These data suggested that the activation of ORL_1 receptor modulates NMDA receptor–dependent spinal facilitation by inhibiting glutamatergic transmission in the spinal cord.

Recently several types of neuropathic pain models were introduced. Self-mutilation (autotomy) induced by total denervation of a hind paw is one of the neuropathic pain models.[12] The chronic constriction injury model[13] and the partial sciatic nerve injury model[14] are the models of neuropathic pain induced by partial denervation of a peripheral nerve. A chronic constriction injury is created by placing four loosely tied ligatures around the sciatic nerve in the rat,[13] and a partial sciatic nerve injury is created by making a tight ligation of one third to one half of the sciatic nerve in the rat.[14] It

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has been reported that NMDA receptor-dependent and wind-up-like spinal facilitation is crucial for the development of autotomy[15] and the maintenance of thermal hyperalgesia induced by chronic constriction injury.[16,17] We recently found that intrathecal injection of nociceptin reduced the level of thermal hyperalgesia induced by chronic constriction injury.[18] There are no data about the role of NMDA receptor-dependent and wind-up-like spinal facilitation in the maintenance of thermal hyperalgesia induced by partial sciatic nerve injury.

The development of autotomy and of thermal hyperalgesia induced by chronic constriction injury have been reported to be inhibited by blocking injury discharge with topically applied local anesthetics.[19-21] In the partial sciatic nerve injury model, blocking injury discharge had no effect on the development of thermal hyperalgesia.[18] These data indicated that mechanisms specific to nerve injury are involved in the development of neuropathic pain.

In the present study, to clarify the role of nociceptin and NMDA receptors in the maintenance of thermal hyperalgesia in the partial sciatic nerve injury model, we studied the effects of intrathecal injection of nociceptin, (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d) cyclohepten-5,10-imine hydrogen maleate (MK-801), a noncompetitive NMDA receptor antagonist; and DL-2-amino-5-phosphonovaleric acid (AP-5), a competitive NMDA receptor antagonist on thermal hyperalgesia induced by partial sciatic nerve injury.

Methods

The following investigations were carried out under a protocol approved by the Institutional Animal Care Committee, Chiba University, Chiba, Japan. Male Sprague-Dawley rats weighing 250-300 g were fitted with long-term intrathecal catheters and studied for the effect of the two agents on thermal hyperalgesia induced by partial sciatic nerve injury.

Intrathecal Catheters

Long-term intrathecal catheters were inserted 3 days before the nerve injury, during isoflurane anesthesia, by passing a PE-10 catheter through an incision in the atlanto-occipital membrane to a position 8 cm caudal to the cisterna at the level of the lumbar enlargement.[22] The external opening of the catheter was fastened to the top of the skull and sealed with a piece of steel wire. Rats showing neurologic deficits were not studied.

Partial Sciatic Nerve Injury

Anesthesia was induced by inhalation of 5% isoflurane and maintained at a concentration of 2-3% as needed. After a local incision, the biceps femoralis of each leg was bluntly dissected at the mid-thigh to expose the sciatic nerve. Each nerve was carefully mobilized, with care taken to avoid undue stretching. An 8-0 silicon-treated silk suture was inserted into the right sciatic nerve just proximal to the sciatic trifurcation with a three-eighths curved, reversed-cutting mini-needle and tightly ligated so that the dorsal one third to two thirds of the nerve thickness was trapped in the ligature.[14] The left nerve was only mobilized. Both incisions were closed layer to layer, with 3-0 silk sutures, and the rats were allowed to recover from the anesthetics.

After the sciatic nerve injury was induced, the animals were maintained individually in clear plastic cages with solid floors covered with 3-6 cm sawdust. All animals ate and drank normally after the procedure.

Thermal Nociceptive Test

Paw withdrawal latency against thermal stimulation was measured with a device similar to that previously described.[23] The rats were placed beneath a clear plastic cage (10 × 20 × 24 cm) on an elevated floor of clear glass (2-mm thick). A radiant heat source (JRC-12 V-100 W eye projector halogen lamp; Iwasaki Electric, Tokyo, Japan) with an aperture diameter of 5 mm was contained in a movable holder placed beneath the glass floor. The voltage to the thermal source was controlled by a constant voltage supply. To reduce the variability in plate surface temperature resulting from minor changes in room temperature, the interior of the box under the animal was prepared with a heat source such that the glass temperature was regulated at 30°C. The calibration of the thermal test system was such that the average response latency in 10 normal untreated rats was maintained at 10 s before the initiation of an experimental series.

To initiate a test, a rat was placed in the box and allowed 5-10 min to habituate. The halogen lamp beneath the floor was then positioned so that it focused on the plantar surface of one hind paw that was in contact with the glass. Care was taken not to focus the lamp on skin that was not in contact with the glass plate. The light was then activated, initiating a timing circuit. The interval between the application of the light beam and a response was measured to the nearest 0.1 s. The trial was terminated and the lamp removed in

the absence of a response within 20 s, and 20 s was then assigned as the response latency time.

Motor Function

Motor function was evaluated when the rats performed two specific behavior tasks, the placing-stepping reflex and the righting reflex. The placing-stepping response is evoked by drawing the dorsum of either hind paw over the edge of a table top. In normal animals, the stimulus elicits an upward lifting of the paw onto the surface of the table, called "stepping." Animals with any degree of hind limb flaccidity will have an altered reflex or none at all. The righting reflex can be demonstrated by placing an animal horizontally with its back on the table. The animal will usually show an immediate coordinated twisting of the body around its longitudinal axis to regain its normal position on its feet. Animals displaying ataxic behavior will show a decreased ability to right themselves. To quantify the extent of motor function, both tasks were scored on a scale of 0-2, in which 0 = absence of function and 2 = normal motor function. Animals that could perform the motor tasks but did so more slowly than normal animals were assigned a score of 1.

Experimental Protocol

A preliminary study carried out in our laboratory revealed that the maximum thermal hyperalgesia occurred 7-14 days after partial sciatic nerve injury. Each hyperalgesic animal received one medication administered intrathecally at each of two time points: 7 and 11 days after the creation of the nerve lesion. Before the drug injection, the hind paws were tested alternately three times, with 5-min intervals between the repeated testing of one paw as the baseline data. Both paws were tested alternately at 5, 15, 30, 60, and 90 min after the injection. To obtain control data, saline was injected intrathecally. To separate groups of rats, to verify that the effects of nociceptin on thermal hyperalgesia induced by partial sciatic nerve injury were due to the interaction at the naloxone sensitive opioid receptor, the highest dose of nociceptin was coadministered with 28 nM or 140 nM of naloxone. Naloxone (140 nM) was also administered intrathecally to show whether 140 nM intrathecally administered naloxone had any effect on thermal hyperalgesia induced by partial sciatic nerve injury. To define the role of other excitatory amino acid receptors in the maintenance of thermal hyperalgesia induced by partial sciatic nerve injury, we also examined the effect of intrathecally administered γ-D-glutamylaminomethyl sulphonate (GAMS), an antagonist of α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) receptor and kainate receptor, and (RS)-α-methyl-4-carboxyphenylglycine (MCPG), a metabotropic glutamate receptor antagonist, on thermal hyperalgesia induced by partial sciatic nerve injury.

Drugs and Injection

The agents used in this study were nociceptin (molecular weight, 1809; Peptide Institute Inc., Osaka, Japan), naloxone hydrochloride (molecular weight, 364 d; Sigma Chemical Co., St. Louis, MO), MK-801 (molecular weight, 337 d; Research Biochemicals, Natik, MA), AP-5 (molecular weight, 197 d; Sigma Chemical), GAMS (molecular weight, 240 d; Sigma Chemical) and MCPG (molecular weight, 209 d; Research Biochemicals). Because of the limitation of the solubility of MCPG, 48 nM MCPG was the highest dose that we used in this study. All drugs were dissolved in saline and administered intrathecally in 10 μl vehicle.

Data Analysis and Statistics

To analyze the magnitude of thermal hyperalgesia, the difference score was calculated by subtracting the paw withdrawal latency of the control side (left side) from the paw withdrawal latency of the injured side (right side). A negative score thus indicates a shorter withdrawal latency to thermal nociceptive stimuli on the injured side; that is, hyperalgesia. To compare the predrug right and left paw withdrawal latencies and difference scores of each group, we used one-way analysis of variance (ANOVA) with Tukey's multiple comparison test. To analyze the effects of drugs on the hyperalgesia, the post-drug difference score was calculated by subtracting the maximum paw withdrawal latency of the control side (left side) from the maximum paw withdrawal latency of the injured side (right side). Maximum paw withdrawal latency was defined as the single longest paw withdrawal latency value during the experiment. To obtain a dose-response curve, the dose was plotted against the maximum paw withdrawal latency or the post-drug difference score. Dose-response curves were established with a least-squares linear regression analysis. To evaluate the dose dependence, we calculated the correlation coefficient. We analyzed the effect of drugs based on the post-drug difference score. Use of the median effective dose was not suitable. Thus we calculated the dose that resulted in a post-drug different score at −3 s (effective dose at −3 s) and its 95% confidence interval, instead of the median effective dose.

Negative 3 s was chosen because the post-drug difference score in the saline-treated group was approximately −5 s, and the post-drug difference score in the highest nociceptin dose group was approximately −1 s. To analyze the effect of MK-801, AP-5, GAMS, and MCPG on the post-drug difference score, we used ANOVA.

Whenever appropriate, results are expressed as mean ± SD. Critical values that reached a $P < 0.05$ level of significance were considered significant.

Results

Motor Function

Ten minutes after the intrathecal injection of 30 nM MK-801 or 51 nM AP-5, the score of both placing-stepping reflex and righting reflex decreased to 1 in 3 of six MK-801-treated rats and in two of five AP-5-treated rats. Intrathecal injection of 3.0 nM MK-801 or 5.1 nM AP-5 had no effect on the motor function. After intrathecal injection of 125 nM GAMS, the score of placing-stepping reflex and righting reflex decreased to 1 in all rats, and intrathecal injection of 42 nM GAMS had no motor effect. Thus 30 nM MK-801, 51 nM AP-5, or 42 nM GAMS was the highest dose used in this study. Intrathecal nociceptin, naloxone, and MCPG had no effect on the placing-stepping reflex and righting reflex at the dose used in this study.

Effects of Drugs on Thermal Hyperalgesia

Before the drug injection, the values of right and left paw withdrawal latencies and difference scores (n = 68) were 6.5 ± 0.9 s, 10.7 ± 0.9 s, and −4.2 ± 1.0 s, respectively, and the right paw withdrawal latency was significantly shorter than the left paw withdrawal latency ($P < 0.001$ by Student's t test). There was no difference between right and left paw withdrawal latencies and difference scores of each group (data not shown; right paw withdrawal latency: $P > 0.9$; left paw withdrawal latency: $P > 0.8$; difference score: $P > 0.9$ by ANOVA).

Intrathecal injection of nociceptin had no effect on the maximum paw withdrawal latencies of the uninjured paw (figs. 1 and 2; $P > 0.8$ by ANOVA). In contrast to the lack of effect on the maximum paw withdrawal latency in the uninjured paw, figure 2 suggests that intrathecally administered nociceptin increased the maximum paw withdrawal latencies of the injured paw to the extent that these paw withdrawal latencies were normalized such that the post-drug difference score was 0. To analyze this further, post-drug difference scores are plotted in figure 2. As indicated, intrathecal injection of nociceptin increased the post-drug difference score in a dose-dependent manner ($r = 0.8$, $P < 0.001$), and the effective dose at −3 s was 2.1 nM (95% confidence interval, 0.9-6.0 nM).

The value of post-drug difference score in the rats given 17 nM nociceptin + 28 nM naloxone is not significantly different from that of the 17 nM-nociceptin-treated rats (fig. 3, table 1; $P > 0.7$ by Student's t test). On the other hand, the value of the post-drug difference score in the rats given 17 nM nociceptin + 140 nM naloxone is significantly more negative than that of the rats given 17 nM nociceptin (fig. 3, table 1; $P < 0.05$ by Student's t test). The post-drug difference score in the rats given 140 nM naloxone was not significantly different from that in the saline-treated rats (table 1; $P > 0.2$ by Student's t test).

Neither MK-801 (30 and 3 nM) nor AP-5 (51 and 5.1 nM) had any effect on the maximum paw withdrawal latencies of the injured and uninjured paw and the post-drug difference score compared with those in the saline-treated rats (fig. 1, table 1; right maximum paw withdrawal latency, $P > 0.4$; left maximum paw withdrawal latency, $P > 0.05$; post-drug difference score, $P > 0.9$ by ANOVA).

Neither GAMS (42 nM) nor MCPG (48 nM) had any effect on the maximum paw withdrawal latencies of the injured and uninjured paws and the post-drug difference score compared with those in the saline-treated rats (table 1; right maximum paw withdrawal latency, $P > 0.3$; left maximum paw withdrawal latency, $P > 0.7$; post-drug difference score, $P > 0.4$ by ANOVA).

Discussion

In the present study, we showed clearly that intrathecal injection of nociceptin, but not NMDA receptor antagonists, attenuated the level of thermal hyperalgesia induced by partial sciatic nerve injury in a dose-dependent manner. In this study, 28 nM naloxone did not antagonize the effect of 17 nM nociceptin, and only a very high dose of naloxone (140 nM) fully reversed the effect. We found previously that 28 nM naloxone fully reversed the effect of 17 nM dynorphin A on the formalin test.[24] This suggested that intrathecal injection of 28 nM naloxone is an adequate dose to antagonize the activation of naloxone-sensitive spinal opioid receptors. Furthermore, intrathecal injection of 140 nM naloxone

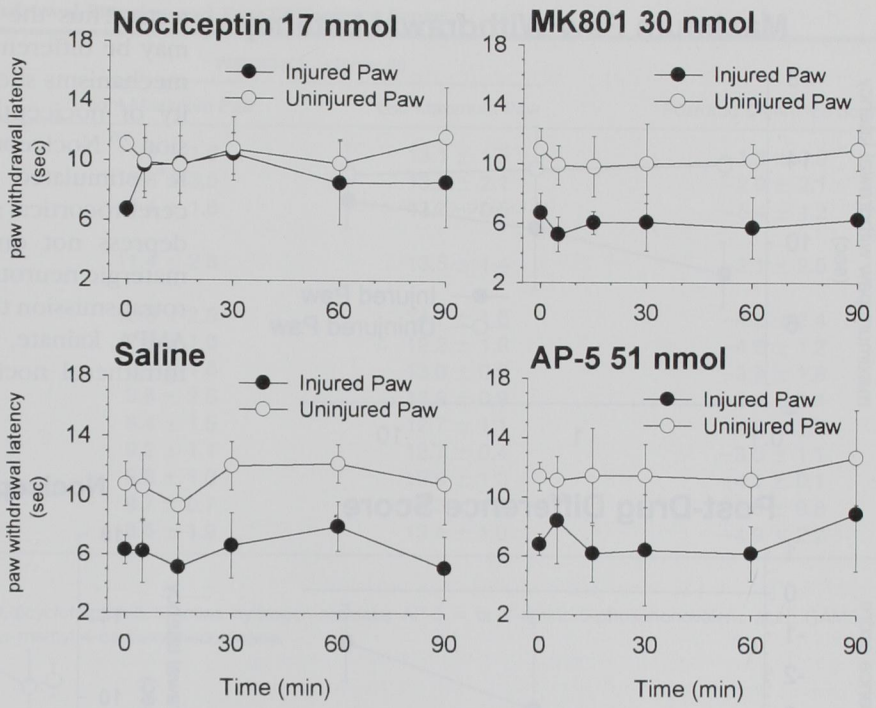

Fig. 1. Effects of intrathecal injection of nociceptin (17 nM), (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,-10-imine hydrogen maleate (MK-801; 30 nM), DL-2-amino-5-phosphonovaleric acid (AP-5; 51 nM), and saline on the thermal nociceptive threshold. Ordinate: paw withdrawal latency (measured in seconds). The abscissa shows time (measured in minutes) after drug injection. Each line represents the mean ± SD determination made in five or six rats. Injured paw = sciatic nerve injured paw (right paw); uninjured paw = sciatic nerve uninjured paw (left paw).

had no effect on the level of thermal hyperalgesia compared with that of saline. These data indicate that intrathecally administered nociceptin produces a strong antihyperalgesic effect, and this effect of nociceptin is not mediated by the activation of the naloxone-sensitive opioid receptors. Nociceptin has been reported to be a natural ligand for the ORL_1 receptor, and naloxone is not active at the ORL_1 receptor.[4] Although no selective ORL_1 receptor antagonist is available and an antagonist study could not be performed to show that the effect of nociceptin on the thermal hyperalgesia induced by partial sciatic nerve injury is mediated through the activation of ORL_1 receptor, we believe that the effect of nociceptin is most likely mediated by the activation of ORL_1 receptor.

Here we analyzed the level of thermal hyperalgesia using the difference score. Seltzer *et al.*[14] reported that partial sciatic nerve injury induced a bilateral decrease in the paw withdrawal threshold to heat pulses from a carbon dioxide laser. We do not know why, in the present study, the paw withdrawal latencies of uninjured paws were not affected by partial sciatic nerve injury. On the other hand, Seltzer *et al.*[14] also reported that the response duration to a supra-threshold noxious carbon dioxide laser heat pulse in the operated paw increased significantly, but there was no change in the response duration of the uninjured paw. We think that the paw withdrawal latency that we measured shares characteristics with the response duration to a supra-threshold noxious carbon dioxide laser heat pulse. Dougherty *et al.*[21] also reported that the paw withdrawal latencies of uninjured paws measured with the device that we used did not change during the first 21 days after partial sciatic nerve injury, and they also analyzed the level of thermal hyperalgesia using difference scores.

Intrathecal injection of both MK-801 and AP-5 had no effect on thermal hyperalgesia induced by partial sciatic nerve injury at the dose that resulted in mild motor dysfunction. We could not examine the effects of doses of MK-801 and AP-5 any higher than those we used in this study because they produced motor dysfunction. In the chronic constriction injury model, the NMDA receptor antagonist has been reported to attenuate the level of thermal hyperalgesia at the dose that we used here.[16,17] This may suggest that the mechanisms producing thermal hyperalgesia in the partial sciatic nerve injury model may be different from those in the chronic constriction injury model. We do not know the precise mechanisms that cause thermal hyperalgesia induced by partial sciatic nerve injury, but thermal hyperalgesia induced by partial sciatic nerve injury is maintained

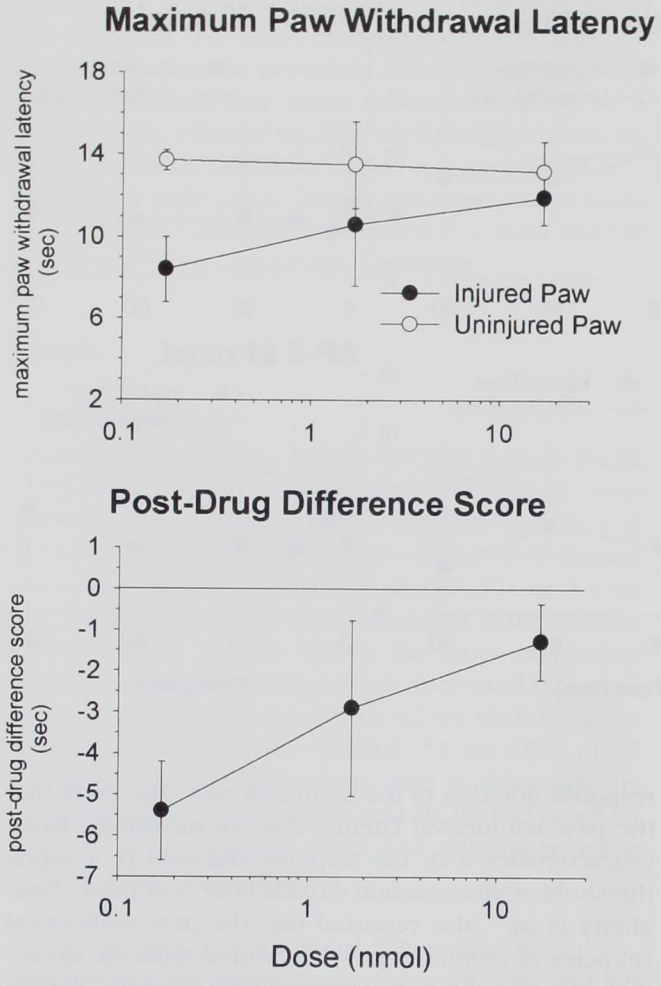

Fig. 2. Log dose–response curve for the effects of nociceptin on maximum paw withdrawal latency (*top*) and post-drug difference score (*bottom*). Maximum paw withdrawal latency is the single longest paw withdrawal latency during the experiment. A post-drug difference score is calculated by subtracting the maximum paw withdrawal latency of the control side (left side) from the maximum paw withdrawal latency of the injured side (right side). Each line represents the mean ± SD of five or six rats. Injured paw = sciatic nerve injured paw (right paw); uninjured paw = sciatic nerve uninjured paw.

by spinal NMDA receptor-independent mechanisms. It has been reported that intravenous administration of ketamine, an NMDA receptor antagonist, did not alleviate all neuropathic pain.[25] These clinical data also indicated that not all neuropathic pain states are maintained by NMDA receptor-dependent mechanisms.

The mechanism of the action of nociceptin in the spinal cord is not clear. It has been reported that intracerebroventricularly injected nociceptin produced hyperalgesia in the mice tail flick[2] and the mice hot plate[1] tests. Thus the role of nociceptin in the spinal cord may be different from that in the brain. Four possible mechanisms should be considered. The first is the ability of nociceptin to depress glutamatergic transmission.[5,6] Nociceptin has been reported to inhibit 46 mM K^+-stimulated glutamate release from rat perfused cerebrocortical slices.[6] Thus intrathecal nociceptin may depress not only NMDA receptor-dependent glutamatergic neurotransmission but also glutamatergic neurotransmission that depends on other receptors, such as AMPA, kainate, and metabotropic glutamate receptors. Intrathecal nociceptin may have depressed all spinal

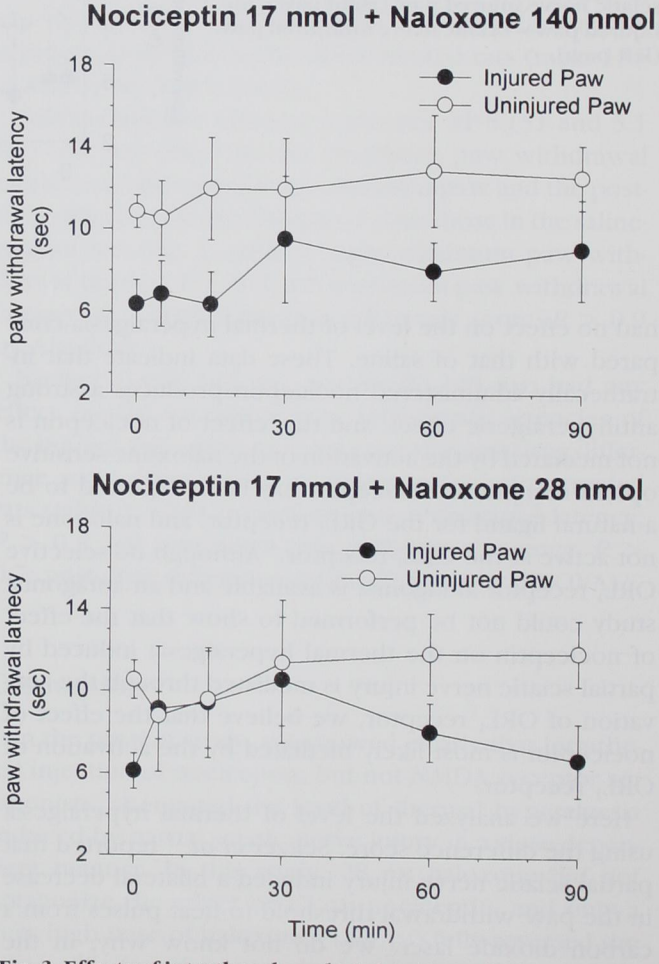

Fig. 3. Effects of intrathecal coadministration of 17 nM nociceptin with 140 nM naloxone (*top*) or 28 nM (*bottom*) on the thermal nociceptive threshold. Ordinate: paw withdrawal latency (measured in seconds). The abscissa shows time (in minutes) after drug injection. Each line represents the mean ± SD determination made in five rats. Injured paw = sciatic nerve injured paw (right paw); uninjured paw = sciatic nerve uninjured paw (left paw).

Table 1. Right and Left Maximum Paw Withdrawal Latency and Post-Difference Score

	Withdrawal Latency (s)		
	Right Maximum Paw	Left Maximum Paw	Postdrug Difference Score
Nociceptin 17 nmol (n = 5)	11.9 ± 1.3	13.1 ± 1.5	−1.3 ± 0.9
Nociceptin 1.7 nmol (n = 5)	10.6 ± 3.0	13.5 ± 2.1	−2.9 ± 2.1
Nociceptin 0.17 nmol (n = 6)	8.4 ± 1.6	13.7 ± 0.5	−5.4 ± 1.2
Nociceptin 17 nmol + naloxone 28 nmol (n = 5)	11.4 ± 2.8	13.5 ± 1.4	−2.1 ± 2.5
Nociceptin 17 nmol + naloxone 140 nmol (n = 5)	10.6 ± 2.5	14.7 ± 1.6	−4.2 ± 2.4
MK-801 30 nmol (n = 6)	7.6 ± 1.0	12.2 ± 1.0	−4.6 ± 1.2
MK-801 3.0 nmol (n = 5)	9.1 ± 1.6	13.0 ± 0.9	−3.9 ± 1.8
AP-5 51 nmol (n = 5)	9.6 ± 2.6	13.9 ± 0.9	−4.4 ± 2.4
AP-5 5.1 nmol (n = 5)	8.4 ± 1.5	12.7 ± 1.1	−4.3 ± 1.7
Naloxone 140 nmol (n = 5)	9.2 ± 1.1	12.7 ± 0.4	−3.5 ± 1.1
GAMS 42 nmol (n = 5)	8.8 ± 1.0	12.9 ± 1.0	−4.1 ± 0.1
MCPG 48 nmol (n = 5)	9.7 ± 0.7	13.3 ± 1.2	−3.6 ± 0.8
Saline (n = 6)	8.5 ± 1.9	13.4 ± 1.0	−4.9 ± 2.2

Values are mean ± SD.

MK-801 = ((+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine hydrogen maleate; AP-5 = DL-2-amino-5-phosphonovaleric acid; GAMS = γ-D-glutamylaminomethyl sulphonate; MCPG = (RS)-α-methyl-4-carboxyphenoglycine.

glutamatergic transmission and decreased the level of thermal hyperalgesia induced by partial sciatic nerve injury. However, in the present study, intrathecal injection of either the AMPA-kainate receptor antagonist or the metabotropic glutamate receptor antagonist had no effect on the level of thermal hyperalgesia induced by partial sciatic nerve injury at the dose applied in this study. The second possible mechanism is the ability of nociceptin to inhibit the voltage-dependent N-type Ca^{2+} channel current.[26] This effect of nociceptin was not antagonized by naloxone.[26] It has been suggested that, in the spinal cord, the release of the primary afferent peptides, such as calcitonin gene-related peptide and substance P, was regulated by the N-type Ca^{2+} channel.[27-29] Furthermore, it has been reported that N-type Ca^{2+} channel blocker depresses glutamatergic transmission.[30,31] However, we recently found that intrathecal injection of the N-type Ca^{2+} channel blocker had no effect on thermal hyperalgesia induced by partial sciatic nerve ligation (T. Yamamoto, unpublished observations). The third possible mechanism is that nociceptin increases inwardly rectifying K^+ conductance.[32] This nociceptin effect also was not antagonized by naloxone.[32] It is possible that nociceptin causes membrane hyperpolarization by an increase in K^+ conductance, and this membrane hyperpolarization reduces cellular excitability and inhibits neurotransmitter release. For example, the activation of μ- and δ-opioid receptors increases an inwardly rectifying K^+ conductance,[33,34] and this effect is thought to be important in allowing opioids to reduce cellular excitability and inhibit neurotransmitter release. The fourth possible mechanism is that nociceptin inhibits adenylate cyclase.[1] The inhibition of adenylate cyclase activity by the κ-opioid agonist has been demonstrated in the rat spinal cord.[35] The coupling of μ- and δ-opioid receptors to the inhibitory system of adenylate cyclase also has been demonstrated in the brain.[36]

In conclusion, NMDA receptor-dependent spinal facilitation is not a common mechanism underlying the maintenance of neuropathic pain, and ORL_1 receptor activation may become one of the key methods for the treatment of neuropathic pain.

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