Anesthesiology
82:1266–1273, 1995

Role of Nitric Oxide in the Development of Thermal Hyperesthesia Induced by Sciatic Nerve Constriction Injury in the Rat

Tatsuo Yamamoto, M.D., Naohito Shimoyama, M.D.**

Background: **Nitric oxide (NO) has been shown to be involved in mediating nociceptive information transmission in the spinal cord. It is known that the N-methyl-D-aspartate receptor plays an important role in the development of the spinal facilitation evoked by a protracted small afferent input and that this effect is mediated at least in part by NO. Recently, it has been found that N-methyl-D-aspartate receptor–mediated spinal facilitation is crucial in the development of thermal hyperesthesia evoked by a nerve constriction injury. In the current study, we investigated the role of NO in the development of thermal hyperesthesia after a nerve constriction injury.**

Methods: **The Bennett and Xie model (four loose chromic gut ligations around the rat sciatic nerve) was used to examine the development of thermal hyperesthesia. An NO synthase inhibitor (Nω-nitro-L-arginine or Nω-nitro-L-arginine methyl ester hydrochloride), rat hemoglobin, or L-arginine was administered intrathecally 10 min before the nerve injury (pretreatment study) or 15 min after the nerve injury (posttreatment study).**

Results: **Pretreatment but not posttreatment administration of NO synthase inhibitor significantly delayed the development of thermal hyperesthesia. The effect of NO synthase inhibitor was reversed by the coadministration of L-arginine but not by the coadministration of D-arginine. Pretreatment with rat hemoglobin also delayed the development of thermal hyperesthesia. L-Arginine itself had no effect on the development of thermal hyperesthesia.**

Conclusions: **NO may play an important role in the development of N-methyl-D-aspartate receptor–mediated spinal facilitation after a nerve constriction injury. (Key words: Nerve(s), injury: neuropathic pain. Neurotransmitters: nitric oxide. Pain: hyperesthesia.)**

DAMAGE to a sensory nerve may induce the development of neuropathic pain. Neuropathic pain may be constant, intermittent, or paroxysmal with a burning, sharp, or aching sensation and is relatively refractory to the standard analgesic agents, such as opioids.[1] A difficulty in defining this clinical state and improving management has been the absence of applicable animal models with correlative validity. It has been shown that thermal hyperesthesia occurs after the constriction injury created by placing four loosely tied ligatures around the sciatic nerve in the rat.[2] Although the specific mechanism underlying the development of the thermal hyperesthesia is unknown, several lines of evidence suggest that N-methyl-D-aspartate (NMDA) receptor–mediated spinal facilitation plays an important role in the development and the maintenance of the thermal hyperesthesia induced by the constriction nerve injury.[3–6] The NMDA receptor is one of the receptors for excitatory amino acids, such as glutamate, and is now thought to be involved in the transmission of nociceptive information in the spinal cord.[7,8]

It has been reported that nitric oxide (NO) plays an important role in synaptic transmission in the central nervous system.[9,10] NO is synthesized from the semiessential amino acid L-arginine by NO synthase and is the endogenous stimulator of soluble guanylate cyclase and acts as a messenger molecule. It is known that the effects of NMDA receptor activation are mediated at least in part by production of NO.[11] For example, both NMDA receptor antagonists[12,13] and NO synthase inhibitors[14,15] depress the agitation behavior induced by a formalin injection into the rat paw. On the other hand, it has been suggested that not all of the NMDA receptor–mediated effects are equally mediated by the production of NO.[14,16] For instance, thermal hyperesthesia induced by the intraplantar injection of carrageenan has been reported to be eliminated by intrathecal injection of NMDA receptor antagonist but not by intrathecal injection of NO synthase inhibitor.† Thus, it is necessary to

---

* Staff Anesthesiologist.

Received from the Department of Anesthesiology, School of Medicine, Chiba University, Chiba, Japan. Submitted for publication August 30, 1994. Accepted for publication February 2, 1995.

Address reprint requests to Dr. Yamamoto: Department of Anesthesiology, School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba-shi, Chiba 260, Japan.

† Yamamoto T, Shimoyama N, Mizuguchi T: Role of nitric oxide on nociceptive transmission in the spinal cord (abstract). Abstracts of the 7th World Congress on Pain. Seattle, IASP Press, 1993, p 226.

determine whether NO acts as an important neural messenger in the establishment of the NMDA receptor–dependent spinal facilitation after a nerve constriction injury.

In this study, we sought to define the effect of intrathecally administered NO synthase inhibitors on the development of thermal hyperesthesia induced by sciatic nerve constriction injury in the rat.

Materials and Methods

The following investigations were carried out according to a protocol approved by the Institutional Animal Care Committee, Chiba University. Male Sprague-Dawley rats (250–300 g) were prepared with chronic intrathecal catheters and examined for the effect of agents on the development of thermal hyperesthesia induced by a chronic constriction injury.

Animal Preparation

Chronic intrathecal catheters were inserted during isoflurane anesthesia by passing a polyethylene-10 catheter through an incision in the atlantooccipital membrane to a position 8 cm caudal to the cisterna at the level of the lumber enlargement.[16] The catheter was externalized on the top of the skull and sealed with a steel wire, and the wound was closed with 3–0 silk sutures. The animals were allowed to recover for 1 week before being used experimentally. All animals displayed normal feeding and drinking behavior postoperatively. No infection was found 1 week after the surgery. Rats showing neurologic deficits postoperatively were discarded.

Nerve Constriction Injury

The hyperesthetic state was induced by chronic constriction of the sciatic nerve with four loose ligatures.[2] Anesthesia was induced by inhalation of isoflurane, maintained at a concentration of 2–3% as needed. After a local incision, the biceps femoralis of each leg was bluntly dissected at midthigh to expose the sciatic nerve. Each nerve was then carefully mobilized, with care taken to avoid undue stretching. Each of four 4–0 chromic gut sutures was tied loosely with a square knot around the right sciatic nerve. We used a brief twitch in the muscle surrounding the exposure as an indicator of the desired degree of constriction. To verify that the hyperesthetic state was induced by the chronic constriction injury itself, a sham operation (left sciatic nerve mobilization) was performed. Both incisions were closed, layer to layer, with 3–0 silk sutures, and the rats were allowed to recover from the anesthetic.

After surgery, the animals were maintained individually in clear plastic cages with solid floors covered with 3–6 cm sawdust. Animals appropriately prepared showed a mild eversion of the affected paw and a mild to moderate degree of foot drop. All animals postoperatively displayed normal feeding and drinking behavior.

Thermal Nociceptive Test

Paw withdrawal latency (PWL) in response to thermal stimulation was measured with a device similar to that previously reported.[17] The rats were placed in a clear plastic cage (10 × 20 × 24 cm) on an elevated floor of clear glass (2 mm thick). A radiant heat source (Eye Projector Halogen Lamp JRC-12V-100W, 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 is such that the average response latency in ten 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 not in contact with the glass floor. The light was then activated, initiating a timing circuit. The interval between the application of the light beam and the brisk PWL of the hind paw was measured to the nearest 0.1 s. The time value was then assigned as the response latency. The trial was terminated and the lamp removed in the absence of a response within 20 s.

Behavioral Analysis

The general behavior of each rat was carefully observed and tested. Motor function was evaluated by the performance of two specific behavioral tasks[18]: the placing or stepping reflex and the righting reflex. The placing or stepping reflex was evoked by drawing the dorsum of either hind paw over the edge of a table top.

In normal animals this stimulus elicits an upward lifting of the paw onto the surface of the table (stepping). In animals with any degree of hind limb flaccidity, this reflex is altered or absent. In the test of the righting reflex, an animal placed horizontally with its back on the table normally shows an immediate coordinated twisting of the body around its longitudinal axis to regain its normal position on its feet. In animals with ataxic behavior, the ability to right the body is decreased. To quantitate the extent of motor function, both tasks were scored on a scale of 0–2, where 0 = absence of function and 2 = normal motor function. Animals that were able to perform the motor tasks but did so more slowly than normal animals were assigned a score of 1.

Experimental Protocol

Before induction of the sciatic nerve injury, the right and left hind paws were tested three times alternately, with 5-min intervals between consecutive tests, to obtain baseline data. The average of three measurements was defined as the PWL. The investigator who measured PWLs was blinded throughout the study to the treatment that the animal had received.

Pretreatment Study

Drugs were administered intrathecally 10 min before the loose ligations were made. The animals were divided into five groups: Nω-nitro-L-arginine (L-NA) 30 μg (n = 10); Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) 100 μg (n = 10); rat hemoglobin 100 μg (n = 9); L-arginine 300 μg (n = 15); or vehicle (n = 10). Thirty micrograms L-NA, 100 μg L-NAME, or 100 μg rat hemoglobin has been reported to depress the agitation behavior evoked by formalin injection into the paw in rats, and 300 μg L-arginine has been reported to reverse the effect of 30 μg L-NA on the formalin-induced agitation behavior.[14,15] L-NA and L-NAME are NO synthase inhibitors, and rat hemoglobin is an NO scavenger. The postsurgery PWLs of the right and left hind paws were measured 3, 7, 14, 21, and 28 days after the nerve lesion.

Two additional groups of rats were used to verify that the effect of L-NA on the development of thermal hyperesthesia induced by the nerve constriction injury was produced by an effect on NO synthase. The groups received 300 μg of L-arginine (n = 8) or 300 μg D-arginine (n = 7) intrathecally coadministered with 30 μg L-NA. NO synthase uses free L-arginine but not D-arginine as a substrate to produce NO, and L-NA or L-NAME acts as an alternate substrate for NO-synthase. Thus, abundant L-arginine but not D-arginine is able to reverse the effect of L-NA or L-NAME. The postsurgery PWLs of right and left hind paws of the two additional groups were measured 3 and 7 days after the nerve lesion.

Posttreatment Study

Thirty micrograms L-NA (n = 8) or vehicle (n = 8) was administered intrathecally 15 min after the nerve constriction injury. The postsurgery PWLs of the right and left hind paws were measured 3 and 7 days after the nerve lesion.

Drugs

The agents administered intrathecally in this study were L-NA (Sigma, St. Louis, MO), L-NAME (Sigma), rat hemoglobin (Sigma), L-arginine (Sigma), and D-arginine (Sigma). All agents were dissolved in 20% 2-hydroxypropyl-β-cyclodextrin (Research Biochemicals, Natick, MA). These drugs were administered intrathecally in 10 μl vehicle.

Data Analysis

To analyze the magnitude of the hyperesthesia, the difference score (DS) was calculated by subtracting the PWL of the control side (left side) from the PWL of the injured side (right side). A negative score thus indicates a lower threshold on the injured side, that is, hyperesthesia. The levels of thermal hyperesthesia and the PWLs of the rats in each group before surgery (day 0) and on days 3, 7, 14, 21, and 28 were compared by one-way analysis of variance. For multiple comparisons, we used Dunnett's test. To compare the PWLs and DSs before surgery (day 0) and on day 3, 7, 14, 21, and 28 between groups, analysis of variance was used. To analyze the general behavior data, we used chi-squared analysis.

Wherever appropriate, results are expressed as means ± SEM. Critical values that reached a $P < 0.05$ level of significance were considered statistically significant.

Results

All drugs used in this study had no effect on the placing, stepping, or righting reflexes 4, 7, 14, 21, or 28 days after the nerve constriction injury.

Pretreatment Study

Table 1 shows the presurgery right and left PWLs and DSs in each group. Analysis of variance showed that

Table 1. Presurgery Right and Left Paw Withdrawal Latencies and Difference Scores in the Pretreatment Study

	Right PWL (s)	Left PWL (s)	DS (s)
L-NA administered group (n = 10)	10.4 ± 0.4	10.2 ± 0.3	0.2 ± 0.2
L-NAME administered group (n = 10)	10.0 ± 0.2	10.1 ± 0.2	−0.1 ± 0.2
Hemoglobin administered group (n = 9)	10.1 ± 0.2	10.2 ± 0.2	−0.1 ± 0.1
L-Arginine administered group (n = 15)	10.3 ± 0.2	10.3 ± 0.3	0.0 ± 0.1
Vehicle administered group (n = 10)	10.1 ± 0.2	10.1 ± 0.2	0.0 ± 0.1
L-NA + L-arginine administered group (n = 8)	10.6 ± 0.2	10.1 ± 0.2	0.4 ± 0.2
L-NA + D-arginine administered group (n = 7)	10.3 ± 0.2	10.4 ± 0.2	−0.1 ± 0.2

Values are mean ± SEM.

PWL = paw withdrawal latency; DS = difference score; L-NA = Nω-nitro-L-arginine; L-NAME = Nω-nitro-L-arginine methyl ester hydrochloride.

there were no differences between presurgery right and left PWLs and DSs of each group (right PWL $P > 0.7$; left PWL $P > 0.9$; DS $P > 0.1$).

In each group, there are no differences between the left PWLs on days 3, 7, 14, 21, and 28 after the nerve constriction injury (table 2). In the vehicle group, the DSs on days 3, 7, 14, 21, and 28 after the nerve constriction injury were significantly more negative than the presurgery values (fig. 1). In the L-NA group and the L-NAME group, the DSs on days 3 and 7 after the nerve constriction injury were not significantly different from the presurgery DSs, but the DSs on days 14, 21, and 28 after the nerve constriction injury were significantly more negative than the presurgery DSs (fig. 1). On days 3, 7, and 14 after the nerve constriction injury, the DSs of the vehicle group were more negative than those of the L-NA group, but on days 21 and 28 after the nerve constriction injury, the DSs of the vehicle group were not significantly different from those of the L-NA group (fig. 1). On days 3 and 7 after the nerve constriction injury, the DSs of the vehicle group were more negative than those of the L-NAME group, but on days 14, 21, and 28 after the nerve constriction injury, the DSs of the vehicle group were not significantly different from those of the L-NA group (fig. 1). When 300 μg of L-arginine or 300 μg D-arginine was coadministered intrathecally with 30 μg of L-NA, L-arginine but not D-arginine reversed the effect of L-NA on the DS on days 3 and 7 after the nerve constriction injury (fig. 2).

In the hemoglobin group, the DSs on day 3 after the nerve constriction injury were not significantly different from the presurgery DSs, but the DSs on days 7 and 14 after the nerve constriction injury were significantly more negative than the presurgery DSs (fig. 3). On days 3 and 7 after the nerve constriction injury, the DSs of the vehicle group were more negative than those of the hemoglobin group, but on day 14 after the nerve constriction injury, the DSs of the vehicle group were not significantly different from those of the hemoglobin group (fig. 3).

In the L-arginine group, the DSs on days 3 and 7 after the nerve constriction injury were significantly more negative than the presurgery DSs, and the DSs of the L-arginine group on days 3 and 7 after the nerve constriction injury were not significantly different from those of the vehicle group (fig. 4).

Posttreatment Study

In the group in which L-NA was administered after nerve constriction injury, the DSs on days 3 and 7 after the injury were significantly more negative than the presurgery DSs, and the DSs of this group on days 3 and 7 after the nerve constriction injury were not significantly different from those of the group given vehicle after injury (fig. 5).

Discussion

The current study clearly demonstrates that pretreatment but not posttreatment with NO synthase inhibitors delays the development of thermal hyperesthesia after a nerve constriction injury. This effect of NO synthase inhibitor was reversed when L-arginine was coadministered with L-NA. D-Arginine coadministered with L-NA had no effect on the delayed development of thermal hyperesthesia. We also found that pretreatment with rat hemoglobin, which contains a heme moiety and acts as an NO scavenger, delayed the development of thermal hyperesthesia induced by the nerve constriction injury. These data strongly suggest that NO plays

Table 2. Postsurgery Left Paw Withdrawal Latencies in the Pretreatment Study

	Day 3	Day 7	Day 14	Day 21	Day 28
L-NA administered group (n = 10)	10.0 ± 0.3	10.1 ± 0.3	10.3 ± 0.3	10.8 ± 0.2	10.4 ± 0.2
L-NAME administered group (n = 10)	10.2 ± 0.2	10.3 ± 0.3	10.2 ± 0.3	10.6 ± 0.2	10.7 ± 0.2
Hemoglobin administered group (n = 9)	10.2 ± 0.3	10.1 ± 0.4	9.9 ± 0.3	—	—
L-Arginine administered group (n = 15)	10.6 ± 0.2	10.2 ± 0.2	—	—	—
Vehicle administered group (n = 10)	10.7 ± 0.2	10.0 ± 0.2	10.7 ± 0.2	10.6 ± 0.3	10.3 ± 0.3
L-NA + L-Arginine administered group (n = 8)	11.0 ± 0.3	10.7 ± 0.2	—	—	—
L-NA + D-arginine administered group (n = 7)	9.9 ± 0.3	10.0 ± 0.6	—	—	—

Values are mean ± SEM.

PWL = paw withdrawal latency; DS = difference score; L-NA = Nω-nitro-L-arginine; L-NAME = Nω-nitro-L-arginine methyl ester hydrochloride.— = not measured.

an important role in the development of thermal hyperesthesia induced by nerve injury.

Time Course of the Development of Thermal Hyperesthesia

In the vehicle group, thermal hyperesthesia developed 3 days after the nerve constriction injury. Maximum thermal hyperesthesia (DS approximately 3.0 s) occurred 7–28 days after the nerve injury. Bennett and Xie reported that the thermal hyperesthesia was evident on the 2nd postoperative day, that it lasted for 2 months, and that the DS during the first 40 days after the nerve injury was about −3.0 s.[2] These data suggest that intrathecal injection of 10 μl vehicle (20% 2-hydroxypropyl-β-cyclodextrin) has no effect on the development and maintenance of thermal hyperesthesia induced by nerve constriction injury in the rat. In each group, there was no difference among the left PWLs on days 3, 7, 14, 21, and 28 after the nerve lesion. This finding indicates that intrathecally administered drugs have no effect on the PWLs of the nonlesioned side.

Role of Nitric Oxide in the N-Methyl-D-Aspartate Receptor–Mediated Spinal Facilitation

We recently demonstrated that pretreatment with MK-801, an NMDA receptor antagonist, delays the development of thermal hyperesthesia induced by a nerve injury but that, 14 days after the nerve injury, significant thermal hyperesthesia is observed.[4] Moreover, we and

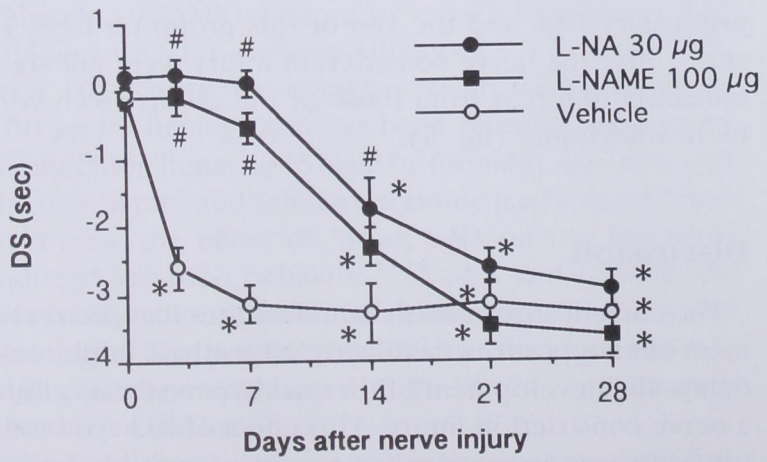

Fig. 1. Effect of 30 μg intrathecal Nω-nitro-L-arginine (L-NA) and 100 μg intrathecal Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) on the development of thermal hyperesthesia induced by a nerve constriction injury in the pretreatment study. The vehicle group is presented for comparison. Each point represents the mean ± SEM of ten rats. Ordinate = difference score (DS); abscissa = days after the nerve constriction injury. *$P < 0.05$ compared with presurgery (day-0) value in the same group. #$P < 0.05$ compared with the vehicle group 3, 7, and 14 days after the nerve injury.

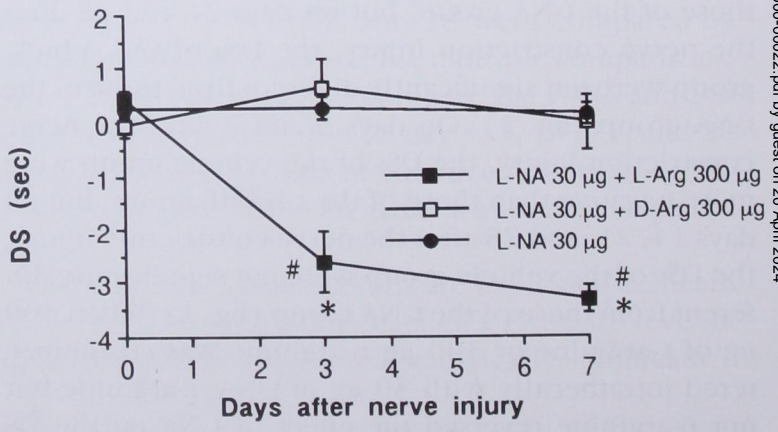

Fig. 2. Effect of intrathecal coadministration of 300 μg L-arginine (L-Arg) with 30 μg Nω-nitro-L-arginine (L-NA) and intrathecal coadministration of 300 μg D-arginine (D-Arg) with 30 μg L-NA on the development of thermal hyperesthesia induced by a nerve constriction injury in the pretreatment study. The L-NA 30 μg group is presented for comparison. Each point represents the mean ± SEM of seven to ten rats. Ordinate = difference score (DS); abscissa = days after a nerve constriction injury. *$P < 0.05$ compared with presurgery (day-0) value in the same group. #$P < 0.05$ compared with the L-NA group 3 and 7 days after the nerve injury.

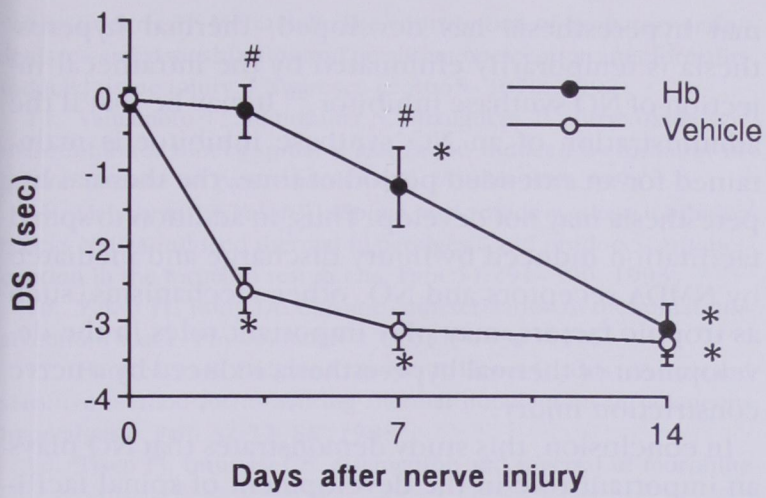

Fig. 3. Effect of intrathecal administration of 100 μg rat hemoglobin (Hb) on the development of thermal hyperesthesia induced by the nerve constriction injury in the pretreatment study. The vehicle group is presented for comparison. Each point represents the mean ± SEM of nine to ten rats. Ordinate = difference score (DS); abscissa = days after the nerve constriction injury. *$P < 0.05$ compared with presurgery (day-0) value in the same group. #$P < 0.05$ compared with the vehicle group 3 and 7 days after the nerve injury.

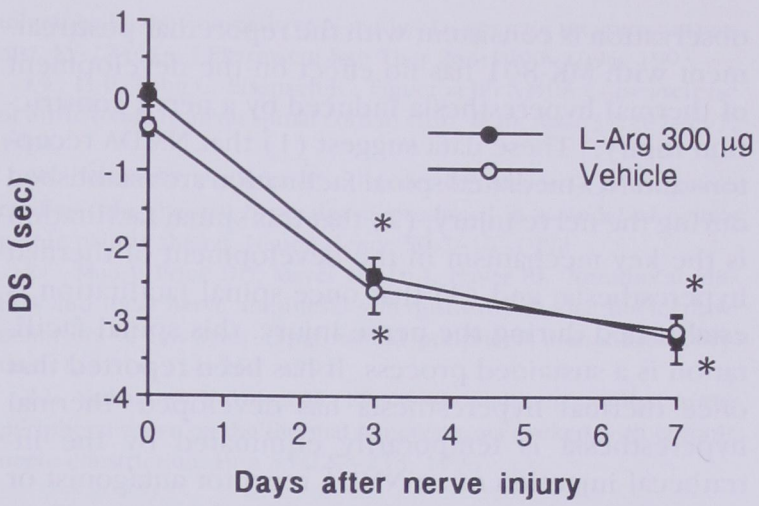

Fig. 4. Effect of intrathecal administration of 300 μg L-arginine on the development of thermal hyperesthesia induced by a nerve constriction injury in the pretreatment study. The vehicle group is presented for comparison. Each point represents the mean ± SEM of nine to ten to fifteen rats. Ordinate = difference score (DS); abscissa = days after the nerve constriction injury. *$P < 0.05$ compared with presurgery (day-0) value in the same group.

others demonstrated that blocking of the injury discharge evoked by the nerve constriction injury with local anesthetics prevents the development of thermal hyperesthesia induced by the nerve constriction injury.[4,6] These data suggest that the injury discharge resulting from the nerve injury may induce NMDA receptor–dependent spinal facilitation and that this spinal facilitation plays an important role in the development of thermal hyperesthesia after sciatic nerve constriction injury.[4] In the current study, pretreatment with NO synthase inhibitor delayed the development of the thermal hyperesthesia evoked by a nerve constriction injury. It has been reported that, *in vitro*, NMDA causes release of NO from the rat spinal cord.[19] NO is thought to act as a second messenger after the activation of the NMDA receptor.[11] These data suggest that nerve injury induces NMDA receptor activation in the spinal cord, that this NMDA receptor activation causes NO release, and that NO may induce the spinal facilitation that causes thermal hyperesthesia.

Pretreatment with L-arginine, a precursor of NO, had no effect on the development of thermal hyperesthesia induced by the nerve injury at the dose that reversed the effect of L-NA on the development of thermal hyperesthesia. One possible explanation is that there is a supply of L-arginine in the spinal cord and that additional L-arginine had no effect on the generation of NO. L-Arginine has been reported to have no effect on the agitation behavior evoked by the formalin injection into the rat paw,[15] but this agitation behavior was reported to be depressed by the intrathecal injection of NO synthase inhibitor.[14,15] These data also suggest that L-arginine itself has no effect on NO-mediated spinal nociceptive transmission.

Posttreatment with NO synthase inhibitor had no effect on the development of thermal hyperesthesia. This

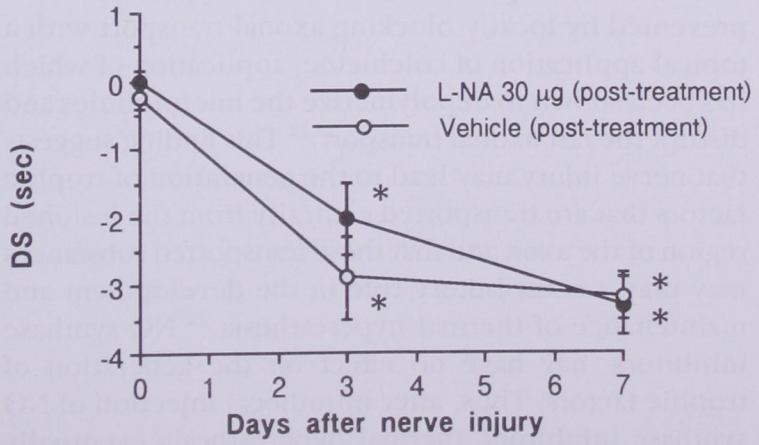

Fig. 5. Effect of 30 μg Nω-nitro-L-arginine (L-NA) administered intrathecally after nerve constriction injury on the development of thermal hyperesthesia induced by the injury in the posttreatment study. The group given vehicle after injury is presented for comparison. Each point represents the mean ± SEM of eight rats. Ordinate = difference score (DS); abscissa = days after the nerve constriction injury. *$P < 0.05$ compared with presurgery (day-0) value in the same group.

observation is consistent with the report that posttreatment with MK-801 has no effect on the development of thermal hyperesthesia induced by a nerve constriction injury.[4] These data suggest (1) that NMDA receptor– and NO-mediated spinal facilitation are established during the nerve injury; (2) that this spinal facilitation is the key mechanism in the development of thermal hyperesthesia; and (3) that once spinal facilitation is established during the nerve injury, this spinal facilitation is a sustained process. It has been reported that once thermal hyperesthesia has developed, thermal hyperesthesia is temporarily eliminated by the intrathecal injection of an NMDA receptor antagonist or an NO synthase inhibitor (the range of duration of action is between 1 h and 2 days).[3,20,21] Thus, after the establishment of the state of facilitation in the spinal cord, the facilitation is temporarily eliminated by the intrathecal injection of NMDA receptor antagonist or NO synthase inhibitor but is reestablished within 2 days after the drug administration. If the level of thermal hyperesthesia was tested on day 1, there may have been a short-term effect on the posttreatment NO synthase inhibitor. However, by testing on day 3, this transient effect was most likely missed.

In the L-NAME and the L-NA groups, the thermal hyperesthesia was not significantly different from that of the vehicle group 21 and 28 days after the nerve injury, respectively. Thus, even when NMDA receptor– and NO-mediated spinal facilitation is blocked by the pretreatment with NO synthase inhibitor, thermal hyperesthesia eventually develops after the nerve injury.

It has been reported that thermal hyperesthesia is prevented by locally blocking axonal transport with a topical application of colchicine, application of which has been shown to depolymerize the microtubules and disrupt the fast axonal transport.[22] This finding suggests that nerve injury may lead to the generation of trophic factors that are transported centrally from the lesioned region of the axon and that these transported substances may play a contributory role in the development and maintenance of thermal hyperesthesia.[22] NO synthase inhibitors may have no effect on the generation of trophic factors. Thus, after intrathecal injection of NO synthase inhibitors, thermal hyperesthesia eventually developed in both the pretreatment and the posttreatment studies. This finding suggests that a single preemptive treatment with an NO synthase inhibitor cannot block the thermal hyperesthesia over the long term, given the ongoing stimulus of the ligatures. As mentioned above, it has been reported that once thermal hyperesthesia has developed, thermal hyperesthesia is temporarily eliminated by the intrathecal injection of NO synthase inhibitor.[20] It may be that if the administration of an NO synthase inhibitor is maintained for an extended period of time, the thermal hyperesthesia may not develop. Thus, in addition to spinal facilitation induced by injury discharge and mediated by NMDA receptors and NO, other mechanisms, such as trophic factors, may play important roles in the development of thermal hyperesthesia induced by a nerve constriction injury.

In conclusion, this study demonstrates that NO plays an important role in the development of spinal facilitation induced by a nerve constriction injury. NO synthase inhibitor has no behavioral effect at the dose that delays development of thermal hyperesthesia induced by a nerve injury.

References

1. Arnér S, Meyerson BA: Lack of analgesia effect of opioids on neuropathic and idiopathic forms of pain. Pain 33:11–23, 1988

2. Bennett GJ, Xie YK: A peripheral mononeuropathy in rat that produces disorder of pain sensation like those seen in man. Pain 33: 87–107, 1988

3. Yamamoto T, Yaksh TL: Spinal pharmacology of thermal hyperesthesia induced by constriction injury of sciatic nerve: Excitatory amino acid antagonists. Pain 49:121–128, 1992

4. Yamamoto T, Shimoyama N, Mizuguchi T: Role of the injury discharge in the development of thermal hyperesthesia after sciatic nerve constriction injury in the rat. ANESTHESIOLOGY 79:993–1002, 1993

5. Yamamoto T, Shimoyama N, Asano H, Mizuguchi T: Time-dependent effect of morphine and time-independent effect of MK-801, an NMDA antagonist, on the thermal hyperesthesia induced by unilateral constriction injury to the sciatic nerve in the rat. ANESTHESIOLOGY 80:1311–1319, 1994

6. Dougherty PM, Garrison CJ, Carlton SM: Differential influence of local anesthetic upon two models of experimentally induced peripheral mononeuropathy in the rat. Brain Res 570:109–115, 1992

7. Watkins JC, Evans RH: Pharmacology of excitatory amino acid transmitters. Annu Rev Pharmacol Toxicol 21:165–204, 1981

8. Salt TE, Hill RG: Neurotransmitter candidates of somatosensory primary afferent fibers. Neuroscience 10:1083–1103, 1983

9. Garthwaite J: Glutamate, nitric oxide and cell-cell signalling in the nervous system. Trends Neurosci 14:60–67, 1991

10. Bredt DS, Snyder SH: Nitric oxide, a novel neuronal messenger. Neuron 8:2–11, 1992

11. Meller ST, Gebhart GF: Nitric oxide (NO) and nociceptive processing in the spinal cord. Pain 52:127–136, 1993

12. Yamamoto T, Yaksh TL: Comparison of the antinociceptive effects of pre- and posttreatment with intrathecal morphine and MK801, an NMDA antagonist, on the formalin test in the rat. ANESTHESIOLOGY 77:757–763, 1992

13. Coderre TJ, Melzack R: The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury. J Neurosci 12:3665–3670, 1992

14. Yamamoto T, Shimoyama N, Mizuguchi T: Nitric oxide synthase inhibitor blocks spinal sensitization induced by formalin injection into the rat paw. Anesth Analg 77:886–890, 1993

15. Malmberg AB, Yaksh TL: Spinal nitric oxide synthase inhibition blocks NMDA-induced thermal hyperalgesia and produces antinociception in the formalin test in rats. Pain 54:291–300, 1993

16. Yaksh TL, Rudy TA: Chronic catheterization of the spinal subarachnoid space. Physiol Behav 17:1031–1036, 1976

17. Hargreaves K, Dubner R, Brown F, Flores C, Joris J: A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 32:77–88, 1988

18. Tiseo PJ, Inturrisi CE: Attenuation and reversal of morphine tolerance by the competitive N-methyl-D-aspartate receptor antagonist, LY-274614. J Pharmacol Exp Ther 264:1090–1096, 1993

19. Li P, Tong C, Eisenach JC, Figueroa JP: NMDA causes release of nitric oxide from rat spinal cord in vitro. Brain Res 637:287–291, 1994

20. Meller ST, Pechman PS, Gebhart GF, Maves TJ: Nitric oxide mediates the thermal hyperalgesia produced in a model of neuropathic pain in the rat. Neuroscience 50:7–10, 1992

21. Mao J, Price DD, Mayer DJ, Lu J, Hayer RL: Intrathecal MK-801 and local nerve anesthesia synergistically reduce nociceptive behaviors in rats with experimental peripheral mononeuropathy. Brain Res 576:254–262, 1992

22. Yamamoto T, Yaksh TL: Effects of colchicine applied to the peripheral nerve on the thermal hyperesthesia evoked with chronic nerve constriction. Pain 55:227–233, 1993