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Role of the Injury Discharge in the Development of Thermal Hyperesthesia after Sciatic Nerve Constriction Injury in the Rat

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Background: **Usually, a barrage of impulses ("injury discharge") is evoked following sensory nerve damage. It has been suggested that injury discharge may produce the hyperexcitatory state in the spinal cord, and this hyperexcitability may cause neurogenic pain. In the present study, the authors examined the role of injury discharge in developing the hyperesthetic state following nerve constriction injury.**

Methods: **A model of thermal hyperesthesia caused by a constriction injury created by making four loose ligations around the rat sciatic nerve was examined. To block the injury discharge, 0.5% bupivacaine was applied to the sciatic nerve before constriction injury. To block the hyperexcitatory state, (+)-MK-801, an N-methyl-D-aspartate antagonist, was administered intrathecally 15 min before the nerve lesion.**

Results: **Blocking injury discharge significantly delayed the development of hyperesthesia. Bupivacaine had no effect on the development of hyperesthesia when bupivacaine was applied to the sciatic nerve 15 min after the nerve constriction injury. Systemic bupivacaine had no effect on the development of thermal hyperesthesia. Intrathecal (+)-MK-801 also delayed the development of hyperesthesia when (+)-MK-801 was administered intrathecally 15 min before the nerve injury. When (+)-MK-801 was administered 15 min after the nerve injury, (+)-MK-801 had no effect on the development of hyperesthesia.**

Conclusion: **These results suggest that injury discharge may induce facilitation of spinal dorsal horn neurons, and this spinal facilitation may play an important role in developing thermal hyperesthesia following sciatic nerve constriction injury. (Key words: Hyperesthesia. Nerve, injury: injury discharge. Pain, neuropathic. Receptor: N-methyl-D-aspartate.**

This article is accompanied by a Highlight. Please see this issue of ANESTHESIOLOGY, page 28A.

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WHEN sensory fibers are damaged, a barrage of impulses (injury discharge) that lasts many seconds or even several minutes is emitted.[1] Self-mutilation (autotomy) induced by total denervation of a hind paw is thought to be one of the neurogenic pain models,[2] and autotomy development has been reported to be inhibited by blocking injury discharge with topically applied local anesthetics.[3,4] Thus, injury discharge is thought to play an important role in triggering autotomy. Though injury discharge is only of short duration, injury discharge in C-fibers sets off a process of long-lasting, widespread hyperexcitability of the dorsal horn interneurons,[5–7] and this hyperexcitability may induce autotomy.[4,8]

The N-methyl-D-aspartate (NMDA) receptor is one of the receptors of excitatory amino acids, such as glutamate, and is now thought to be involved in the transmission of nociceptive information in the spinal cord.[9,10] Activation of chemosensitive afferents with chemical irritants, such as mustard oil, generates a state of central sensitization in the spinal cord,[6,11] and this hyperexcitability is blocked by the NMDA antagonist MK-801.[12] Intrathecal MK-801 administered just before neurectomy suppressed autotomy development.[8] This suggested that the NMDA antagonist, administered intrathecally just before the nerve injury, modified the hyperexcitability level of the spinal cord neurons that was induced by injury discharge.[8]

The autotomy model represents one of the sensory disorders associated with complete deafferentation. The disorders of pain sensation associated with complete deafferentation do not include all of those that occur in neuropathies that spare some of the nerve's normal connections with the periphery. For example, hyperalgesia and allodynia are the prominent symptoms in many peripheral neuropathies, but in cases of complete

deafferentation, hyperalgesia and allodynia are not observed. Recently, it has been shown that ipsilateral thermal and mechanical hyperesthesia occur 5–7 days following the constriction injury created by making four loose ligations around the sciatic nerve in the rat.[13,14] Though the specific mechanisms underlying the thermal hyperesthesia following this constriction injury are not known, several lines of evidence have indicated that the peripheral nerve lesion may evoke a major reorganization of dorsal horn function. Histologic examination of the nerve after constriction injury showed that all of the large Aβ axons and a large percentage of the Aδ axons were damaged, but most of the C-fibers appeared anatomically intact.[15–17] Electrophysiologic studies revealed that ectopic discharges occur in the damaged myelinated primary afferent nerves after loose sciatic nerve constriction injury,[18,19] and this spontaneous discharge was reported to originate in the dorsal root ganglion.[20] In the spinal dorsal horn, there are time-dependent, transsynaptic changes in morphology.[21]

Nerve constriction injury appears to induce injury discharge. Brief twitching of the muscle surrounding the exposed nerve was observed during nerve constriction injury surgery,[14] and this muscle twitching may be caused by injury discharge. If injury discharge induces the hyperexcitability in the spinal cord and this hyperexcitability produces thermal hyperesthesia following nerve constriction injury, then it may be prevented by either blocking injury discharge with a topical application of an local anesthetic or modifying the hyperexcitability level with an intrathecal injection of an NMDA antagonist. In the present study, therefore, we sought to define the role of injury discharge in the development of the thermal hyperesthesia induced by sciatic nerve constriction injury, by topical application of a local anesthetic to the sciatic nerve or intrathecal injection of an NMDA antagonist.

Methods

The following investigations were carried out under a protocol approved by the Institutional Animal Care Committee of Chiba University.

Animal Preparation

The hyperesthetic state was induced by chronic constriction of the sciatic nerve using four loose ligatures.[14] Anesthesia was induced by inhalation with 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 mid thigh to expose the sciatic nerves. Each nerve was carefully mobilized, with care taken to avoid undue stretching. Four 4-0 chromic gut sutures were each tied loosely with a square knot around the right sciatic nerve. The left sciatic nerve was only mobilized. Both incisions were closed layer to layer with 3-0 silk sutures, and the rats were allowed to recover from anesthetics.

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

Nociceptive Threshold

The thermal nociceptive threshold was measured with a device similar to that used previously.[22] The rats were placed beneath a clear plastic cage (10 × 20 × 24 cm) upon an elevated floor of clear glass (2 mm thick). A radiant heat source (Eye projector halogen lamp JRC-12V-100W, Iwasaki Electric, Tokyo, Japan) 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 temperatures 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 plate temperature was regulated at 30° C. The calibration of the thermal test system is such that the average response latency (±SD) in ten normal untreated rats was maintained at 10 ± 0.5 s prior to 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 heat source beneath the floor was then positioned so that it focused at the plantar surface of one hind paw that was in contact with the glass. Care was taken not to focus the light source on the skin that was off the glass plate. The light was activated, which initiated a timing circuit. The interval between the application of the light beam and the brisk hind paw withdrawal 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. This value was assigned as its response latency.

General Behavior

General behavior was evaluated every day during the study by a scoring system of two types of behavior; normal and mildly to severely impaired.

1. Placing/stepping reflex: This response was evoked by drawing the dorsum of either hind paw across the edge of a table. This stimulus elicits an upward lifting of the paw from the surface of the table (stepping).
2. Righting reflex: A rat placed horizontally with its back on a table normally will show an immediate coordinated twisting of the body around its longitudinal axis to regain its normal posture.

Injury discharge during the nerve constriction injury was evaluated by the presence (+) or absence (−) of a brief twitching of the muscle surrounding the exposed nerve.

Experimental Protocol

Before sciatic nerve constriction injury, both right and left hind paws were tested three times alternately, with 5-min intervals between consecutive tests, to obtain the baseline data. The average of three measurements was defined as paw withdrawal latency (PWL).

Pretreatment Study.

Local Anesthetic Study. In the blocked nerve (BLOCK) group (n = 15), 0.5 ml 0.5% bupivacaine (Fujisawa, Osaka, Japan) was locally infiltrated intra- and subcutaneously and in deep subdermal structures before the skin incision. After the right sciatic nerve was mobilized and before the sciatic nerve loose ligations, a small pad of Gelfoam (Upjohn, Kalamazoo, MI) soaked in 0.5 ml 0.5% bupivacaine was placed around the intact epineurium of the right sciatic nerve, forming a continuous meniscus, for 10 min. In the control (CONT) group (n = 15), 0.5 ml normal saline was locally infiltrated intra- and subcutaneously and in deep subdermal structures before the skin incision, and a small pad of Gelfoam soaked in 0.5 ml normal saline was placed around the intact epineurium, forming a continuous meniscus, for 10 min, after the right sciatic nerve was mobilized and before the sciatic nerve loose ligations.

The post-surgery PWLs of right and left hind paws were measured alternately 7 days after the nerve lesion. In 12 rats of each group, the post-surgery PWLs of right and left paws were also tested 14 days after the nerve injury, and in 6 rats of each group, the post-surgery PWLs of right and left hind paws were measured 4 and 21 days after the nerve injury.

Two additional groups of rats were used to define the effect of systemic bupivacaine on the development of thermal hyperesthesia. In one group (n = 6), 1.0 ml 0.5% bupivacaine was injected intraperitoneally just before the nerve constriction injury (IP BUP group). In the other group (n = 6), 1.0 ml saline was injected intraperitoneally just before the nerve constriction injury (IP SAL group). The post-surgery PWLs of right and left hind paws were measured alternately 7 days after the nerve lesion.

NMDA Antagonist Study. Animals were prepared with chronic catheters in the lumbar subarachnoid space 7 days before the nerve constriction injury.[23] Briefly, under isoflurane anesthesia, a PE-10 catheter was advanced through an incision in the atlanto-occipital membrane to a position 8 cm caudal to the cisterna, placing the tip at the level of the lumbar enlargement. The catheter was externalized on the top of the skull and sealed with a piece of steel wire. The wound was closed with 3-0 silk sutures. Rats showing neurologic deficits postoperatively were discarded.

In the group administered (+)-MK-801 (MK group, n = 19), 10 μg (+)-MK-801 (Research Biochemicals, Natick, MA) was administered intrathecally 15 min before the nerve constriction injury. This dose was selected on the basis of previous studies.[24,25] (+)MK-801 was dissolved in normal saline at a concentration of 1 mg/ml and was administered intrathecally in a volume of 10 μl of vehicle. In the group administered saline (SALINE group, n = 18), 10 μl of normal saline was injected intrathecally 15 min before the nerve constriction injury.

The post-surgery PWLs of right and left hind paws were measured alternately 7 days after the nerve lesion. In 13 rats of each group, the post-surgery PWLs of right and left paws were also tested 14 and 21 days after the nerve injury, and in 6 rats of each group, the post-surgery PWLs of right and left hind paws were measured 4 and 28 days after the nerve injury.

An additional group of rats was used to define the stereospecificity of (−)-MK-801 effects on the development of thermal hyperesthesia ((−)-MK group, n = 6). Ten micrograms (−)-MK-801 (Research Biochemicals), an inactive isomer of (+)-MK-801, was administered intrathecally 15 min before the nerve constriction injury in a volume of 10 μl of vehicle. The post-

surgery PWLs of right and left paws were measured alternately 7 days after the nerve lesion.

Post-treatment Study.

Local Anesthetic Study. In the blocked nerve (BLOCK-post) group (n = 8), 15 min after the nerve injury, a small pad of Gelfoam soaked in 0.5 ml 0.5% bupivacaine was placed around the nerve constriction injury, forming a continuous meniscus, for 10 min. In the control (CONT-post) group (n = 8), a small pad of Gelfoam soaked in 0.5 ml normal saline was placed around the nerve constriction injury 15 min after the nerve injury, forming a continuous meniscus, for 10 min. The post-surgery PWLs of right and left hind paws were tested alternately 7 days after the nerve lesion.

NMDA Antagonist Study. Animals were prepared with chronic catheters in the lumbar subarachnoid space 7 days before the nerve constriction injury.[23] In the group administered (+)-MK-801 (MK-post group, n = 8), 10 μg (+)-MK-801 was administered intrathecally 15 min after the nerve constriction injury in a volume of 10 μl of vehicle. In the group administered saline (SALINE-post group, n = 8), 10 μl of normal saline was injected intrathecally 15 min after the nerve constriction injury. The post-surgery PWLs of right and left hind paws were tested alternately 7 days after the nerve lesion.

Electrophysiologic Study. To determine whether the injury discharge was evoked by nerve constriction injury and whether the topical application of bupivacaine blocked the generation of the injury discharge evoked by nerve constriction injury, the animals were prepared for acute electrophysiologic study under isoflurane anesthesia.

Anesthesia was induced by inhalation of 5% isoflurane, maintained at a concentration of 2%. A tracheostomy was performed. The animal was anesthetized with oxygen and 2% isoflurane, paralyzed with pancuronium bromide (1 mg/kg intraperitoneally), and artificially respired with continuous monitoring of heart rate and expired carbon dioxide. After local incision, the biceps femoralis of the right leg was bluntly dissected at mid thigh to expose the right sciatic nerve. The right sciatic nerve was carefully mobilized. After mobilizing the right sciatic nerve, a small pad of Gelfoam soaked in either 0.5 ml 0.5% bupivacaine (n = 3) or 0.5 ml saline (n = 5) was placed around the intact epineurium of right sciatic nerve, forming a continuous meniscus, for 10 min. An oil pool was made to cover the mobilized right sciatic nerve. A bipolar platinum recording electrode was placed on the mobilized right sciatic nerve which was covered with an oil pool. One 4-0 chromic gut suture was tied loosely with a square knot around the right sciatic nerve 5 mm distal from the recording electrode.

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, *i.e.,* hyperesthesia. In the pretreatment study, the presurgery (day 0) and days 4, 7, 14, 21, and 28 levels of thermal hyperesthesia were compared among the rats in each group with one-way analysis of variance. For multiple comparisons, we used the Dunnett test. In the post-treatment study, the presurgery (day 0) and day 7 levels of thermal hyperesthesia were compared among the rats in each group with the *t* test. To compare the presurgery (day 0) and days 4, 7, 14, 21, and 28 PWLs and DSs between groups, the *t* test was used. To analyze the general behavior data, we used chi-square analysis. Critical values that reached a $P < 0.05$ level of significance were considered statistically significant.

Results

Pretreatment Study

Local Anesthetic Study.

General Behavior. In the BLOCK group, no brief twitching of the muscle surrounding the exposed nerve was observed during nerve constriction injury surgery. In the CONT group, brief twitching of the muscle surrounding the exposed nerve was observed during surgery. In both the BLOCK and the CONT groups, no placing, stepping, or righting reflex impairment was found 4, 7, 14, or 21 days after the nerve constriction injury.

In the IP BUP and the IP SAL groups, brief twitching of the muscle surrounding the exposed nerve was observed during nerve constriction injury surgery. Neither intraperitoneal bupivacaine nor intraperitoneal saline had an effect on placing, stepping, or righting reflexes 7 days after the nerve constriction injury.

Thermal Hyperesthesia. Table 1 shows the levels of presurgery right and left PWLs and DSs in both the

Table 1. Presurgery Right- and Left-paw Withdrawal Latency and Difference Scores

	BLOCK (n = 15)	CONT (n = 15)	MK-801 (n = 19)	SALINE (n = 18)
Right PWL(s)	10.0 ± 1.1	10.0 ± 0.8	9.8 ± 0.8	10.0 ± 0.9
Left PWL(s)	9.9 ± 0.9	10.1 ± 0.7	9.9 ± 0.7	10.0 ± 0.9
Difference score(s)	0.0 ± 0.5	−0.1 ± 0.3	−0.1 ± 0.3	−0.1 ± 0.4

Values are mean ± SD.

PWL = paw withdrawal latency.

BLOCK and the CONT groups. There is no difference between presurgery right and left PWLs and DSs of the BLOCK group and that of the CONT group.

In the BLOCK group, the DS levels on days 4 and 7 are the same as the presurgery DS level, but the DS levels on days 14 and 21 are significantly more negative than the presurgery DS level (fig. 1). In the CONT group, DS levels on days 4, 7, 14, and 21 are significantly more negative than the presurgery levels (fig. 1). On days 4 and 7 after the nerve constriction injury, the DS levels of the CONT group are significantly more negative than that of the BLOCK group, but on days 14 and 21 after the nerve injury, there is no difference between the DS levels (±SD) of the CONT group (day 14 −2.8 ± 0.7 s, day 21 −2.6 ± 1.1 s) and those of the BLOCK group (day 14 −1.8 ± 1.6 s, day 21 −2.6 ± 1.7 s; fig. 1).

The presurgery DS level of the IP BUP group is the same as that of the IP SAL group. Seven days after the nerve lesion, thermal hyperesthesia developed, and the DS levels of the IP BUP group and the IP SAL group are the same (fig. 2).

NMDA Antagonist Study.

General Behavior. In the MK, SALINE, and (−)-MK groups, brief twitching of the muscle surrounding the exposed nerve was observed during surgery. Neither intrathecal (+)-MK-801 (10 μg) nor intrathecal (−)-MK-801 (10 μg) had an effect on the placing, stepping, or righting reflexes 4, 7, 14, 21, or 28 days after the nerve constriction injury.

Thermal Hyperesthesia. Table 1 shows the levels of presurgery right and left PWLs and DSs in both the MK and the SALINE groups. There is no difference between presurgery right and left PWLs and DSs of the MK group and that of the SALINE group.

In the MK group, the DS levels on days 4 and 7 are the same as the presurgery DS level, but the DS levels on days 14, 21, and 28 are significantly more negative than the presurgery DS level (fig. 3). In the SALINE group, the DS levels on days 4, 7, 14, 21, and 28 are significantly more negative than the presurgery levels (fig. 3). On days 4, 7, 14, and 21 after nerve constriction injury, the DS levels of the SALINE group are significantly more negative than those of the MK group, but on day 28 after the nerve injury, there is no difference between the DS level (±SD) of the SALINE group (−3.0 ± 1.2 s) and that of the MK group (−2.8 ± 1.3 s; fig. 3).

The presurgery DS level of the (−)-MK group is the same as that of the SALINE group. Seven days after the nerve lesion, thermal hyperesthesia developed, and the DS level (±SD) of the (−)-MK group (−3.2 ± 1.2 s) is the same as that of the SALINE group (−3.0 ± 0.9 s).

Post-treatment Study

Local Anesthetic Study.

General Behavior. In the BLOCK-post group and CONT-post group, brief twitching of the muscle surrounding the exposed nerve was observed during nerve constriction injury surgery. In both the BLOCK-post and the CONT-post groups, no placing, stepping, or righting reflex impairment was found 7 days after the nerve constriction injury.

Thermal Hyperesthesia. The presurgery DS level of the BLOCK-post group is the same as that of the CONT-post group. Seven days after the nerve lesion, thermal hyperesthesia developed, and the DS level of the BLOCK-post group is the same as that of the CONT-post group (fig. 4).

NMDA Antagonist Study.

General Behavior. In the MK-post and the SALINE-post groups, brief twitching of the muscle surrounding the exposed nerve was observed during nerve constriction injury surgery. In both the MK-post and the SALINE-post groups, no placing, stepping, or righting reflex

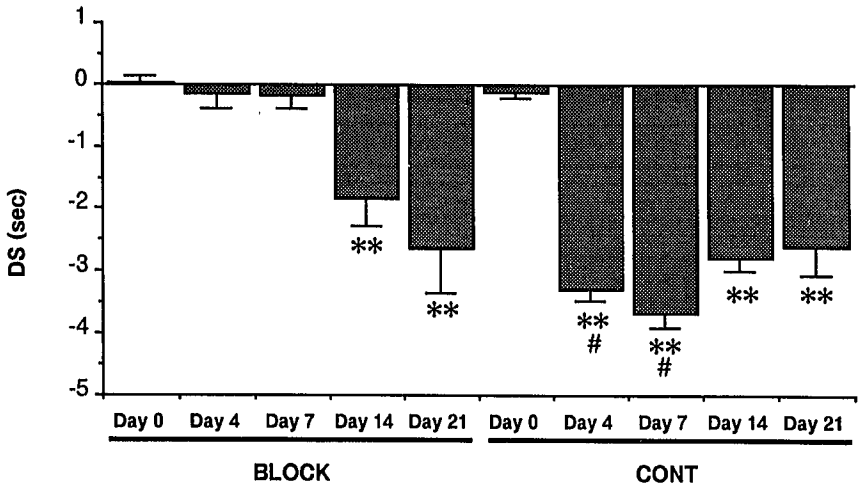

Fig. 1. Effects of local application of bupivacaine to the sciatic nerve on thermal hyperesthesia in the pretreatment study. The bars show the group mean ± SEM of difference scores (DSs). BLOCK = BLOCK group; CONT = control group. Day 0 = presurgery; Day 4 = 4 days after the nerve lesion; Day 7 = 7 days after the nerve lesion; Day 14 = 14 days after the nerve lesion; Day 21 = 21 days after the nerve lesion. **$P < 0.01$ as compared to day 0 of the same group. #$P < 0.05$ as compared to days 4 and 7 of the BLOCK group.

impairment was found 7 days after the nerve constriction injury.

Thermal Hyperesthesia. The presurgery DS level of the MK-post group is the same as that of the SALINE-post group. Seven days after the nerve lesion, thermal hyperesthesia developed, and the DS level of the MK-post group is the same as that of the SALINE-post group (fig. 5).

Electrophysiologic Study. After saline treatment, the constriction injury evoked injury discharge. Figure 6 illustrated a typical result. The mean (±SD) duration of injury discharge was 26 ± 18 s, and the mean number of spikes (±SD) was 1,420 ± 740. After bupivacaine treatment, no injury discharge was evoked by the nerve constriction injury.

Discussion

Results from the present study demonstrate that topical application of bupivacaine, but not systemic administration of bupivacaine, blocks the development of thermal hyperesthesia 4 and 7 days after nerve constriction injury when bupivacaine was applied 15 min before the nerve injury. We also found that intrathecal (+)-MK-801, but not (−)-MK-801, administered 15 min before the nerve injury, prevents the development of thermal hyperesthesia 4 and 7 days after the nerve lesion. In this study, blinding was not included. We recognized that the absence of blinding decreased the level of objectivity of the behavioral assessment, though our data clearly demonstrated the effect of the MK-801 or bupivacaine on the development of the thermal hyperesthesia.

Local Anesthetic Study

Bupivacaine has been reported to block the injury discharge resulting from sciatic nerve injury.[26] In the present study, we found no brief twitching of the muscle surrounding the exposed nerve in the BLOCK group. In the electrophysiologic study, an electrode that was placed 5 mm proximal from the nerve constriction injury detected the injury discharge evoked

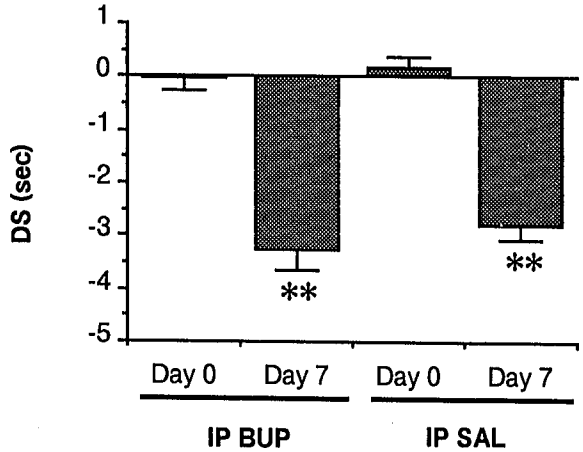

Fig. 2. Effects of intraperitoneal bupivacaine on thermal hyperesthesia. The bars show the group mean ± SEM of difference scores (DSs). IP BUP = IP BUP group; IP SAL = IP SAL group. Day 0 = presurgery; Day 7 = 7 days after the nerve lesion. **$P < 0.01$ as compared to day 0 of the same group.

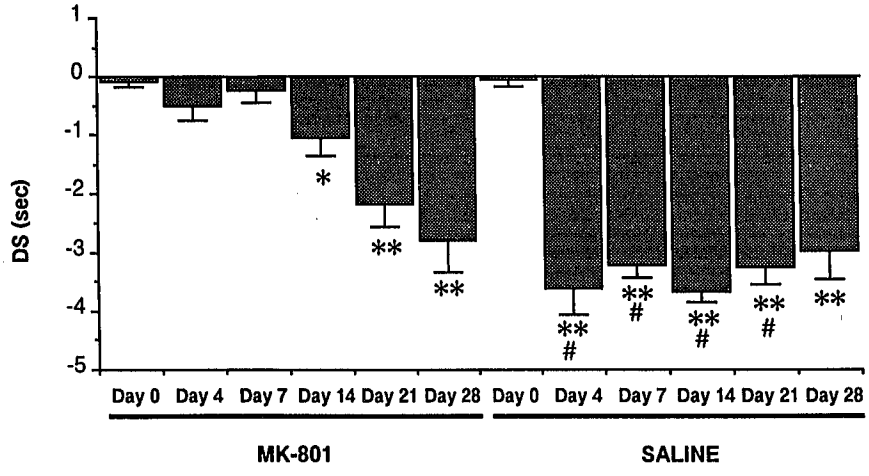

Fig. 3. Effects of intrathecal (+)-MK-801 on thermal hyperesthesia in the pretreatment study. The bars show the group mean ± SEM of difference scores (DSs). MK-801 = MK group; SALINE = SALINE group. Day 0 = presurgery; Day 4 = 4 days after the nerve lesion; Day 7 = 7 days after the nerve lesion; Day 14 = 14 days after the nerve lesion; Day 21 = 21 days after the nerve lesion; Day 28 = 28 days after the nerve lesion. *$P < 0.05$ as compared to day 0 of the same group. **$P < 0.01$ as compared to day 0 of the same group. #$P < 0.05$ as compared to days 4, 7, 14, and 21 of the MK group.

by constriction injury, and bupivacaine topically applied to the sciatic nerve blocked the generation of the injury discharge. Thus, we think that the nerve constriction injury evoked the injury discharge, that the injury discharge may be transmitted to the spinal cord, and that the topical application of bupivacaine blocked the injury discharge evoked by the nerve constriction injury in the present study.

In the IP BUP group, thermal hyperesthesia developed after the nerve injury, and the level of the thermal hyperesthesia observed in the IP BUP group 7 days after the nerve constriction injury was the same as that in the IP SAL group. The amount of bupivacaine applied to the sciatic nerve in the BLOCK group was the same as that injected intraperitoneally in the IP BUP group. The discrepancy between the effect of topically applied bupivacaine and that of intraperitoneally injected bupivacaine was due to the different route of drug administration. Thus, we conclude that bupivacaine applied to the sciatic nerve worked at the sciatic nerve and delayed the development of thermal hyperesthesia in the BLOCK group. In the post-treatment study, top-

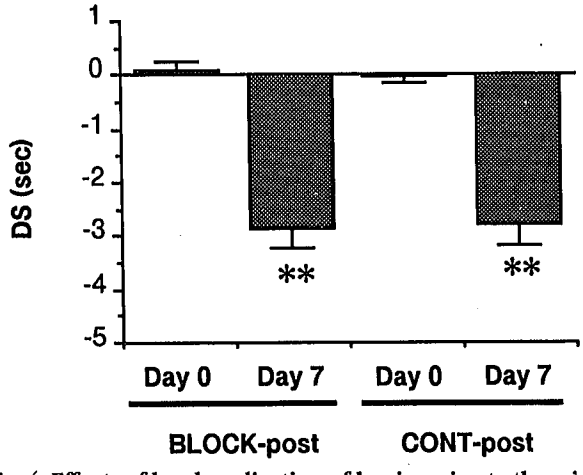

Fig. 4. Effects of local application of bupivacaine to the sciatic nerve on thermal hyperesthesia in the post-treatment study. The bars show the group mean ± SEM of difference scores (DSs). BLOCK-post = BLOCK-post group; CONT-post = CONT-post group. Day 0 = presurgery; Day 7 = 7 days after the nerve lesion. **$P < 0.01$ as compared to day 0 of the same group.

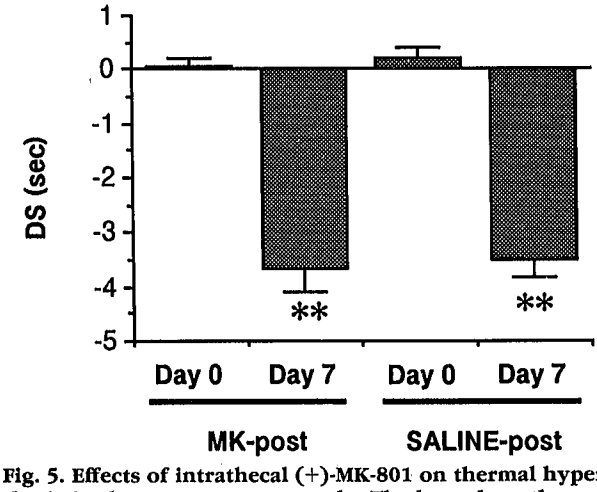

Fig. 5. Effects of intrathecal (+)-MK-801 on thermal hyperesthesia in the post-treatment study. The bars show the group mean ± SEM of difference scores (DSs). MK-post = MK-post group; SALINE-post = SALINE-post group. Day 0 = presurgery; Day 7 = 7 days after the nerve lesion. **$P < 0.01$ as compared to day 0 of the same group.

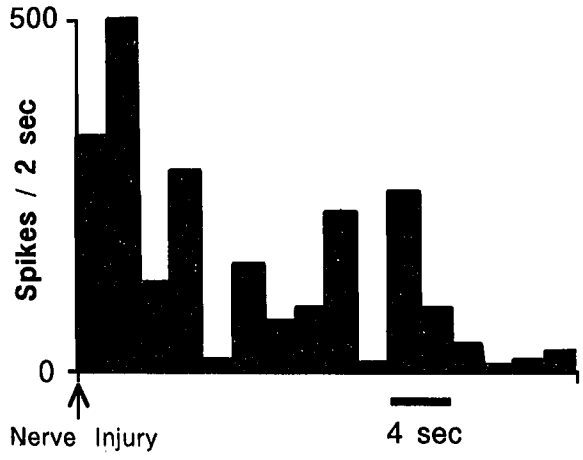

Fig. 6. Typical histogram of the discharge induced by the nerve constriction injury.

ical application of bupivacaine did not affect the development of thermal hyperesthesia. These data suggest that the topically applied bupivacaine in the BLOCK group worked during the first 15 min after the nerve injury. The electrophysiologic study revealed that the duration of the injury discharge evoked by nerve constriction injury was 26 s. Thus, we conclude that the effect of bupivacaine on the hyperesthetic state in the BLOCK group was due to the effect of bupivacaine on the injury discharge and that the injury discharge during the nerve constriction injury is an important trigger of the thermal hyperesthesia following nerve constriction injury. Dougherty *et al.*[27] reported that, when topically applied to the sciatic nerve before the nerve constriction injury, lidocaine reduced both the duration and the magnitude of the thermal hyperesthesia. This observation is consistent with our finding.

It has been reported that bupivacaine (0.5%, 0.6 ml), injected to the sciatic nerve ipsilateral to the injury 3 days after constriction injury, attenuated thermal hyperesthesia for less than 2 days.[28] These data suggest that, after the development of thermal hyperesthesia, ongoing abnormal peripheral input may be derived from the injured sciatic nerve because of the continuous constriction, and this input may play an important role in maintaining the hyperesthetic state after nerve constriction injury.[28] In the present study, development of thermal hyperesthesia was blocked for more than 7 days after nerve constriction injury when bupivacaine was topically applied during the surgery. Thus, we think that injury discharge may be critical for developing the hyperesthetic state and that an ongoing abnormal peripheral input from the injured sciatic nerve may be necessary for maintaining it.

NMDA Antagonist Study

It has been reported that intrathecal MK-801 temporarily eliminates thermal hyperesthesia following nerve constriction injury (the range of duration of action is between 1 h and 2 days), once thermal hyperesthesia is established.[24,28] Davar *et al.*[29] reported that, when MK-801 (1.0 mg/kg) was injected intraperitoneally before and then for 7 days after the nerve constriction injury, thermal hyperesthesia did not develop in the animals treated with MK-801. In the present study, intrathecal (+)-MK-801 prevented the development of thermal hyperesthesia for more than 7 days after nerve constriction injury when (+)-MK-801 was administered 15 min before the nerve lesion, and this MK-801 effect was stereospecific. When (+)-MK-801 was administered intrathecally 15 min after the nerve injury, (+)-MK-801 had no effect on the development of thermal hyperesthesia following the nerve constriction injury. These findings showed that the nerve constriction injury is critical in the development of thermal hyperesthesia. Repetitive input from C-fibers can evoke a powerful and spinally mediated facilitation (wind-up) of the dorsal horn wide dynamic range neurons,[30] and NMDA antagonists block this wind-up phenomenon.[31] We think that injury discharge during constriction injury may induce the "wind-up"-like facilitation in the spinal cord, and this facilitation may be the major cause of the development of thermal hyperesthesia following nerve constriction injury. Though intrathecal (+)-MK-801 appears to block the established injury discharge-dependent facilitation of dorsal horn neurons, the blockage is only transient. Conversely, when the development of the facilitation is suppressed by a single preinjury administration of (+)-MK-801, additional post-injury administration is not necessary for the maintenance of the normoesthetic state. Thus, either blocking the "wind-up"-like facilitation by intrathecal administration of an NMDA antagonist 15 min before nerve injury or blocking the injury discharge by topical application of local anesthetics during the surgery prevents the development of thermal hyperesthesia 7 days after nerve constriction injury.

In the post-treatment study, intrathecal MK-801 had no effect on the development of thermal hyperesthesia.

These results suggest that, once an injury discharge-evoked spinal facilitation is initiated, subsequent NMDA antagonism does not block the development of thermal hyperesthesia. This suggested that the spinal facilitation, following initiation by injury discharge, reflects a sustained process. This observation is consistent with a recent report that the intrathecal injection of NMDA will evoke a significant dose-dependent hyperesthesia that is antagonized by pre- but not post-treatment with NMDA antagonists.[32]

In both the BLOCK and the MK groups, thermal hyperesthesia developed 14 days after the nerve injury. In the local anesthetic study, there is no significant difference between the DS levels of the BLOCK group and that of the CONT group on days 14 and 21. In the NMDA antagonist study, there is no significant difference between the DS level of the MK group and that of the SALINE group on day 28. It has been reported that the thermal hyperesthesia secondary to nerve constriction injury was prevented by locally blocking axonal transport with a topical application of colchicine, which has been shown to depolymerize the microtubules and disrupt the fast axonal transport to the nerve central, but not peripheral, to the injury.[17] This suggests that nerve injury may lead to the generation of trophic factors that are transported centrally from the lesioned region of the axon, and these transported substances may play a contributory role in developing and maintaining thermal hyperesthesia.[17] Intrathecal strychnine (glycine antagonist) administered just after the nerve lesion and on days 1 and 2 after the nerve lesion significantly enhanced the thermal hyperesthesia normally observed on day 7, as compared to intrathecal saline.[17] This suggests that the loss of a spinal strychnine-sensitive inhibition augments the development of hyperesthesia induced by chronic nerve constriction.[17] Thus, not only injury discharge but also other mechanisms, such as trophic factors and/or spinal glycine inhibition, may play important roles in developing thermal hyperesthesia following nerve constriction injury.

It has been reported that spinal facilitation induced by surgical incision and other noxious inputs during surgical operation aggravate postoperative pain and that preemptive analgesia attenuates or prevents the development of spinal facilitation.[33] This observation suggests that the spinal facilitation induced by tissue injury plays an important role in perception of pain in a clinical situation.

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