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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

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Background: **It is known that peripheral nerve injury induces time-dependent changes in dorsal horn function. The current study investigated the time dependency of the effects of intrathecal morphine and MK-801, an N-methyl-D-aspartate antagonist, on the thermal hyperesthesia evoked by unilateral constriction injury to the sciatic nerve in the rat.**

Methods: **In rats with a unilateral constriction injury to the sciatic nerve, paw withdrawal latency against thermal stimulation for the injured paw was typically 3 s less than that for the uninjured paw during the first 5 weeks after the injury. Drugs were administered intrathecally 1 or 5 weeks after the nerve injury.**

Results: **Intrathecal morphine increased the paw withdrawal latencies of both the injured paw and the uninjured paw in an equally dose dependent manner in the 1-week study. In the 5-week study, morphine increased the paw withdrawal latency of the uninjured paw in a dose-dependent manner, but not that of the injured paw. Intrathecal MK-801 increased the paw withdrawal latency of the injured paw to the level of the uninjured paw in a dose-dependent manner in both the 1- and 5-week studies.**

Conclusions: **These data indicate that (1) an N-methyl-D-aspartate receptor–mediated spinal facilitation may be the common mechanism maintaining the thermal hyperesthesia evoked by the constriction injury, and (2) the effects of intrathecal morphine on this thermal hyperesthesia are time-dependent. (Key words: Analgesia, opioid: morphine. Antagonists, N-methyl-D-aspartate: MK-801. Nerve, injury: hyperesthesia.)**

IN humans, incomplete injury to a peripheral nerve can give rise to neuropathic pain, which may be constant, intermittent, or paroxysmal, with a burning, sharp, or aching sensation. The effectiveness of opioids on neuropathic pain is still controversial. Although Arnér and Meyerson[1] reported that neuropathic pain appears to be relatively refractory to standard analgesics such as opioids, Portenoy *et al.*[2] reported that some patients with neuropathic pain were effectively treated with opioids.

Self-mutilation (autotomy) after peripheral nerve section in the rat has been presented as a model of neuropathic pain in humans,[3] and the effect of oral morphine on autotomy changes in a time-dependent manner.[4,5] Although 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.

It has been shown that time-dependent thermal and mechanical hyperesthesia occurs after the creation of a local injury by unilateral constriction of the sciatic nerve in the rat.[6,7] In this constriction injury model, histologic examination revealed that all of the large Aβ axons and a large percentage of the Aδ axons were damaged, and unmyelinated fiber was also damaged markedly.[8–12] We have observed that, 1 week after the nerve constriction injury, μ and δ agonists have no selective effect upon the observed hyperesthesia.[13] On the other hand, thermal hyperesthesia, but not the normal thermal escape response, can be selectively blocked by the intrathecal injection of N-methyl-D-aspartate (NMDA) antagonists, such as MK-801 and ketamine, 1 week after nerve constriction injury.[14,15]

Although the specific mechanisms underlying the thermal hyperesthesia after this constriction injury are not known, several sets of data have indicated that the peripheral nerve injury evokes a time-dependent reorganization of the spinal dorsal horn functions and of the peripheral nerve. In the spinal dorsal horn, there

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are time-dependent, transsynaptic changes in morphology.[16] The substance P staining density in the spinal dorsal horn is reduced 7 and 14 days after creation of the nerve lesion,[17] but the level of calcitonin gene–related peptide is unchanged 7 and 14 days after the nerve lesion.[17,18] In the peripheral nerve, it has been reported that time-dependent alterations in the sympathetic innervation of the injured paw occurred in the constriction injury model.[19] Thus, it is possible that the effects of opioids and NMDA antagonists on the thermal hyperesthesia after nerve constriction injury may change in a time-dependent manner. In the current study, to clarify whether the effects of morphine and MK-801 on the hyperesthesia after nerve constriction injury are time-dependent or not, we studied the effects of intrathecally administered morphine and MK-801 1 and 5 weeks after the nerve constriction injury.

Materials and Methods

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

Intrathecal Catheters

Chronic intrathecal catheters were inserted, during isoflurane anesthesia by passing a PE-10 catheter through an incision in the atlantooccipital membrane to a position 8 cm caudal to the cisterna at the level of the lumbar enlargement.[20] 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.

Nerve Constriction Injury

The hyperesthetic state was induced by chronic constriction of the sciatic nerve with four loose ligatures.[6,7] Anesthesia was induced by inhalation of 5% 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 were then 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 receiving the 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 showed a mild eversion of the affected paw and a mild to moderate degree of foot drop (*i.e.*, weakness of the hind paw's dorsiflexors). All animals postoperatively displayed normal feeding and drinking.

Thermal Nociceptive Test

Paw withdrawal latency (PWL) against thermal stimulation was measured with a device similar to that previously used.[21] 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) 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 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 the skin that was off of the glass plate. The light was then activated, initiating a timing circuit. The interval between the application of the light beam and the brisk hind paw withdrawal response was manually 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 then assigned as the response latency.

Motor Function

Motor function was evaluated by the performance of two specific behavioral tasks.[22] (1) Placing–stepping reflex: this response 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). Animals with any degree of hind limb flaccidity demonstrate an altered reflex or no reflex. (2) 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 the legs. Animals displaying ataxic behavior show a decreased ability to right themselves. 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 more slowly than normal animals were assigned a score of 1.

Experimental Protocol

In these experiments, the effects of intrathecally administered agents on the PWL was assessed in the normal (left sciatic nerve uninjured) and hyperesthetic (right sciatic nerve injured) paws of operated rats. The PWLs of the injured and uninjured paws were measured before the nerve constriction injury and 1, 4, and 5 weeks after the nerve injury to determine the time course of the level of thermal hyperesthesia evoked by the nerve constriction injury.

Consistent with a previous report,[6] a preliminary study carried out in our laboratory revealed that the maximum thermal hyperesthesia occurred between 7 and 42 days after the nerve constriction injury. The animals were divided into six groups: 1- and 5-week studies for testing morphine; 1- and 5-week studies for testing MK-801; and 1- and 5-week studies for testing naloxone on the morphine analgesia effect. Four to six rats were tested for the effects of drugs administered intrathecally at each dose of the drugs. Each hyperesthetic animal received one medication administered intrathecally at two time points: 7 and 11 days after creation of the nerve lesion in the 1-week study or 35 and 39 days after the nerve lesion in the 5-week study. In the 1-week study, an intrathecal catheter was inserted 3 days before the nerve constriction injury. In the 5-week study, an intrathecal catheter was inserted 4 weeks after the nerve lesion. Before 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 once at 5, 15, 30, 60, and 90 min after the injection.

To verify that the analgesic effects of the intrathecally administered morphine was due to the interaction with an opioid receptor, the highest dose of morphine was administered intrathecally, followed 30 min later by intraperitoneal injection of naloxone. To assess the effect of naloxone on morphine analgesia, left and right paws were tested 5 min after naloxone injection.

Drugs and Injection

The agents administered intrathecally in this study were morphine hydrochloride (Takeda, Osaka, Japan) and (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine (MK-801; Research Biochemicals, Natick, MA). These agents were dissolved in normal saline and administered intrathecally in 10 μl vehicle. For the antagonism study, the following agents were administered: 10 μg morphine followed at 30 min by naloxone hydrochloride (1 mg/kg intraperitoneally; Sankyo, Tokyo, Japan).

Data Analysis and Statistics

To define the magnitude of the thermal hyperesthesia in a given rat, the difference score (DS) was computed by subtracting the latency of the control side (left paw) from the latency of the injured side (right paw). Negative DS values thus indicate a lower threshold on the injured side, *i.e.,* hyperesthesia.

To analyze the effects of drugs on the hyperesthesia, the postdrug difference score (post-DS) was calculated by subtracting the maximum PWL of the control side (left side) from the maximum PWL of the injured side (right side). Maximum PWL was defined as the single longest PWL value during the first 30 min after intrathecal administration of the drug. In the current study, we used maximum PWL to estimate the drug effect on the hyperesthesia. The duration of action is different for each drug. If the mean PWL is used for analyzing the drug effect, we underestimate the effects of a drug whose duration of action is short or overestimate the effect of one whose duration of action is long. Thus, we think that maximum PWL is more appropriate for evaluating the effect of a drug than the mean PWL.

To obtain a dose–response curve, the dose was plotted against the maximum PWL or the post-DS. Dose response curves were established with a least-squares linear regression analysis. Dose dependency was analyzed by one-way analysis of variance (ANOVA). In the naloxone study, we used a paired *t* test. ANOVA was carried out with Dunnett's test for multiple comparisons. To compare the slopes and elevations of the

regression lines, we used a t test.[23] $P < 0.05$ levels were considered significant.

Results

Motor Function

Intrathecal morphine had no effect on the placing–stepping reflex or the righting reflex. Intrathecal injection of 1 μg MK-801 had no effect upon the placing–stepping reflex or the righting reflex. At 10 μg, the score of both placing–stepping reflex and righting reflex fell to 1 in ten of fifteen rats. Thus, 10 μg MK-801 was the highest dose employed in this study.

Time Course of the Development of Thermal Hyperesthesia

Presurgery DS (± SEM) was −0.07 ± 0.1 s (n = 43). The DS values (± SEM) at 1, 4, and 5 weeks after the nerve constriction injury were −3.4 ± 0.2 (n = 43), −3.3 ± 0.3 (n = 25), and −3.1 ± 0.3 (n = 16) s, respectively, and these DS values were significantly more negative than those before surgery. There was no difference between the DS values at 1, 4, and 5 weeks after the nerve lesion ($P > 0.6$, ANOVA). The presurgery PWL (± SEM) of uninjured paws was 10.1 ± 0.2 s (n = 43). The PWLs (± SEM) of uninjured paws at 1, 4, and 5 weeks after the nerve constriction injury were 10.9 ± 0.2 (n = 43), 11.0 ± 0.2 (n = 25), and 10.9 ± 0.2 (n = 16) s, respectively. The PWLs of uninjured paws at 1, 4, and 5 weeks after the nerve constriction injury were significantly longer than the presurgery PWL of uninjured paws ($P < 0.001$, ANOVA).

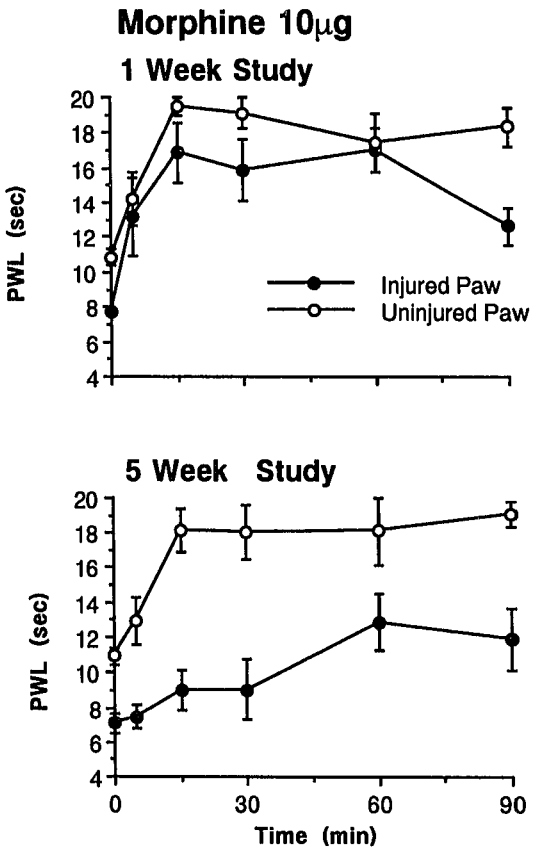

Fig. 1. Effects of intrathecal morphine (10 μg) on the paw withdrawal latency (PWL) from noxious thermal stimulation in the 1- and 5-week studies. Ordinate = PWL; abscissa = time (minutes) after drug injection. Each line represents the mean ± SEM determination made in four to six rats. Injured paw = sciatic nerve–injured paw (right paw); uninjured paw = sciatic nerve–uninjured paw (left paw).

Hyperesthetic Rat Study

Morphine. In the 1-week study, morphine produced proportionally equal increases in PWLs of the injured paw and the uninjured paw in a dose-dependent manner (figs. 1 and 2, $P < 0.05$, ANOVA), and the morphine dose–response curve for the maximum PWL of the injured paw was shifted to the right from that for the maximum PWL of the uninjured paw in a parallel fashion (fig. 2, $P < 0.005$, t test). Thus, the post-DS was preserved even as the PWLs on each side increased due to intrathecal morphine in the 1-week study (fig. 3, $P > 0.6$, ANOVA).

In the 5-week study, morphine produced an increase in the PWL of the uninjured paw in a dose-dependent manner (figs. 1 and 2, $P < 0.001$, ANOVA), but morphine did not produce an elevation in the PWL of the injured paw in a dose-dependent manner (figs. 1 and 2, $P > 0.05$, ANOVA). Thus the dose–response curve for the maximum PWL of the injured paw is not parallel to that for the maximum PWL of the uninjured paw in the 5-week study (fig. 2, $P < 0.005$, t test), and intrathecal morphine made the post-DS more negative in a dose-dependent manner in the 5-week study (fig. 3, $P < 0.01$, ANOVA).

Naloxone antagonized the morphine antinociceptive effect significantly in the 1- and 5-week studies (fig. 4, $P < 0.05$, paired t test), reversing the increased PWLs of both the injured and uninjured paws.

MK-801. MK-801 had no effect on the PWLs of the uninjured paw in either the 1- or 5-week study (figs. 5 and 6, $P > 0.1$, ANOVA). In contrast to the lack of effect upon the maximum PWL in the uninjured paw,

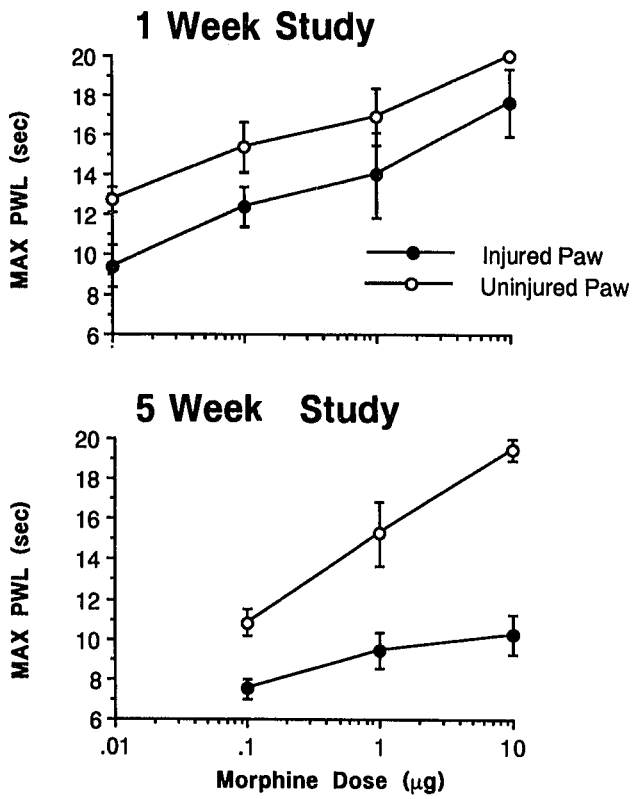

Fig. 2. Log dose–response curve of the effect of morphine on maximum paw withdrawal latency (MAX PWL) in the 1- and 5-week studies, where MAX PWL = the single longest PWL value during the first 30 min after intrathecal administration of the drug. Ordinate = MAX PWL; abscissa = log dose (micrograms). Each point represents the mean ± SEM of four to six rats. Injured paw = sciatic nerve–injured paw (right paw); uninjured paw = sciatic nerve–uninjured paw (left paw).

inspection of figure 6 suggests that intrathecally injected MK-801 produced an increase in the maximum PWLs of the injured paw to the extent that these maximum PWL values were normalized such that post-DS = 0 in both the 1- and 5-week studies. To analyze this further, post-DS values are plotted in figure 7. As indicated, intrathecal MK-801 increased the post-DS in a dose-dependent manner in both the 1- and 5-week studies ($P < 0.01$, ANOVA), and the dose–response curve of post-DS values for the 1-week study overlapped that for the 5-week study ($P > 0.2$, t test).

Discussion

Results from the current study demonstrate that the effects of morphine on the PWLs of the injured and the uninjured paw in rats with a constriction injury are time-dependent, and intrathecal morphine increased the PWLs of injured paws 1 week after the nerve lesion but not 5 weeks after the nerve lesion. This may help explain the seemingly unpredictable analgesic effect of opioids in patients with neuropathic pain. On the other hand, the effects of MK-801 on the post-DS values of the injured and the uninjured paw 1 week after the nerve constriction injury are the same as those 5 weeks after the nerve injury. Thus, MK-801 selectively abolished the hyperesthetic state evoked by the constriction injury both 1 and 5 weeks after the nerve injury. In the current study, we manually measured PWLs to the nearest 0.1 s. We recognized that the manual measurement may decrease the level of the accuracy, but our data clearly demonstrated the effects of intrathecally administered morphine or MK-801 on the PWLs in both the 1- and 5-week studies.

Time Course of the Development of Thermal Hyperesthesia

We found that the level of thermal hyperesthesia 1 week after the nerve injury is the same as that 5 weeks after the nerve lesion and that the DS between 1 and 5 weeks after the nerve injury is approximately −3.0 s. Bennett and Xie[6] reported that the hyperalgesic response to noxious radiant heat was evident on the 2nd postoperative day and lasted for 2 months and that the DS during the first 40 days after the nerve injury is

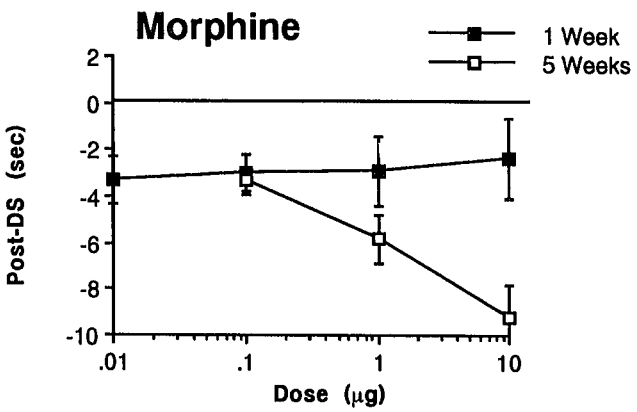

Fig. 3. Log dose–response curve of the effects of morphine on the postdrug difference score (post-DS) in the 1- and 5-week studies, where post-DS = maximum paw withdrawal latency (MAX PWL) of the sciatic nerve–injured paw − MAX PWL of the sciatic nerve–uninjured paw. Each point represents the mean ± SEM of four to six rats. 1 Week = 1-week study; 5 Weeks = 5-week study.

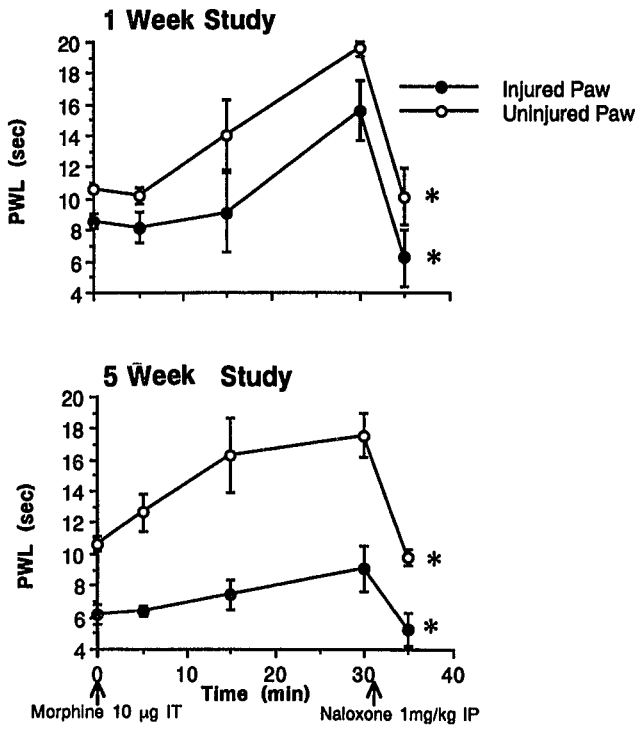

Fig. 4. Time course of paw withdrawal latency (PWL) of the morphine (10 μg intrathecally) plus naloxone (1 mg/kg intraperitoneally) study. Ordinate = PWL; abscissa = time (minutes). Each line represents the mean ± SEM of determinations made in four rats. Injured paw = sciatic nerve-injured paw (right paw); uninjured paw = sciatic nerve-uninjured paw (left paw); IT = intrathecal injection; IP = intraperitoneal injection. *$P < 0.05$ compared to PWL at 30 min after the morphine injection (paired t test).

about −3.0 s. These results are consistent with our current results. On the other hand, two groups have reported that the duration of the thermal hyperesthesia evoked by the nerve constriction injury is less than 5 weeks.[12,24] In the current study, all of the animals retain thermal hyperesthesia for 5 weeks. We think that the duration of the thermal hyperesthesia is depend on the level of the constriction injury. Although we have not systematically studied this relationship, we found that the more tightly the constriction injury was made, the longer the hyperesthesia lasted. Of course, too tight a constriction injury causes hypoalgesia. PWLs of uninjured paws at 1, 4, and 5 weeks after the nerve constriction injury are about 0.8 s longer than presurgery PWLs of uninjured paws. We think that this increase of PWLs of uninjured paws is, at least partly, due to the stress of operation of the nerve constriction injury.

It has been reported that significant axonopathy, with simultaneous degeneration and regeneration, is observed during the first 3 weeks after the nerve constriction injury and that progressive recovery begins between 3 and 4 weeks after the nerve lesion.[11] Wakisaka *et al.*[19] reported that the decrease in the sympathetic vasoconstrictor innervation to the affected hind paw was first noted on postoperative day 5, was very marked by postoperative days 10–14, and progressed to a complete or nearly complete loss by postoperative day 30. These results suggested that anatomic changes begin during the 1st week after the nerve injury and are completed by about 4 weeks after the nerve injury. Although Coggeshall *et al.*[12] showed a lack of correlation between thermal hyperesthesia and anatomic change in

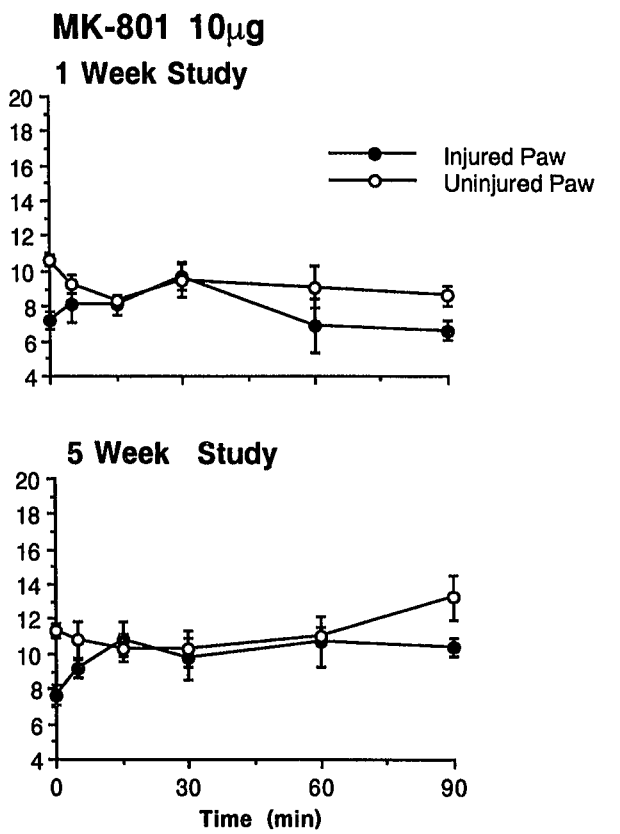

Fig. 5. Effects of intrathecal MK-801 (10 μg) on paw withdrawal latency (PWL) against thermal stimulation in the 1- and 5-week studies. Ordinate = PWL; abscissa = time (minutes) after drug injection. Each line represents the mean ± SEM determination made in four to six rats. Injured paw = sciatic nerve-injured paw (right paw); uninjured paw = sciatic nerve-uninjured paw (left paw).

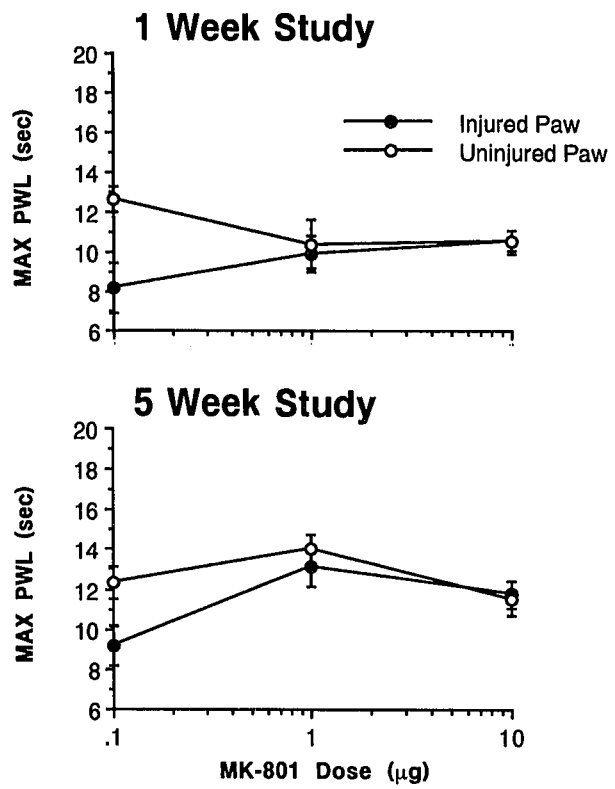

Fig. 6. Log dose–response curve of the effect of MK-801 on maximum paw withdrawal latency (MAX PWL) in the 1- and 5-week studies, where MAX PWL = the single longest PWL value during the first 30 min after intrathecal administration of the drug. Ordinate = MAX PWL; abscissa = log dose (micrograms). Each point represents the mean ± SEM of four to six rats. Injured paw = sciatic nerve–injured paw (right paw); uninjured paw = sciatic nerve–uninjured paw (left paw).

the sciatic nerve except during the initial development of the hyperesthesia after the nerve constriction injury, we believe that the appropriate times to verify the time-dependent effects of drugs on the thermal hyperesthesia evoked by the constriction nerve injury are 1 and 5 weeks after the nerve injury.

In the 5-week study, an intrathecal catheter was inserted 4 weeks after the nerve constriction injury. The DS values at 4 weeks are the same as that at 5 weeks after the nerve injury. We think that the operation to insert the intrathecal catheter has no effect on the thermal hyperesthesia evoked by the nerve constriction injury.

Morphine

In the current study, effects of intrathecally administered morphine on the PWLs are antagonized with intraperitoneally injected naloxone. These data clearly indicate that the effects of morphine on PWLs are caused by the interaction between the opioid receptor and morphine. Stevens *et al.*[25] reported that, in rats with a constriction injury, μ opioid binding in the spinal L4 segment was significantly increased 2–5 days after injury, that the binding was bilateral to the injury in laminae V and X but only ipsilateral in laminae I–II, and that μ opioid binding in all laminae gradually declined toward control values by day 10. Lombard *et al.*[26] reported that, in laminae I–II of the L3–L4 spinal segments, a significant decrease in the ipsilateral μ opioid binding was observed with a maximum decrease occurring 2 weeks after the nerve constriction injury (ipsilateral μ opioid binding 2 weeks after the nerve lesion is 87.6% of the contralateral μ opioid binding). This decrease is followed by a restoration toward the control value beginning as early as 4 weeks after the nerve lesion and the level of μ opioid binding in the ipsilateral dorsal horn 4 weeks after the nerve lesion is 94.6% of that in the contralateral dorsal horn.[24] These μ opioid binding studies suggested that, 5 weeks after the nerve constriction injury, the level of the ipsilateral μ opioid binding in the spinal cord is almost the same as that of the contralateral μ opioid binding. In the 5-week study, intrathecal morphine increased the PWL of the uninjured paw, but not the injured paw, in a dose-dependent manner. These data indicate that μ opioid bindings in the dorsal horn do not match the effect of morphine on the PWL of the injured paw at

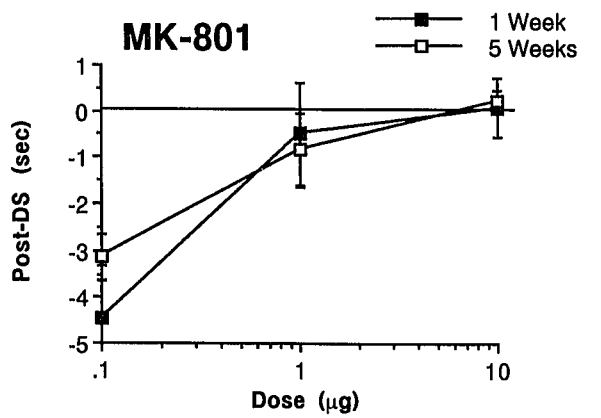

Fig. 7. Log dose–response curve of the effects of MK-801 on the postdrug difference score (post-DS) in the 1- and 5-week studies, where post-DS = maximum paw withdrawal latency (MAX PWL) of the sciatic nerve–injured paw − MAX PWL of the sciatic nerve–uninjured paw. Each point represents the mean ± SEM of four to six rats. 1 Week = 1-week study; 5 Weeks = 5-week study.

4–5 weeks. Thus, the time-dependent effects of intrathecal morphine may not be due to the time-dependent changes of μ opioid binding in the spinal cord. As noted in the introduction, time-dependent changes in morphology and biochemistry occurred in the spinal cord. Although the specific mechanisms that result in the time-dependent changes of the effects of intrathecal morphine on the PWLs of the injured paws are unknown, it is possible that the time-dependent effect of intrathecal morphine may due to these morphologic and biochemical changes in the spinal cord.

In the naloxone study, naloxone antagonizes the effect of morphine on the PWL of the injured paw in the 5-week study. In the dose response study, intrathecally injected morphine could not increase the PWLs of the injured paw in a dose-dependent manner in the 5-week study, but we think that intrathecally administered morphine may still have some effect on the PWL of the injured paw in the 5-week study. Thus, we think that naloxone antagonized any small effect of morphine on the PWL of the injured paw in the 5-week study.

MK-801

We have previously reported that intrathecally administered MK-801 had no effect on the PWL of the uninjured paw but increased the PWLs of the injured paws to the level of the uninjured paw 1 week after the nerve constriction injury.[14] These results are comparable to the current results. In the current study, the effect of MK801 on the PWLs of the injured and the uninjured paw 5 weeks after the nerve lesion is the same as that 1 week after the nerve lesion. One possible mechanism underlying the hyperesthetic state evoked by the constriction injury is the property whereby repetitive C-, but not A-fiber stimulation yields a central facilitation (wind-up).[27] NMDA antagonists have been reported to block this wind-up phenomena.[28] It has been reported that thermal hyperesthesia did not develop after nerve constriction injury in rats given neonatal capsaicin treatment.[29] We have recently reported that the injury discharge evoked by the constriction injury induces NMDA receptor dependent spinal facilitation and this spinal facilitation may play an important role in developing thermal hyperesthesia.[30] These results suggested that the injury discharge induces C-fiber dependent wind-up-like facilitation in the spinal cord and this spinal facilitation may cause the development of the thermal hyperesthesia. We have also reported that, when capsaicin was administered intrathecally 2 days after the nerve injury, capsaicin did not abolish the thermal hyperesthesia 7 days after the nerve injury.[18] The thermal hyperesthesia was temporarily eliminated by the intrathecal injection of NMDA receptor antagonists.[14] Thus, once thermal hyperesthesia is developed, the thermal hyperesthesia is not mediated by the capsaicin sensitive C fiber. These results suggested that C-fiber dependent and NMDA receptor dependent spinal facilitation plays an important role in the development of thermal hyperesthesia and that C-fiber independent and NMDA receptor dependent spinal facilitation plays an important role in the maintenance of thermal hyperesthesia. Thus, we think that this C-fiber independent and NMDA receptor dependent spinal facilitation may play an important role in maintaining the hyperesthetic state 1 and 5 weeks after the nerve lesion. In the current study, we examined the effects of MK-801 1 and 5 weeks after the nerve constriction injury, and it is possible that different results could occur at different times after the nerve injury. We believe that the C-fiber independent and NMDA receptor dependent spinal facilitation may be the common mechanism maintaining the hyperesthetic state after sciatic nerve constriction injury in the rat.

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