Effect of Methylprednisolone on Neuropathic Pain and Spinal Glial Activation in Rats

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Background: Basic data are lacking regarding the efficacy and mechanisms of action of corticosteroids in neuropathic pain. Because recent studies indicate that spinal glial activation mediates the pathologic pain states, the authors sought to determine the effects of systemic and intrathecal methylprednisolone on the development and maintenance of neuropathic pain and spinal glial activation in a rat model.

Methods: Rats were anesthetized, and L5 and L6 spinal nerves were tightly ligated. Then, continuous infusion of systemic ($4 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) or intrathecal ($80 \text{ } \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) methylprednisolone or saline was started. Mechanical allodynia and thermal hyperalgesia were evaluated on days 4 and 7 postoperatively with von Frey and Hargreaves tests, respectively. Spinal astrocytic activation was evaluated with glial fibrillary acidic protein immunoreactivity on day 7. In other groups of rats, continuous 3-day treatment with intrathecal methylprednisolone or saline was started 7 days after spinal nerve ligation, when neuropathic pain had already developed. Behavioral tests and immunostaining were performed up to 3 weeks after the treatment.

Results: Spinal nerve ligation induced mechanical allodynia and thermal hyperalgesia on days 4 and 7 postoperatively. Glial fibrillary acidic protein immunoreactivity was remarkably enhanced on day 7. Both systemic and intrathecal methylprednisolone inhibited the development of neuropathic pain states and glial activation. Three-day treatment with intrathecal methylprednisolone reversed existing neuropathic pain state and glial activation up to 3 weeks after the treatment.

Conclusion: Systemic and intrathecal methylprednisolone inhibited spinal glial activation and the development and maintenance of a neuropathic pain state in a rat model of spinal nerve ligation.

ALTHOUGH mechanisms of the development and maintenance of neuropathic pain are still only partially understood, activation of spinal glia has been implicated in recent studies. Immunohistochemical studies have shown that spinal glia are activated in various animal models of pathologic pain, including subcutaneous formalin injection[1,2] and spinal nerve injuries.[3,4] It is likely that glia are activated by a variety of pain-transmitting substances and prostaglandins. This activation of spinal glia are causally related to the pathologic pain states because pharmacologic inhibition of glial activation prevents the development of pain.[5,6] Activated glia can possibly release pain-enhancing substances, such as prostaglandins, excitatory amino acids, growth factors, and proinflammatory cytokines, which may lead to pathologic pain states.

Systemic and intrathecal corticosteroid treatments have been clinically used with various efficacies in patients with intractable pain.[7–10] However, basic data are lacking regarding the efficacy and mechanisms of action of corticosteroids in neuropathic pain. Recent studies have shown that nerve injuries induce spinal production of prostaglandins[11,12] and other inflammatory mediators,[13] which are involved in enhanced pain states.[14–16] Corticosteroids are known to inhibit prostaglandin production by suppressing phospholipase A_2 activity. Evidence also indicates that corticosteroids inhibit production of inflammatory mediators.[17] Therefore, corticosteroid therapy can be effective in the prevention and treatment of neuropathic pain.

One of the authors (S. S.) previously indicated that continuous systemic methylprednisolone reversed neuropathic hyperalgesia in rats.[18] A recent clinical study also showed that intrathecal methylprednisolone reversed postherpetic neuralgia.[10] Using a rat model of spinal nerve ligation, we tested whether methylprednisolone inhibits the development or maintenance of neuropathic pain and whether it suppresses spinal astrocytic activation as indicated by glial fibrillary acidic protein (GFAP) immunoreactivity. We first examined the efficacy of continuous systemic administration of methylprednisolone on the development of mechanical allodynia and thermal hyperalgesia and its effects on spinal astrocytic activation. To clarify the site of action of methylprednisolone, we next examined the effect of continuous intrathecal administration of methylprednisolone. Finally, we tested the efficacy of intrathecal methylprednisolone in rats in which neuropathic pain states had already developed.

Materials and Methods

Animals

All experiments were performed using male Sprague-Dawley rats, each weighing 150–200 g on the day of surgery. Rats were housed individually in plastic cages with soft bedding at room temperature and maintained on a 12-h light/12-h dark cycle with free access to food and water. The following studies were performed under a protocol approved by the Institutional Animal Care Committee of the University of Tokyo (Tokyo, Japan).

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Surgical Procedure

All the surgical procedures were performed under inhalational anesthesia with isoflurane in 100% oxygen, induced at 5% and maintained at 2%. Animals showing neurologic deficits were excluded from the following experiments.

Spinal Nerve Ligation. Neuropathic pain was induced following the methods of Kim and Chung.[19] Rats were anesthetized and placed under a microsurgical apparatus in a prone position. A midline incision was made on the back, and the left paraspinal muscles were separated from the spinous processes at the L4–S2 levels. The L6 transverse process was carefully removed, and the L4–L6 spinal nerves were identified. Careful teasing of the underlying fascia exposed the left L4 and L5 spinal nerves. The nerves were gently separated, and the L5 nerve was tightly ligated with a 6-0 silk thread. The left L6 spinal nerve was then located just caudal and medial to the sacroiliac junction and tightly ligated with a silk thread. In sham operations, similar exposure was performed without nerve ligations.

Intrathecal Catheterization. A chronic intrathecal catheter was introduced during isoflurane anesthesia.[20] Approximately 7 cm polyethylene PE-10 catheter was prepared and sterilized. The fascia and ligaments at the L4–L5 interspace were carefully removed. The dura was tensed and incised with a 22-gauge needle until cerebrospinal fluid leaked out and the cauda equina was identified. The catheter was inserted 1.5 cm in the cervical direction and sutured to the overlying fascia.

Implantation of an Infusion Osmotic Pump. Infusion osmotic pumps with a flow moderator (ALZET, Cupertino, CA) were used for continuous systemic or intrathecal drug administration. For systemic administration, a pump with a flow rate of 10 μl/h was filled with the drug to be delivered and was implanted subcutaneously. For intrathecal administration, a pump with a flow rate of 1 μl/h was filled with the drug to be delivered and was connected to the catheter. After intrathecal insertion of the catheter, the pump was implanted subcutaneously and gently sutured to the surrounding tissues.

Drugs

Methylprednisolone (6α-methylprednisolone 21-hemisuccinate sodium salt) was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in distilled water. The doses for systemic and intrathecal methylprednisolone were selected according to a previous study[18] and our pilot study, respectively.

Behavioral Assessment

All the behavioral tests were performed between 10 AM and 3 PM by an examiner blinded to the treatment groups.

Mechanical Threshold. To quantify mechanical sensitivity of the foot, the threshold of foot withdrawal in response to normally innocuous mechanical stimuli was determined by using the von Frey filaments and the up–down method.[21] Each rat was placed in a transparent plastic dome with a metal-mesh floor allowing access to the plantar surface of the hind paw and was habituated for 10 min to this environment. The von Frey hair was pressed perpendicular to the plantar surface of the hind paw with sufficient force to cause slight buckling and was held for approximately 6–8 s. Stimuli were presented at intervals of several seconds, allowing for apparent resolution of any behavioral response to previous stimuli. A positive response was noted if the hind paw was sharply withdrawn. Flinching immediately on removal of the hair was also considered a positive response.

Thermal Threshold. The latency of foot withdrawal to noxious heat stimuli was measured using the paw withdrawal apparatus.[22] Rats were placed separately on a temperature-controlled, 3-mm-thick glass floor under which a light box was located. They were habituated to the environment for approximately 10 min before testing. The movable radiant heat source beneath the glass floor was focused on the planter surface of the hind paw. Withdrawal latencies were measured automatically with photocell light. A cutoff time was set at 20 s to avoid tissue damage. Light intensity was preset to obtain a baseline latency of approximately 10 s. Ten withdrawal latencies were collected with at least 5-min intervals, and the middle 6 of the 10 latencies were averaged.

Immunohistochemistry

Animals were deeply anesthetized with intraperitoneal sodium pentobarbital (100 mg/kg) and were perfused through the ascending aorta with 100 ml heparinized normal saline, followed by 300 ml ice-cold paraformaldehyde, 4%, in 0.1 M phosphate buffer (pH 7.4). After perfusion, the spinal cord around L5 and L6 was removed and postfixed in the same fixative for 4 h at room temperature. Tissues were then stored in 30% sucrose solution in 0.1 M phosphate buffer overnight at 4°C for cryoprotection. A thin slit was placed on the ventral horn contralateral to the spinal nerve ligation. Then, 40-μm-thick transverse sections were sliced with a cryotome (CM1800; Leica, Heidelberg, Germany) at −15°C. Every fifth section of the spinal cord was retained in 0.1 M phosphate buffer solution. Approximately 20 sections were obtained from each animal. Immunostaining was performed on the free-floating sections. The sections were first incubated for 30 min in 0.3% hydrogen peroxide for endogenous peroxidase blocking and were rinsed with 0.1 M phosphate-buffered saline. The sections were incubated for 60 min in blocking solution (0.1 M phosphate-buffered saline containing 0.3% Triton X-100 and 5.0% normal rabbit serum) and were incubated overnight in goat primary antibody for GFAP (1:2,000; Santa-Cruz Biotechnology, Santa-Cruz, CA) in

0.1 M phosphate-buffered saline containing 0.3% Triton X-100 and 1.0% normal rabbit serum (buffer 1). The sections were washed with buffer 1 and incubated for 120 min in the biotinylated rabbit antibody to goat immunoglobulin G (1:5,000; Vector Laboratories, Burlingame, CA) diluted in buffer 1. The sections then were washed with 0.1 M phosphate-buffered saline containing 0.3% Triton X-100 (buffer 2) and were incubated for 120 min in avidin-biotin-peroxidase complex (Vectrastain ABC-Elite Kit; Vector Laboratories) in buffer 2. Visualization of the reaction product was achieved by incubation for 4 min with diaminobenzidine and nickel-ammonium sulfate to which hydrogen peroxide was added (DAB kit; Vector Laboratories). Sections were washed with 0.1 M phosphate buffer several times. All the incubations were performed at room temperature. After these staining procedures, the sections were placed on a slide glass and dried overnight. The cover glass was slipped with histologic mounting liquid (Permount; Fisher Scientific, Fair Lawn, NJ).

Astrocytic Activation Responses

Assessments of astrocytic activation responses were performed in three sections chosen at random from each animal.

Number of Positive Cells. Astrocytes with positive GFAP immunoreactivity in the left dorsal horn of the spinal cord were counted under ×200 magnification and totaled for the three sections.

Number of Pixels. The area of GFAP immunostaining was measured in the dorsal horn of the spinal cord with a computer-assisted image analysis system (NIH Image; US National Institutes of Health, Bethesda, MD). Images of the spinal cord were digitally captured with gray scales ranging from 0 to 255, and the number of pixels above a predetermined threshold was automatically counted.

Morphologic Classification. The sections were surveyed under ×400 magnification and scored following classification by Colburn *et al.*[23] Criteria for each class were as follows: baseline staining (−): astrocytes exhibit extensive fine projections, cells were well spaced and neatly arranged; mild response (+): astrocytes still exhibit numerous long but thickening projections, less area between individual astrocytes, GFAP immunoreactivity more apparent; moderate response (++): astrocytes were less ramified/exhibit bold projections, increased density of astrocytic cells now overlapping, prominent GFAP immunoreactivity; intense response (+++): astrocytes becoming rounded with few projections, densely arranged/overlapping, intense GFAP immunoreactivity.

Protocols

Systemic Methylprednisolone and Development of Neuropathic Pain. We first examined the effect of continuous systemic administration of methylpred-nisolone on the development of neuropathic pain and spinal astrocytic activation responses. Rats were anesthetized with isoflurane, and the left L5 and L6 spinal nerves were tightly ligated or sham operated. Then, in the ligated rats, an infusion osmotic pump was implanted subcutaneously, and systemic methylprednisolone (4 mg/kg/day) or saline was delivered continuously until the time of death (n = 6 for each group). At 4 and 7 days postoperatively, mechanical allodynia and thermal hyperalgesia were assessed with tactile sensitivity to von Frey hairs and paw withdrawal latency to heat stimulus, respectively. After the behavioral tests on day 7, rats were perfused with 4% paraformaldehyde, and the lumbar spinal cord was removed for immunohistochemical processing with GFAP antibody.

Intrathecal Methylprednisolone and Development of Neuropathic Pain. The effect of continuous intrathecal administration of methylprednisolone on the development of neuropathic pain and spinal astrocytic activation responses were examined. Rats were anesthetized, and the left L5 and L6 spinal nerves were tightly ligated. Then, a catheter was inserted intrathecally through the L4–L5 interspace, and methylprednisolone (80 μg/kg/day) or saline was delivered continuously with an osmotic pump until the time of death (n = 6 for each group). Mechanical allodynia and thermal hyperalgesia were assessed on days 4 and 7 postoperatively. After the behavioral tests on day 7, rats were perfused with the fixative, and the lumbar spinal cord was removed for immunohistochemical processing.

Intrathecal Methylprednisolone and Maintenance of Neuropathic Pain. The effect of continuous intrathecal administration of methylprednisolone on existing neuropathic pain and spinal astrocytic activation responses were examined. Seven days after spinal nerve ligation, development of neuropathic pain was confirmed with behavioral tests, and a second surgical procedure was performed to place an intrathecal catheter. Then, methylprednisolone (80 μg · kg^{-1} · day^{-1}) or saline was delivered continuously with an osmotic pump (n = 6 for each group). Three days later, *i.e.*, 10 days after the nerve ligation, the catheter and the pump were removed surgically, and the rats were observed for the next 3 weeks. Mechanical allodynia and thermal hyperalgesia were assessed on days 4, 7, 10, 13, 17, 24, and 31 after the spinal nerve ligation. In different groups of rats, the lumbar spinal cord was removed for GFAP immunoreactivity on day 10, *i.e.*, immediately after the methylprednisolone treatment, or day 31, *i.e.*, 3 weeks after the treatment (n = 6 for each group).

Statistical Analysis

Data were expressed as mean ± SD. In the preventive paradigm, all the behavioral data were analyzed by one-way analysis of variance at each time point followed by Bonferroni multiple comparison tests. In the mainte-

nance paradigm, temporal change in each group was analyzed with repeated-measures analysis of variance and the Dunnett test. Differences between the groups at each time point were determined by unpaired *t* tests. Image analysis data were compared with one-way analysis of variance and the Bonferroni test. Data of morphologic classification were analyzed with the Mann–Whitney test. *P* values less than 0.05 were considered significant in each test.

Results

All the rats maintained good health and continued to gain weight throughout the experimental period. No infection or motor dysfunction was observed in any of the animals. There was no significant difference in weight between the groups. No abnormalities were observed on visual inspection of the spinal cords.

Systemic Methylprednisolone and Development of Neuropathic Pain

Figure 1A illustrates the changes in mechanical sensitivity to von Frey filaments after spinal nerve ligation and the effect of systemic methylprednisolone. Remarkable decreases in the tactile threshold were observed on days 4 and 7 (1.3 ± 0.6 and 1.1 ± 0.3 g, respectively) in the saline group as compared with the sham-operated group (12.1 ± 1.5 and 12.3 ± 1.5 g), indicating the development of mechanical allodynia. Decreases in the tactile threshold were significantly inhibited on days 4 and 7 in the methylprednisolone group (10.1 ± 1.2 and 7.5 ± 1.9 g) as compared with the saline group, although there were still significant differences between the methylprednisolone and the sham-operated groups. Figure 1B demonstrates the changes in the paw-flick latency to heat stimuli after spinal nerve ligation. Remarkable decreases in latency were observed on days 4 and 7 in the saline group (5.5 ± 0.8 and 5.3 ± 1.0 s, respectively) as compared with the sham-operated group (10.9 ± 1.0 and 11.2 ± 1.0 s), indicating the development of thermal hyperalgesia. Decreases in latency were significantly inhibited on days 4 and 7 in the methylprednisolone group (9.8 ± 0.8 and 9.9 ± 0.8 s) as compared with the saline group.

Figure 2 shows the GFAP immunoreactivity in the spinal dorsal horn 7 days after the nerve ligation. In contrast to the normal (*A*) and sham-operated (*B*) rats, remarkable GFAP immunostaining (indicating astrocytic activation responses) was observed in rats after spinal nerve ligation (*C*). GFAP immunoreactivity was obviously inhibited in rats treated with continuous systemic methylprednisolone administration started immediately after the nerve ligation (*D*).

Table 1 shows the image-analysis data on the astrocytic responses to spinal nerve ligation and effects of systemic
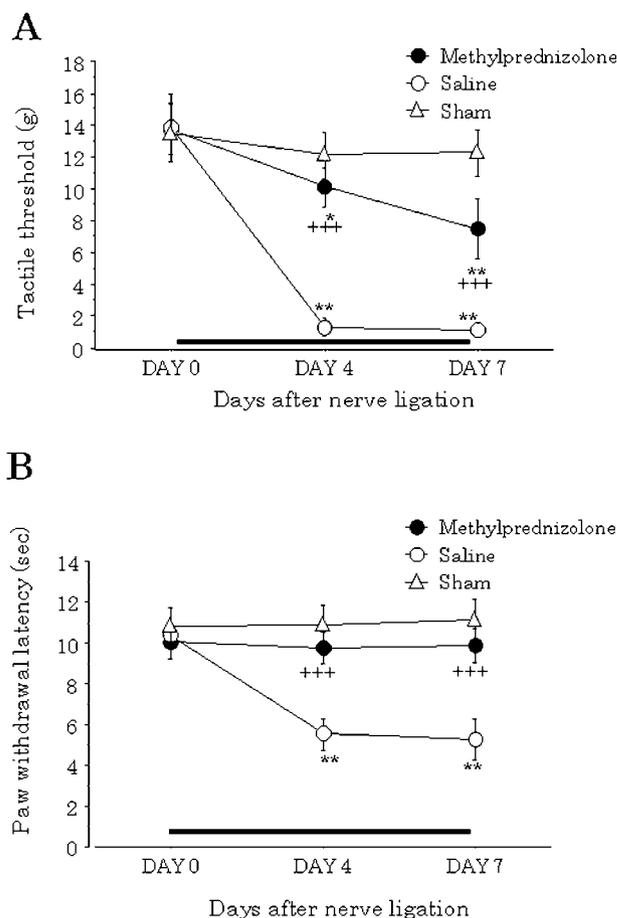

Fig. 1. (*A*) Changes in the mechanical sensitivity to von Frey filaments after spinal nerve ligation and effect of continuous systemic administration of methylprednisolone. Methylprednisolone significantly inhibited the development of mechanical allodynia, although there were still significant differences between the methylprednisolone and the sham-operated groups. (*B*) Changes in the paw withdrawal latency to heat stimuli after spinal nerve ligation and effect of systemic methylprednisolone. Methylprednisolone significantly inhibited the development of thermal hyperalgesia. *Bar* above the x-axis represents systemic treatment with methylprednisolone or saline. * $P < 0.01$, ** $P < 0.0001$ *versus* sham operation; +++ $P < 0.0001$ *versus* saline.

methylprednisolone. Indices of astrocytic activation, namely, the number of GFAP-positive astrocytes and the area of GFAP staining as indicated by the number of pixels, were significantly increased by spinal nerve ligation (216 ± 10 cells, $78,259 \pm 3,047$ pixels) as compared with sham operation (11 ± 1 cells, $1,485 \pm 56$ pixels), and these effects were significantly inhibited by systemic methylprednisolone treatment (44 ± 4 cells, $15,365 \pm 151$ pixels). Morphologic data also indicated that spinal nerve ligation induced astrocytic activation (sham operated *vs.* saline), and this effect was inhibited by systemic methylprednisolone (saline *vs.* methylprednisolone).

Intrathecal Methylprednisolone and Development of Neuropathic Pain

Figure 3A shows the changes in mechanical sensitivity after spinal nerve ligation and the effect of intrathecal
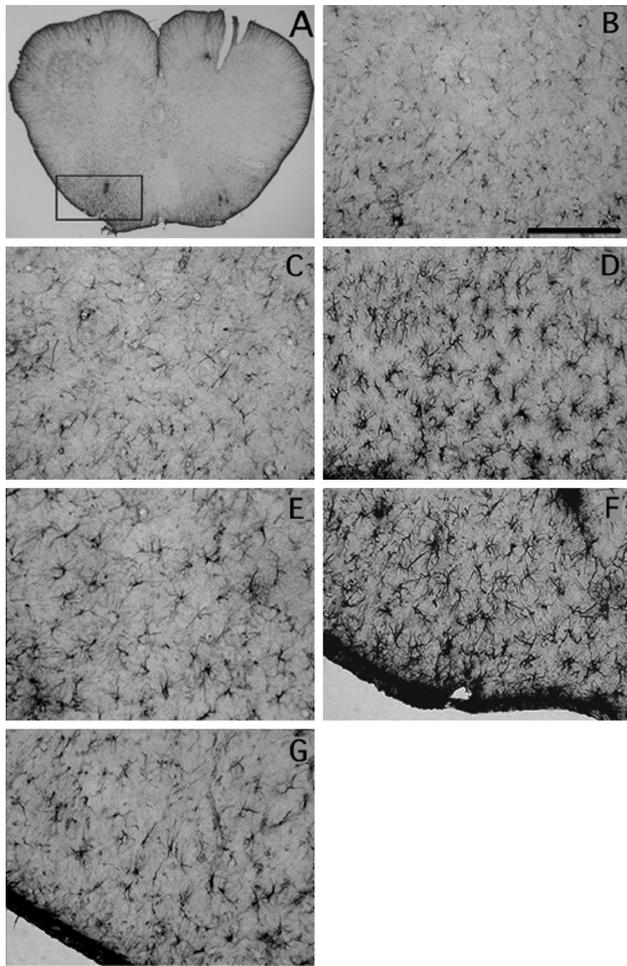
Fig. 2. Spinal immunoreactivity to glial fibrillary acidic protein 7 days after spinal nerve ligation and effect of methylprednisolone. (*A*) Lower magnification image showing the area of the dorsal horn analyzed. Astrocytic activation was not observed in normal (*B*) or sham-operated rats (*C*). Prominent astrocytic activation was observed in rats treated with systemic saline after spinal nerve ligation (*D*). This response was remarkably inhibited in rats treated with continuous systemic methylprednisolone immediately after spinal nerve ligation (*E*). Prominent astrocytic activation was also observed in rats treated with intrathecal saline after spinal nerve ligation (*F*). This activation was remarkably inhibited in rats treated with continuous intrathecal methylprednisolone immediately after spinal nerve ligation (*G*). *Bar* = 100 μm.

Table 1. Effect of Continuous Systemic Methylprednisolone on Spinal Astrocytic Responses 7 Days after Spinal Nerve Ligation

| Group	No. of Positive Cells	No. of Pixels	Morphologic Classification	
Sham operation	11 ± 1	1,485 ± 56	−	13
			+	5
			+ +	0
			+ + +	0
Saline	216 ± 10*	78,259 ± 3,047*	−	0
			+	0
			+ +	3
			+ + +	15
Methylprednisolone	44 ± 4*†	15,365 ± 151*†	−	2
			+	14
			+ +	2
			+ + +	0

Data are presented as mean ± SD. See text for details of morphologic classification: − = baseline staining; + = mild response; + + = moderate response; + + + = intense response.

* $P < 0.001$ *vs.* sham operation.   †$P < 0.001$ *vs.* saline.

methylprednisolone. Tactile thresholds were significantly longer on days 4 and 7 in the methylprednisolone group (13.1 ± 1.6 and 13.5 ± 1.8 g) as compared with the saline group (1.3 ± 1.1 and 1.3 ± 0.8 g). Figure 3B shows the changes in the paw withdrawal latency after spinal nerve ligation. Latencies were significantly longer on days 4 and 7 in the methylprednisolone group (11.0 ± 0.8 and 10.7 ± 1.3 s) as compared with the saline group (5.2 ± 0.9 and 3.9 ± 1.1 s). Spinal GFAP immunoreactivity indicated that the astrocytic activation responses 7 days after the nerve ligation was obviously inhibited in rats treated with continuous intrathecal methylprednisolone (fig. 2F) as compared with saline (fig. 2E). The number of GFAP-positive astrocytes and

the area of GFAP staining were significantly decreased in the methylprednisolone group (17 ± 2 cells, 7,370 ± 457 pixels) as compared with the saline group (147 ± 11 cells, 40,187 ± 3,871 pixels; table 2). Morphologic data also indicated that spinal nerve ligation–induced astrocytic activation was inhibited by intrathecal methylprednisolone. Differences between the saline-treated groups in tables 1 and 2 represent variations in the intensity of staining. Note that differences between the treatment groups in each experimental session are far more remarkable.

Intrathecal Methylprednisolone and Maintenance of Neuropathic Pain

Figure 4A illustrates the temporal changes in mechanical sensitivity to von Frey filaments after spinal nerve ligation and the effect of 3-day treatment with intrathecal methylprednisolone. Significant decreases in the tactile threshold were observed between days 4 and 31 (0.6 ± 0.3 to 7.0 ± 1.7 g) as compared with day 0 (12.3 ± 0.7 g) in the saline group, indicating the maintenance of mechanical allodynia. Tactile thresholds were significantly higher in the methylprednisolone group as compared with the saline group between days 10 and 31, indicating that the 3-day treatment with intrathecal methylprednisolone reversed existing mechanical allodynia and that the effect persisted for at least 3 weeks. Figure 4B shows the temporal changes in paw withdrawal latency to heat stimuli after spinal nerve ligation. Significant decreases in the latency were observed between days 4 and 31 (3.1 ± 0.7 to 8.3 ± 0.5 s) as compared with day 0 (10.9 ± 0.4 s) in the saline group, indicating the maintenance of thermal hyperalgesia. The latencies were significantly higher in the methylprednisolone group as compared with the saline group between days 10 and

A

B
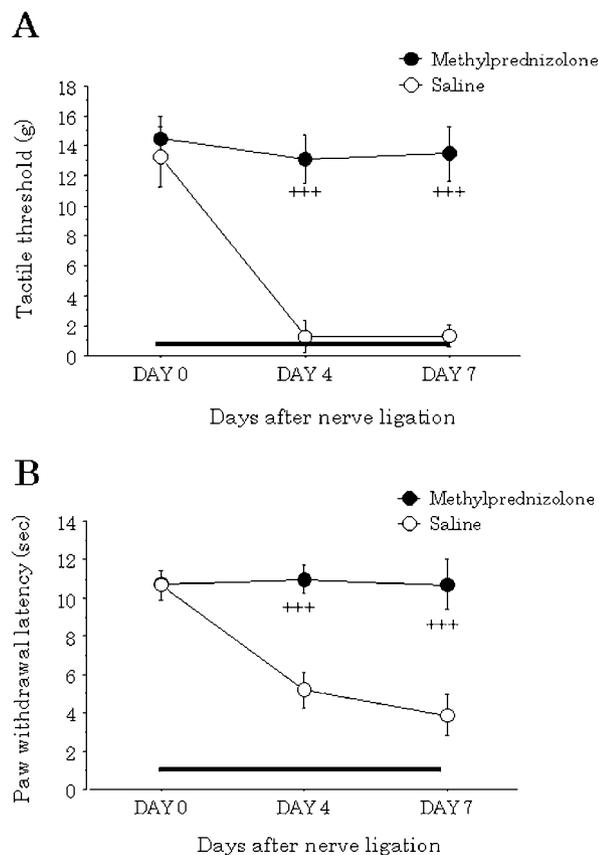
Fig. 3. (*A*) Changes in the mechanical sensitivity to von Frey filaments after spinal nerve ligation and effect of continuous intrathecal administration of methylprednisolone. Methylprednisolone significantly inhibited the development of mechanical allodynia. (*B*) Changes in the paw withdrawal latency to heat stimuli after spinal nerve ligation and effect of intrathecal methylprednisolone. Methylprednisolone significantly inhibited the development of thermal hyperalgesia. *Bar* above the x-axis represents intrathecal treatment with methylprednisolone or saline. +++ *P* < 0.0001 *versus* saline.

31, indicating that the 3-day treatment with intrathecal methylprednisolone reversed existing thermal hyperalgesia and that the effect persisted for at least 3 weeks.

Figure 5 shows the spinal GFAP immunoreactivity after

Table 2. Effect of Continuous Intrathecal Methylprednisolone on Spinal Astrocytic Responses 7 Days after Spinal Nerve Ligation

Group	No. of Positive Cells	No. of Pixels	Morphologic Classification	
Saline	147 ± 11	40,187 ± 3,871	−	0
			+	5
			++	7
			+++	6
Methylprednisolone	17 ± 2*	7,370 ± 457*	−	8
			+	10
			++	0
			+++	0

Data are presented as mean ± SD. See text for details of morphologic classification: − = baseline staining; + = mild response; ++ = moderate response; +++ = intense response.

* *P* < 0.001 *vs.* saline.

A

B
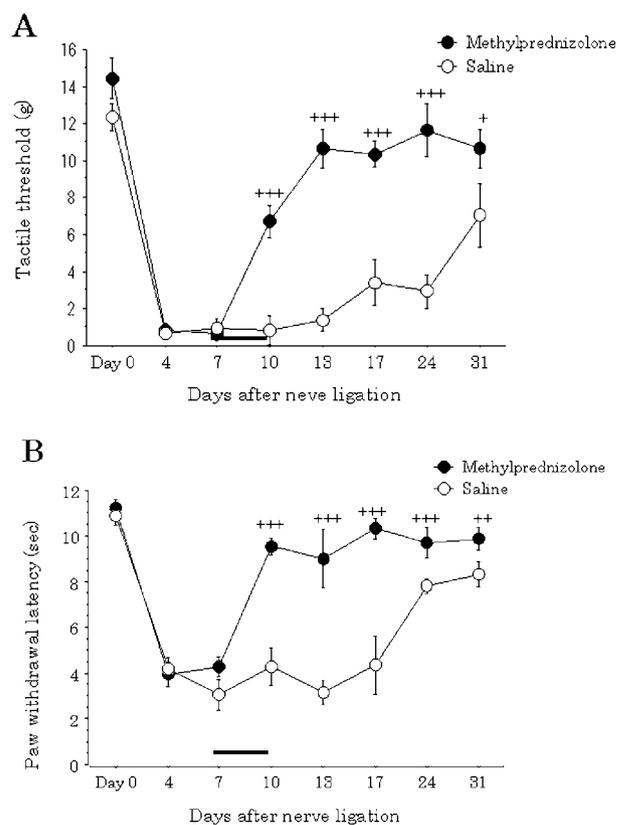
Fig. 4. (*A*) Temporal changes in the mechanical sensitivity to von Frey filaments after spinal nerve ligation and effect of continuous intrathecal methylprednisolone on existing mechanical allodynia. Methylprednisolone significantly reversed mechanical allodynia, and this effect of persisted for at least 3 weeks. (*B*) Temporal changes in the paw withdrawal latency to heat stimuli after spinal nerve ligation and effect of intrathecal methylprednisolone on existing thermal hyperalgesia. Methylprednisolone significantly reversed thermal hyperalgesia, and this effect persisted for at least 3 weeks. *Bar* above the x-axis represents intrathecal treatment with methylprednisolone or saline. + *P* < 0.005, ++ *P* < 0.0005, +++ *P* < 0.0001 *versus* saline.

neuropathic pain had already developed and the effect of intrathecal methylprednisolone. GFAP immunoreactivity was obviously inhibited in rats treated for 3 days with continuous intrathecal administration of methylprednisolone (fig. 5B) as compared with saline (fig. 5A). Astrocytic activation was no longer prominent at 31 days after spinal nerve ligation with either saline (fig. 5C) or methylprednisolone (fig. 5D) treatment.

Effects of intrathecal methylprednisolone on the astrocytic activation in rats with neuropathic pain are shown in table 3. On day 10, *i.e.*, immediately after the 3-day treatment, all the indices of astrocytic activation were significantly inhibited by intrathecal methylprednisolone as compared with saline. On day 31, *i.e.*, 3 weeks after the treatment, the inhibitory effects of intrathecal methylprednisolone on astrocytic responses were still observed except the morphologic scores.
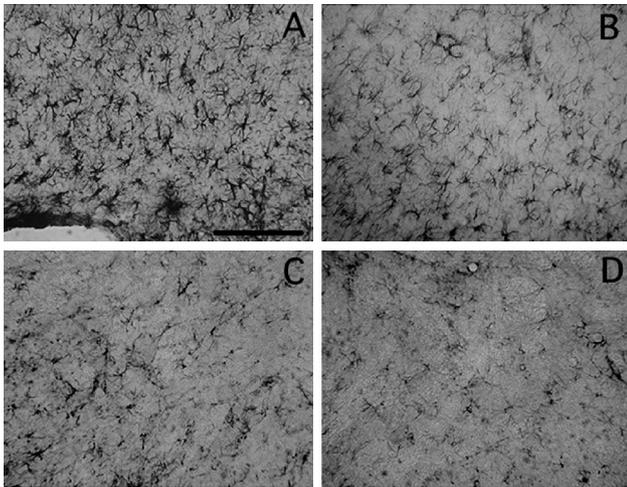
Fig. 5. Spinal glial fibrillary acidic protein immunoreactivity immediately (*A* and *B*) or 3 weeks (*C* and *D*) after the 3-day treatment with continuous intrathecal methylprednisolone or saline started 7 days after nerve ligation. Astrocytic activation was remarkably inhibited in rats treated with intrathecal methylprednisolone (*B*) as compared with saline (*A*). The astrocytic activation responses were weak in both the saline (*C*) and the methylprednisolone groups (*D*) 3 weeks after the treatment, *i.e.*, 31 days after spinal nerve ligation. *Bar = 100 μm.*

Discussion

We have demonstrated that continuous systemic or intrathecal administration of methylprednisolone inhibited spinal glial activation and development of neuropathic pain in the spinal nerve ligation model of rats. Intrathecal methylprednisolone also reversed glial acti-

Table 3. Effect of Continuous Intrathecal Methylprednisolone on Spinal Astrocytic Responses in Rats with Existing Neuropathic Pain

Group	No. of Positive Cells	No. of Pixels	Morphologic Classification	
Day 10				
Saline	18.2 ± 9	45,827 ± 2,201	−	0
			+	1
			++	5
			+++	9
Methylprednisolone	53 ± 3*	9,543 ± 524*	−	1
			+	6
			++	5
			+++	0
Day 31				
Saline	26 ± 2	5,641 ± 439	−	10
			+	5
			++	0
			+++	0
Methylprednisolone	5 ± 0*	963 ± 104*	−	14
			+	1
			++	0
			+++	0

Data are presented as mean ± S.D. Day 10 and 31 represent immediately and 3 weeks after the treatment, respectively. See text for details of morphologic classification: − = baseline staining; + = mild response; ++ = moderate response; +++ = intense response.

* $P < 0.001$ *vs.* saline.

vation and pain behaviors in rats with existing neuropathic pain. Because local infiltration of corticosteroid at the site of nerve ligation inhibited neuropathic pain,[24] systemic methylprednisolone might have acted at the site of nerve ligation in our study. However, considering that the small intrathecal dose of methylprednisolone also prevented and alleviated neuropathic pain, the principal site of action is most likely to be in the spinal cord.

Evidence has accumulated indicating that spinal glia mediate pathologic pain states of diverse etiologies.[25] Activation of spinal glia (astrocytes and microglia) has been observed with immunohistochemical techniques in various animal models of inflammatory[1,2] and neuropathic[3,4] pain. Glia are known to be activated by a variety of substances, including substance P,[26] excitatory amino acids,[27] adenosine triphosphate,[28] nitric oxide, and prostaglandins.[29] Activated glia in turn release substances that excite spinal pain–responsive neurons, such as prostaglandins, excitatory amino acids, growth factors, and proinflammatory cytokines.[30] Tumor necrosis factor, interleukin 1, and interleukin 6 have all been implicated in creating exaggerated pain states in the spinal cord.[15,16,31,32] Evidence is also available showing that glial activation is necessary and sufficient to produce enhanced pain.[5,6,33]

Considering the previous studies indicating that spinal glial activation is causally related to pathologic pain states, we speculate that inhibition of the astrocytic activation by methylprednisolone led to prevention and alleviation of neuropathic pain in our study. Although precise mechanisms of glial suppression by methylprednisolone are still unclear, inhibition of prostaglandin production may contribute because prostaglandins are known to induce glial activation[29] and corticosteroids inhibit prostaglandin production by inhibiting phospholipase A_2 activity. Likewise, suppression of cytokine production by corticosteroids[17] may also contribute to reduced glial activation. Although we have focused on astrocytic activation in this study, microglial responses can also be involved in the development and maintenance of neuropathic pain and the inhibitory effect of methylprednisolone.

We cannot conclude from our study whether there is a causal relation between the suppression of glial activation and the inhibition of neuropathic pain. Methylprednisolone may inhibit neuropathic pain independently from its suppressive effects on spinal glia, or, rather, inhibition of neuropathic pain may lead to avoidance of glial activation. Up-regulation of spinal prostaglandin[11,12] and cytokine[1,3,13] synthesis has been reported in various models of pathologic pain states. Also, evidence is available indicating that these substances are involved in development or maintenance of hyperalgesia, allodynia, or both.[11,12,14–16,31,32] Corticosteroids can affect pain processing in part by interfering with the formation of prostaglandins and other inflammatory mediators in the

spinal cord. Corticosteroids can also suppress local inflammatory responses that produce edema and swelling and worsen neuronal injury. These effects of corticosteroids may lead to alleviation of neuropathic pain.

Consistent data are lacking regarding the efficacy of corticosteroid treatment in neuropathic pain. In clinical studies, systemic methylprednisolone was effective in patients with neuropathic pain,[7,8] whereas it was not effective in preventing postherpetic neuralgia.[9] Intrathecal methylprednisolone proved to be remarkably effective in patients with intractable postherpetic pain and the effect persisted 4 weeks after the treatment.[10] In animal models, intermittent systemic methylprednisolone failed to increase pain thresholds in a nerve transection model of rats.[34] Intermittent intrathecal methylprednisolone also failed to suppress spinal sensitization in a rat formalin model.[35] However, neuropathic pain state was reversed by continuous intrathecal methylprednisolone in our animal study. It is likely that continuous administration of steroid is more advantageous compared with intermittent administration because interruption of drug effects can be avoided.[18] Furthermore, considering that the spinal bioavailability of intrathecal methylprednisolone is much higher than that of the systemic route,[36] intrathecal methylprednisolone can be advantageous because of the lack of systemic side effects. Based on these data, continuous intrathecal corticosteroid therapy may be an effective strategy in the treatment of neuropathic pain when its safety has been established.

Intrathecal catheters are clinically used for continuous opioid administration in patients with intractable pain. Formation of aseptic inflammatory masses at the catheter tip during morphine treatment has been reported.[37,38] Resultant neurologic deficits have also been reported in patients receiving intrathecal analgesic therapy.[39] We need to examine whether the continuous intrathecal corticosteroid administration can also induce mass formation or other harmful responses. Although there were no histologic or behavioral signs of neurotoxicity in one study,[35] there have been anecdotal claims that intrathecal corticosteroid is the cause of arachnoiditis and prolonged neurologic sequelae.[40]

Drawbacks of our study are as follows. We did not observe contralateral behavioral effects after drug administration. This would show whether methylprednisolone has any general antinociceptive effects or any other side effects on the uninjured paw. Image analysis in the contralateral side would further elucidate correlation between astrocytic activation and behavior. Long-term study in rats with sham operation plus intrathecal methylprednisolone or sham operation plus intrathecal saline would also have helped to examine the stability of behavior as well as the specificity of glial activation to the ligation rather than to the intrathecal injection. Further-

more, it is not clear whether the saline-treated rats recovered with a normal time course.

In summary, we have demonstrated that continuous systemic and intrathecal administration of methylprednisolone inhibited spinal glial activation and the development and maintenance of neuropathic pain in a rat model. This study may provide a basis of a clinical strategy for the prevention and treatment of neuropathic pain.

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