[11] J.H. Hwang, K.S. Hwang, J.K. Leem, P.H. Park, S.M. Han, D.H. Lee, The antiallodynic effects of intrathecal cholinesterase inhibitors in a rat model of neuropathic pain, Anesthesiology 90 (1999) 492–499.
[12] S.H. Kim, J.M. Chung, An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat, Pain 50 (1992) 355–363.
[13] W. Klimscha, C. Tong, J.C. Eisenach, Intrathecal alpha 2-adrenergic agonists stimulate acetylcholine and norepinephrine release from the spinal cord dorsal horn in sheep, an in vivo microdialysis study, Anesthesiology 87 (1997) 110–116.
[14] P. Lavand'homme, H.L. Pan, J.C. Eisenach, Intrathecal neostigmine, but not sympathectomy, relieves mechanical allodynia in a rat model of neuropathic pain, Anesthesiology 89 (1998) 493–499.
[15] B. Mesnage, S. Gaillard, A.G. Godin, J.L. Rodeau, M. Hammer, J. Von Engelhardt, P.W. Wiseman, Y. De Koninck, R. Schlichter, M. Cordero-Erausquin., Morphological and functional characterization of cholinergic interneurons in the dorsal horn of the mouse spinal cord, Journal of Comparative Neurology 519 (2011) 3139–3158.
[16] M.J. Millan, Descending control of pain, Progress in Neurobiology 66 (2002) 355–474.
[17] H. Obata, M. Kimura, K. Nakajima, M. Tobe, K. Nishikawa, S. Saito, Monoamine-dependent, opioid-independent antihypersensitivity effects of intrathecally administered milnacipran, a serotonin noradrenaline reuptake inhibitor, in a postoperative pain model in rats, Journal of Pharmacology and Experimental Therapeutics 334 (2010) 1059–1065.
[18] H. Obata, X. Li, J.C. Eisenach, Alpha2-Adrenoceptor activation by clonidine enhances stimulation-evoked acetylcholine release from spinal cord tissue after nerve ligation in rats, Anesthesiology 102 (2005) 657–662.
[19] H. Obata, S. Saito, S. Koizuka, K. Nishikawa, F. Goto, The monoamine-mediated antiallodynic effects of intrathecally administered milnacipran, a serotonin noradrenaline reuptake inhibitor, in a rat model of neuropathic pain, Anesthesia and Analgesia 100 (2005) 1406–1410.
[20] X. Paqueron, D. Conklin, J.C. Eisenach, Plasticity in action of intrathecal clonidine to mechanical but not thermal nociception after peripheral nerve injury, Anesthesiology 99 (2003) 199–204.
[21] L.O. Randall, J.J. Selitto, A method for measurement of analgesic activity on inflamed tissue, Archives Internationales de Pharmacodynamie et de Therapie 111 (1957) 409–419.
[22] M. Sonohata, H. Furue, T. Katafuchi, T. Yasaka, A. Doi, E. Kumamoto, M. Yoshimura, Actions of noradrenaline on substantia gelatinosa neurones in the rat spinal cord revealed by in vivo patch recording, The Journal of Physiology 555 (2004) 515–526.
[23] T.L. Yaksh, R. Dirksen, G.I. Harty, Antinociceptive effects of intrathecally injected cholinomimetic drugs in the rat and cat, European Journal of Pharmacology 117 (1985) 81–88.

Antihypersensitivity Effects of Tramadol Hydrochloride in a Rat Model of Postoperative Pain

Masafumi Kimura, MD, Hideaki Obata, MD, PhD, and Shigeru Saito, MD, PhD

BACKGROUND: Tramadol is used to treat a wide range of acute and chronic pain. This drug induces analgesia by 2 mechanisms of action: opioid receptor activation and enhancement of noradrenaline (NA) and serotonin (5-HT) transmission. The effect of tramadol on NA and 5-HT concentrations in the spinal cord, however, have not been assessed. In the present study, we investigated the antihypersensitivity effect of tramadol using a rat model of postoperative pain. We also evaluated the increase in NA and 5-HT levels in the spinal cord after tramadol injection using in vivo microdialysis.

METHODS: We made a hindpaw incision in male Sprague-Dawley rats (postoperative pain model). Tramadol was administered intraperitoneally and intrathecally 24 hours after paw incision. Mechanical hypersensitivity was measured by determining the withdrawal threshold using von Frey filaments. Microdialysis studies from the dorsal horn of the lumbar spinal cord were performed to measure NA and 5-HT levels after intraperitoneal injection of tramadol. We also measured the NA and 5-HT content in the spinal cord in normal rats and rats with paw incision.

RESULTS: Intraperitoneal (10, 20, and 40 mg/kg) and intrathecal (125, 250, and 500 μg) injection of tramadol produced an antihyperalgesic effect in a dose-dependent manner. The antihypersensitivity effect of tramadol was prevented by intrathecal pretreatment with methysergide (30 μg), a serotonin receptor antagonist; idazoxane (30 μg), a noradrenaline receptor antagonist; and naloxone (30 μg), a nonselective opioid receptor antagonist. Microdialysis study revealed that 5-HT and NA concentrations at the spinal dorsal horn were increased, peaking at 30 minutes after intraperitoneal injection of 20 mg/kg tramadol. Furthermore, the NA and 5-HT content in the ipsilateral dorsal half of the lumbar spinal cord was increased 1 day and 3 days after paw incision, respectively.

CONCLUSIONS: These findings indicate that tramadol inhibits postoperative hypersensitivity by increasing NA and 5-HT levels in the spinal cord and activating opioid receptors. Tramadol might be more effective in the early postoperative period when spinal NA and 5-HT levels are increased. (Anesth Analg 2012;115:443–9)

Tramadol, (1RS; 2RS)-2–[(dimethylamino)methyl]-1–(3-methoxyphenyl)-cyclohexanol hydrochloride, a racemic mixture of 2 enantiomers, is widely used as an analgesic drug for acute and chronic pain, such as postoperative pain, neuropathic pain, and cancer pain. (+)-Tramadol has low affinity for opioid receptors without selectivity,[1] and its principal metabolite (+)-O-desmethyltramadol (M1) also binds to μ-opioid receptors with relatively high affinity.[2,3] One report using μ-opioid–receptor knockout mice suggests that μ-opioid receptors have an important role in the analgesic effects of tramadol.[4] However, actions other than opioid mechanisms might contribute to its analgesic efficacy, because some experiments show that the antinociceptive effects of tramadol are not fully antagonized by naloxone.[5–7]

(−)-Tramadol and its principal metabolite (−)-O-desmethyltramadol inhibits noradrenaline (NA) uptake in a synaptosomal preparation in a specific and stereoselective fashion.[8] In a similar way, (+)-tramadol increases the extraneuronal concentration of serotonin (5-HT) by inhibiting the 5-HT transporter.[9–11] Therefore, tramadol is also a 5-HT and NA reuptake inhibitor. Recent studies demonstrated that tramadol increases the extracellular levels of NA and 5-HT as measured by in vivo microdialysis in the ventral hippocampus.[12] The neurotransmitters NA and 5-HT have important roles in suppressing nociceptive transmission in the spinal cord.[13–14] In vivo measurement of the NA and 5-HT concentrations in the spinal cord after tramadol administration has yet to be conducted. We hypothesized that an increase in 5-HT and NA levels in the spinal cord contributes to the antinociceptive effect of tramadol. Hindpaw incision in rats produces hypersensitivity that mimics postoperative pain in humans.[15] Therefore, we evaluated NA and 5-HT levels in the spinal cord using in vivo microdialysis, and the role of NA, 5-HT, and opioid receptors in the antihypersensitivity effects of tramadol using a rat model of postoperative pain.

From the Department of Anesthesiology, Gunma University Graduate School of Medicine, Gunma, Japan.

Accepted for publication March 20, 2012.

This work was supported by Grants-in-Aid for Scientific Research (grant number 20591823, to H.O.) from the Ministry of Education, Culture, Sport, Science, and Technology of Japan (Tokyo, Japan).

The authors declare no conflicts of interest.

Reprints will not be available from the authors.

This report was previously presented, in part, at the Society for Neuroscience Annual Meeting 2010.

Address correspondence to Hideaki Obata, MD, PhD, Department of Anesthesiology, Gunma University Graduate School of Medicine, 3-39-22, Showa, Maebashi, Gunma 371-8511, Japan. Address e-mail to hobata@showa.gunma-u.ac.jp.

DOI: 10.1213/ANE.0b013e31825683c3

METHODS

Surgical Preparation

The study was approved by the Animal Care and Use Committee of the Gunma University School of Medicine (Maebashi, Japan). Male Sprague-Dawley rats (250 g) were used in all experiments. Animals were housed under a 12-h light-dark cycle, with food and water available ad libitum. For intrathecal administration, a sterilized 32-gauge polyethylene catheter (RecathCo, Allison Park, PA) connected to 8.5-cm Tygon external tubing (Saint-Gobain Performance Plastics, Akron, OH) was inserted under isoflurane anesthesia, as previously described.[16] The catheter was passed caudally 7.5 cm from the cisterna magnum to the lumbar enlargement. Only animals without evidence of neurologic dysfunction after catheter insertion were used for studies. Paw incision, as described by Brennan et al.,[15] was performed 5 days after intrathecal catheter implantation. Briefly, rats were anesthetized with isoflurane and, after sterile preparation with 70% ethanol, a 1-cm long incision was made in the plantar aspect of the right hindpaw, starting 0.5 cm from the edge of the heel toward the toe. The plantaris muscle was elevated and incised longitudinally. The wound was closed with 2 mattress sutures using 5.0 silk.

Behavioral Testing

Rats were placed individually in a plastic cage with a plastic mesh floor and allowed to acclimate to the environment for 20 minutes. Withdrawal threshold was determined using calibrated von Frey filaments (Stoelting, Wood Dale, IL), beginning with the 2.0-g filament. Filaments were applied vertically to an area adjacent to the wound for 6 seconds, using just enough pressure to gently bend the filament. In the absence of a response, a filament of the next greater force was applied. In the presence of a response, a filament of the next lower force was applied. The tactile stimulus producing a 50% likelihood of withdrawal was determined using the up-down method, as described by Chaplan et al.[17] General behavior, including ambulation and activity level, was assessed throughout the testing period. The investigator was blinded to the drug treatment for all studies.

Drugs and Their Administration

Drug testing was performed 24 hours after the paw incision. Rats received intraperitoneal (10, 20, 40 mg/kg) or intrathecal (125, 250, 500 μg) injection of tramadol. The withdrawal threshold was determined before (prepaw incision threshold) and 24 hours after incision (baseline), then at 15, 30, 60, 90, 120, and 180 minutes after intraperitoneal injection, and at 15, 30, 60, and 90 minutes after intrathecal injection using the up-down method with von Frey filaments. Antagonist studies were performed to test whether the effect of tramadol in the postoperative pain model is mediated through α2-adrenergic receptors (idazoxan), 5-HT receptors (methysergide), and opioid receptors (naloxone) in the spinal cord. Saline or 30 μg of each antagonist was administered intrathecally 15 minutes before tramadol injection. The dose of the antagonist was selected according to a previous study.[18] Tramadol was administered intraperitoneally in a volume of 0.5 mL. For intrathecal administration, all drugs were in a volume of 5 μL, followed by a 10-μL saline injection to flush the catheter. All drugs were dissolved in normal saline. Tramadol was donated by the Nihon Shinyaku Corporation (Kyoto, Japan). Other drugs were purchased from Sigma Chemical Co. (St. Louis, MO).

Microdialysis Studies

According to a previous study, microdialysis studies were performed to measure NA and 5-HT levels in the spinal dorsal horn using rats with a paw incision (24 hours after incision).[19] Anesthesia was induced with urethane (1.2 to 1.5 g/kg, intraperitoneal), and maintained with 0.5% isoflurane in 100% oxygen through a nose cone. The left femoral vein was cannulated for transfusion of saline at a rate of 1 mL/hour. A 24G indwelling catheter was placed into the peritoneal cavity for drug administration. Rectal temperature was maintained at 37° to 38°C by a heating pad placed beneath the animal. The L3 to L5 level of the spinal cord was exposed by a thoraco-lumbar laminectomy, and the rat was placed in a stereotaxic apparatus. The probe was inserted from just lateral to the dorsal root and advanced at a 15° angle to a depth of 1 mm using a micromanipulator (model WR-88, Narishige, Japan). The surface of the spinal cord was covered with mineral oil. Microdialysis probes comprised a 1-mm length of cylinder-shaped dialysis membrane (OD = 0.22 mm, ID = 0.20 mm), and the membrane was attached to a 1-cm silica double-lumen tube (OD = 0.35 mm; Eicom Co., Kyoto, Japan). The microdialysis probe was perfused with Ringer's solution (147 mM NaCl, 4 mM KCl, 2.3 mM CaCl2) at a constant flow rate (1 μL/min) using a syringe pump (ESP-64, Eicom Co.). After 120 minutes of constant perfusion, 2 consecutive samples were collected to determine basal NA and 5-HT concentrations in the dialysate. Saline (0.5 mL) or tramadol (20 mg/kg) was administered intraperitoneally through an indwelling catheter and 15-minute perfusate fractions were collected into an autoinjector (EAS-20, Eicom Co.). Samples (15 μL) were automatically injected and analyzed for NA and 5-HT concentration using high-performance liquid chromatography with electrochemical detection by an HTEC-500 analyzing system (Eicom Co.). The chromatographic conditions were as follows: the mobile phase consisted of 0.1 M ammonium acetate buffer (pH 6.0) and methanol (7:3 v/v) containing 0.05 M sodium sulfonate and 50 mg/l EDTA-2Na. The column was a EICOMPAC CAX (2.0 mm × 200 mm; Eicom Co.). The detection limit of this assay in the current study was 30 fg per injection (information from Eicom Co.).

NA and 5-HT Content in the Spinal Cord

We also measured the NA and 5-HT content in the spinal cord in normal rats as a control and rats with paw incision as previously described.[20] The dorsal lumbar spinal cords (L4 to L6) were dissected out and divided into the left and right halves. The spinal cords were weighed and kept on ice. Each spinal cord was homogenized in 500 μL of 0.2 M perchloric acid containing 0.1 mM Na2-EDTA and isoproteronol (0.02 mg/mL) as an internal standard, and centrifuged at 20,000g at 0°C for 15 minutes. Supernatants were

kept at pH 3.0 by adding 1 M sodium acetate, and filtered through a 0.45-μm pore size centrifugal filter (Millipore Co., Bedford, MA). Samples (10 μL) were injected into an HTEC-500 analyzing system (Eicom Co.) and the concentrations of NA and 5-HT were analyzed using high-performance liquid chromatography with electrochemical detection. The chromatographic conditions were as follows: The mobile phase comprised 0.1 M phosphate buffer (pH 6.0) containing 5 mg/l Na2-EDTA, 190 mg/l sodium 1-octanesulphate acid, and 17% methanol, and the column was an EICOMPAK SC-5ODS (3.0 mm × 150 mm, Eicom Co.). The detection limit of this assay in the current study was 30 fg per injection.

Rotarod Test

Sedation and motor coordination were tested using the accelerating rotarod (ENV577, Med Associates Inc., St. Albans, VT) in which rats were required to walk against the motion of a rotating drum with the speed accelerating from 4 to 40 rpm/minutes over 300 seconds. The time on the rod from the start of acceleration until the animal fell from the drum onto the countertrip plate was recorded. A 300-second cutoff was used. One training period per day was performed for 2 days before the drug treatment. Animals were acclimated to the device and habituated to handling to minimize stress during testing.

Statistics

Statistical analysis was conducted using Sigma Plot (Version 11.0, Systat Software, San Jose, CA). The effects of treatment on withdrawal thresholds were examined at each time point using the Kruskal-Wallis test. Significant effects were further evaluated by pairwise comparisons of the mean ranks of the treatment groups at each time point using Student-Newman–Keuls post hoc test. The effects of treatment over time on withdrawal thresholds were examined using the Friedman repeated-measures ANOVA on ranks test. Other data were analyzed using a 1-way or 2-way ANOVA. Post hoc tests were performed for between-group comparisons at time points using a Student-Newman-Keuls post hoc test for multiple comparisons. A P value of <0.05 was considered to indicate statistical significance.

RESULTS

Behavioral Studies

Paw incision induced mechanical hypersensitivity as indicated by a reduced paw withdrawal threshold (51.9 ± 0.9 g before paw incision and 9.0 ± 0.4 g after paw incision, n = 112). A previous study demonstrated robust and stable mechanical hypersensitivity for a few days after the paw incision; thresholds returned to presurgical values by 6 days.[15] Therefore, we performed behavioral studies 24 hours after the paw incision.

Intraperitoneal administration of tramadol (10, 20, and 40 mg/kg) produced antihypersensitivity effects in a dose-dependent fashion ($P < 0.05$ by Friedman repeated-measures ANOVA on ranks test; Fig. 1A). The withdrawal threshold increased at 15 minutes, and the effect continued for 120 minutes after administration of 40 mg/kg tramadol compared with the saline-treated group ($P < 0.05$ by Student-Newman–Keuls post hoc test after the Kruskal-Wallis test;

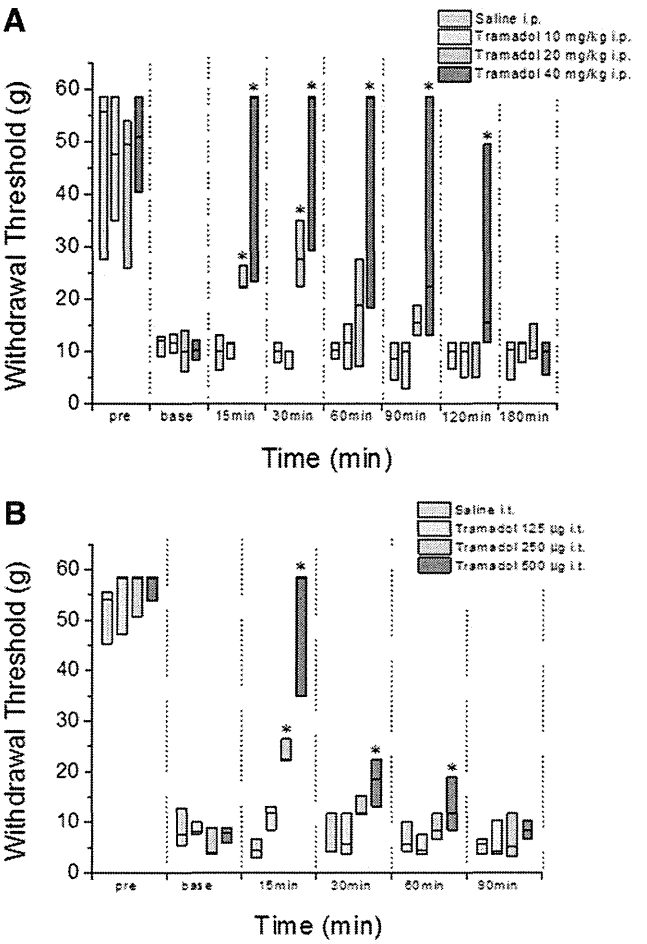

Figure 1. Time-course of the antihypersensitivity effects of intraperitoneally (A) or intrathecally (B) administered tramadol in rats 1 day after paw incision surgery. Withdrawal thresholds are expressed as the median and 25th and 75th percentiles for 7 rats in each group. *$P < 0.05$ compared with the saline-treated group at each time point by Kruskal-Wallis ANOVA on ranks followed by Student Newman Keuls post hoc test.

Fig. 1A). In the 20 mg/kg-treated group, the withdrawal threshold increased 15 and 30 minutes after administration compared with the saline-treated group ($P < 0.05$ by Student-Newman–Keuls post hoc test after the Kruskal-Wallis test; Fig. 1A).

Intrathecal administration of tramadol (125, 250, and 500 μg) produced antihypersensitivity effects in a dose-dependent fashion ($P < 0.05$ by Friedman repeated-measures ANOVA on ranks test; Fig. 1B). The withdrawal threshold increased at 15 minutes after tramadol injection in 250 and 500 μg-treated groups, and the effect continued for 60 minutes after administration of 500 μg tramadol compared with the saline-treated group ($P < 0.05$ by Student-Newman–Keuls post hoc test after Kruskal-Wallis test; Fig. 1B). No adverse behavioral effects, such as motor effects, sedation, or agitation, were observed.

Intrathecal pretreatment with naloxone attenuated the antihypersensitivity effect of 20 mg/kg tramadol after intraperitoneal administration ($P < 0.05$ by Friedman repeated-measures ANOVA on ranks test; Fig. 2A). Although methysergide and idazoxane reversed the antihypersensitivity effect of 20 mg/kg tramadol ($P < 0.05$ by

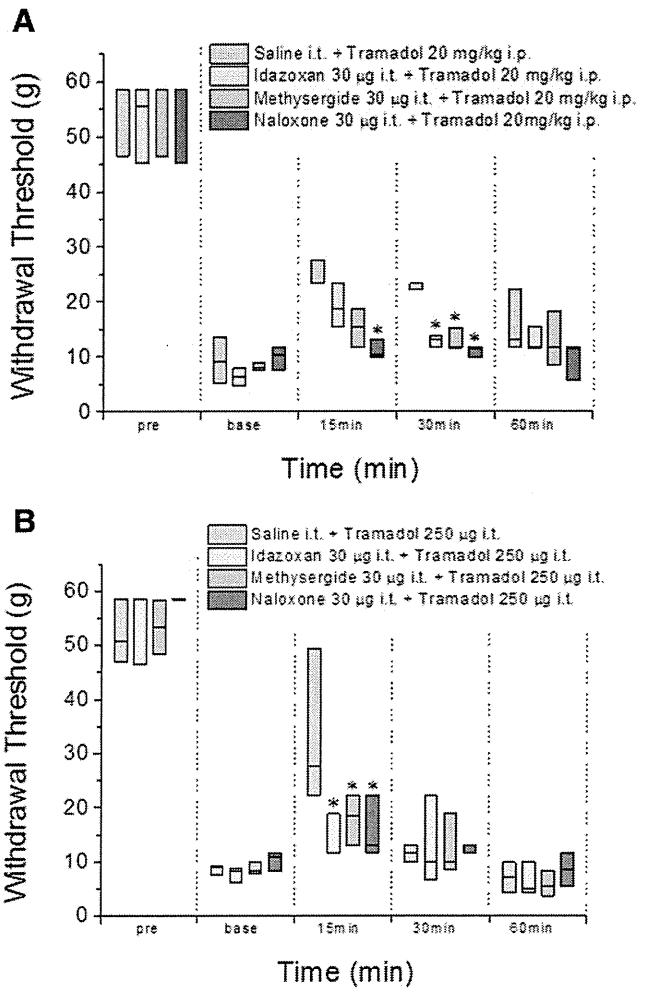

Figure 2. Effects of intrathecal pretreatment with idazoxan, a selective α2-adrenoceptor antagonist; methysergide, a 5-HT receptor antagonist; and naloxone, a μ-opioid receptor antagonist, on the antihypersensitivity effect of 20 mg/kg of tramadol. Saline or 30 μg of each antagonist was administered intrathecally 15 minutes before intraperitoneal (A) or intrathecal (B) injection of tramadol. Withdrawal thresholds are expressed as the median and 25th and 75th percentiles for 7 rats in each group. $*P < 0.05$ compared with the saline-treated group at each time point by Kruskal-Wallis ANOVA on ranks followed by Student Newman Keuls post hoc test.

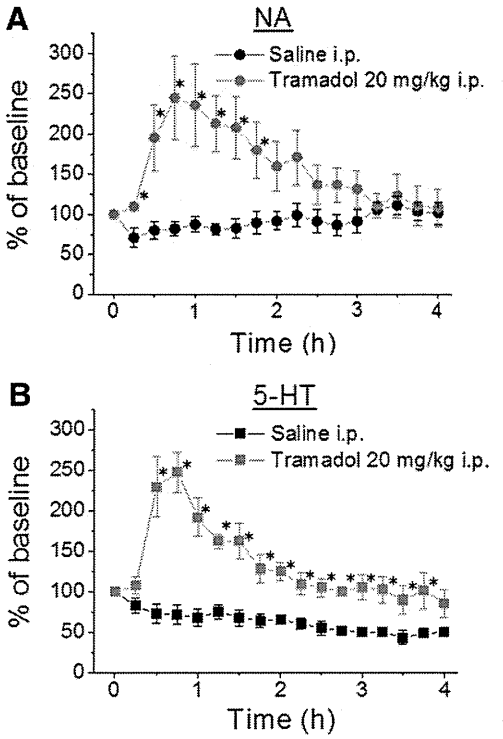

Figure 3. Microdialysis to detect increased spinal noradrenaline (NA; A) and serotonin (5-HT; B) levels. Rats with paw incision ($n = 6$) received intraperitoneal saline or tramadol (20 mg/kg). Data are presented over time as a percentage of the baseline. $*P < 0.05$ compared with the saline-treated group at each time point by 2-way ANOVA followed by Student Newman Keuls post hoc test.

Friedman repeated-measures ANOVA on ranks test; Fig. 2A), both antagonists did not attenuate the effect of tramadol at 15 minutes (Student-Newman-Keuls post hoc test after the Kruskal-Wallis test; Fig. 2A). The antihypersensitivity effect of 250 μg intrathecal tramadol was attenuated by idazoxan, methysergide, and naloxone ($P < 0.05$ by Friedman repeated-measures ANOVA on ranks test; Fig. 2B). The antihypersensitivity effects of the maximum doses of intraperitoneal (40 mg/kg) and intrathecal (500 μg) tramadol were not reversed by these antagonists. Therefore, we selected 20 mg/kg (intraperitoneal administration) and 250 μg (intrathecal administration) of tramadol for antagonist studies.

Microdialysis Study

The baseline NA concentration before drug injection was not different between the saline-treated group and the tramadol-treated group (0.76 ± 0.203 pg/15 μL in the saline-treated group, and 0.87 ± 0.124 pg/15 μL in the tramadol-treated group, respectively). Baseline 5-HT concentrations before drug injection were also similar (0.64 ± 0.065 pg/15 μL in the saline-treated group, and 0.57 ± 0.29 pg/15 μL in the tramadol-treated group, respectively). In the saline-treated group, the NA and 5-HT concentrations in the dialysates did not change over time (Fig. 3). In the tramadol (20 mg/kg intraperitoneally) treated group, NA concentration increased at 15 minutes after the drug injection and reached approximately 250% of the baseline value, and the increase continued for 105 minutes after drug injection ($P < 0.05$ by Student-Newman–Keuls post hoc test after 2-way ANOVA; Fig. 3A). The 5-HT concentration also increased at 30 minutes after the drug injection and reached approximately 250% of the baseline value, and the increase continued for 225 minutes after drug injection ($P < 0.05$ by Student-Newman-Keuls post hoc test after 2-way ANOVA; Fig. 3B).

NA and 5-HT Contents in the Spinal Cord After Paw Incision

NA and 5-HT contents in the homogenized tissue from the dorsal half of the spinal cord of normal rats (day 0) and 1 and 3 days after paw incision were also determined (Fig. 4). The NA concentration was larger on the ipsilateral dorsal half of the spinal cord in rats 1 day after paw incision (505.5 ± 24.3 pg/mg, $n = 8$) compared with normal rats (418.9 ± 31.0 pg/mg, $n = 8$, $P < 0.05$ by 1-way ANOVA).

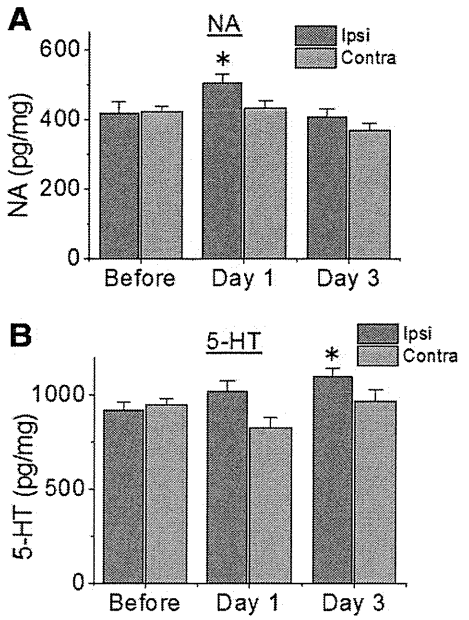

Figure 4. The contents (pg/mg wet tissue) of noradrenaline (NA; A) and serotonin (5-HT; B) in the ipsilateral (Ipsi) and contralateral (Contra) dorsal lumbar spinal cord were measured in rats before paw incision (normal rats), and 1 d (day 1) and 3 d (day 3) after paw incision. The ipsilateral and contralateral halves of the dorsal lumbar spinal cord were measured. All values represent the mean ± SEM for 8 rats. $*P < 0.05$ compared with the normal rats.

The 5-HT concentration was larger in the ipsilateral dorsal half of the spinal cord in rats 3 days after paw incision (1099.2 ± 39.0 pg/mg, $n = 8$) compared with normal rats (919.9 ± 42.8 pg/mg, $n = 8$, $P < 0.05$ by 1-way ANOVA). In contrast, the NA and 5-HT concentrations did not change until 3 days after paw incision on the contralateral side.

Motor Coordination

In the rotarod test, neither intraperitoneal administration of saline (129.8 ± 15.4 seconds before and 134.2 ± 18.2 seconds after administration) nor intraperitoneal administration of 40 mg/kg of tramadol (129.0 ± 18.0 seconds before and 135.4 ± 15.8 seconds after administration) affected performance in normal animals.

DISCUSSION

In the present study, we examined the antihypersensitivity effect of tramadol in a rat model of postoperative pain produced by paw incision. Intraperitoneal or intrathecal tramadol produced a dose-dependent antihypersensitivity effect with no adverse behavioral effects when injected 24 hours after paw incision. The antihypersensitivity effects were attenuated by intrathecal pretreatment with idazoxan, a selective α2-adrenoceptor antagonist; methysergide, a 5-HT receptor antagonist; and naloxone, a nonselective opioid receptor antagonist. Direct measurements of NA and 5-HT from the spinal dorsal horn with microdialysis revealed that tramadol increased both NA and 5-HT levels. Furthermore, the NA content 1d after paw incision and the 5-HT content 3d after paw incision in the ipsilatereal dorsal lumbar spinal cord were larger than in normal animals. These results indicate that NA and 5-HT reuptake inhibition in the spinal cord is an important mechanism underlying tramadol-mediated antihypersensitivity effect in rats with paw incision. Our results also suggest that paw incision increases spinal NA and 5-HT levels and these plastic changes contribute to the antihypersensitivity effect of tramadol.

The neurotransmitters NA and 5-HT have important roles in suppressing nociceptive transmission in the spinal cord. Bulbospinal descending NA and 5-HT systems suppress nociceptive signals from primary afferent neurons to the spinal dorsal horn neurons. Intrathecal administration of adrenoceptor agonists and 5-HT receptor agonists produce antinociceptive effects on acute pain in rodents[13,14] and suppress allodynia in a rat model of neuropathic pain.[21,22] (−)-Tramadol and its principal metabolite (−)-O–desmethyltramadol inhibit NA reuptake,[8] and (+)-tramadol blocks 5-HT reputake.[9–11] Previous studies demonstrated that tramadol inhibits the reuptake of NA and 5-HT in vitro.[8,10–12] The microdialysis experiments of the present study support these findings by showing that tramadol increases NA and 5-HT levels in the spinal cord. Both NA and 5-HT increased to approximately 250% of their baseline value after intraperitoneal injection of tramadol (20 mg/kg).

A previous study showed that NA increase in the spinal cord by a selective NA reuptake inhibitor produced a powerful antihypersensitivity effect through spinal α2-adrenergic receptors in the postoperative pain model.[23] In the present study, intrathecal pretreatment with idazoxan, an α2-adrenoceptor antagonist, reversed the effect of intrathecally administered tramadol. Further support for the assumption of the participation of the NA reuptake inhibition in the analgesic effect of tramadol is derived from the observation that the spinal antinociceptive effects of tramadol are antagonized by the α2-adrenoceptor blocker yohimbine.[7]

Descending serotonergic pathways have been implicated in both inhibitory and facilitatory modulation of spinal neuronal activity. Several 5-HT receptor subtypes, including the 5-HT1A, 5-HT1B, 5-HT2A, 5-HT2C, and 5-HT3 receptors, contribute to the antinociceptive action of 5-HT in the spinal cord.[24] However, there is some controversy because the effects observed are highly dependent on the animal model and behavioral testing paradigm used as well as the dose of agents administered. Methysergide has affinity for both 5-HT1 and 5-HT2 receptor subtypes.[25] We previously demonstrated that 30 μg methysergide reverses the antihypersensitivity effect of intrathecally administered milnacipran, a 5-HT and NA reuptake inhibitor, in a neuropathic pain model.[18] In the present study, intrathecal pretreatment with methysergide attenuated the peak effect of intrathecally administered tramadol to a similar degree as idazoxan. Thus, our results strongly suggest that the increase of 5-HT in the spinal cord plays a crucial role in the antihypersensitivity effects of tramadol. It could be argued that 5-HT in the spinal cord enhances descending facilitation mediated by spinal 5-HT3 receptors as observed in the neuropathic pain state.[26,27] However, this mechanism may not occur in postoperative pain because the analgesic effects of tramadol for postoperative pain in humans are inhibited by the 5-HT3 receptor antagonist ondansetron.[28,29]

(+)-Tramadol has a low affinity for opioid receptors,[1] and the major metabolite of the (+)-tramadol, (+)-O-desmethyltramadol (M1), is a potent agonist of μ-opioid receptors.[30] In the present study, intrathecal pretreatment with a nonselective opioid receptor antagonist naloxone almost reversed the effects of tramadol after intraperitoneal (20 mg/kg) and intrathecal (250 μg) administration. Our results suggest that the antihypersensitivity effect of tramadol is mainly mediated by spinal opioid receptors.

Our results strongly suggest that tramadol produces antihypersensitivity effects in the spinal cord. A previous study revealed that intrathecal administration of a 5-HT and NA reuptake inhibitor has antihypersensitivity effects in a postoperative pain model.[19] Tramadol might reduce hypersensitivity by acting as a 5-HT and NA reuptake inhibitor as well as a opioid receptor agonist in the local spinal cord. In behavioral studies with intrathecal administration, we injected all drugs in a small volume of saline (5 μL) to examine spinal mechanisms of tramadol for postoperative pain. Although we cannot exclude drug effects at the brain or dorsal root ganglia locations, drugs mainly act at the spinal level after intrathecal injection.[16]

Tramadol is widely used as an analgesic drug in the treatment of postoperative, neuropathic, and cancer pain.[31–33] We demonstrate that tramadol mimics activation of descending inhibition by increasing NA and 5-HT in the spinal cord. Antidepressants such as tricyclic antidepressants and 5-HT and NA reuptake inhibitors that increase NA and 5-HT in the spinal cord[34] are recommended as first-line drugs for treatment of neuropathic pain.[35] Therefore, our results suggest that tramadol affects not only postoperative pain, but also chronic pain including neuropathic pain.

In conclusion, our results indicate that tramadol produces antihyperalgesic effects by increasing 5-HT and NA levels in the spinal cord, as well as opioid receptor activation. The spinal cord is an important site of antinociceptive action of tramadol, spinal, or epidural administration of tramadol might be a promising method to suppress postoperative pain. ■

DISCLOSURES

Name: Masafumi Kimura, MD.
Contribution: This author helped conduct the study, analyze the data, and write the manuscript.
Attestation: Masafumi Kimura has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.
Name: Hideaki Obata, MD, PhD.
Contribution: This author helped design the study, conduct the study, and write the manuscript.
Attestation: Hideaki Obata has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.
Name: Shigeru Saito, MD, PhD.
Contribution: This author helped write the manuscript.
Attestation: Shigeru Saito has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.
This manuscript was handled by: Steven L. Shafer, MD.

REFERENCES

1. Hennies HH, Friderichs E, Schneider J. Receptor binding, analgesic and antitussive potency of tramadol and other selected opioids. Arzneimittelforschung 1988;38:877–80
2. Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, Vaught JL, Jacoby HI, Selve N. Complementary and synergistic antinociceptive interaction between the enantiomers of tramadol. J Pharmacol Exp Ther 1993;267:331–40
3. Paar WD, Poche S, Gerloff J, Dengler HJ. Polymorphic CYP2D6 mediates O-demethylation of the opioid analgesic tramadol. Eur J Clin Pharmacol 1997;53:235–9
4. Ide S, Minami M, Ishihara K, Uhl GR, Sora I, Ikeda K. Mu opioid receptor-dependent and independent components in effects of tramadol. Neuropharmacology 2006;51:651–8
5. Carlsson KH, Jurna I. Effects of tramadol on motor and sensory responses of the spinal nociceptive system in the rat. Eur J Pharmacol 1987;139:1–10
6. Kayser V, Besson JM, Guilbaud G. Effects of the analgesic agent tramadol in normal and arthritic rats: comparison with the effects of different opioids, including tolerance and cross-tolerance to morphine. Eur J Pharmacol 1991;195:37–45
7. Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, Vaught JL. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an 'atypical' opioid analgesic. J Pharmacol Exp Ther 1992;260:275–85
8. Driessen B, Reimann W, Giertz H. Effects of the central analgesic tramadol on the uptake and release of noradrenaline, and dopamine in vitro. Br J Pharmacol 1993;108:806–11
9. Driessen B, Reimann W. Interaction of the central analgesic, tramadol, with the uptake and release of 5-hydroxytryptamine in the rat brain in vitro. Br J Pharmacol 1992;105:147–51
10. Frink MC, Hennies HH, Englberger W, Haurand M, Wilffert B. Influence of tramadol on neurotransmitter systems of the rat brain. Arzneimittelforschung 1996;46:1029–36
11. Raffa RB. Basic pharmacology relevant to drug abuse assessment: tramadol as example. J Clin Pharm Ther 2008;33:101–8
12. Bloms-Funke P, Dremencov E, Cremers TI, Tzschentke TM. Tramadol increases extracellular levels of serotonin and noradrenaline as measured by in vivo microdialysis in the ventral hippocampus of freely-moving rats. Neurosci Lett 2011; 490:191–5
13. Yaksh TL, Wilson PR. Spinal serotonin terminal system mediates antinociception. J Pharmacol Exp Ther 1979;208:446–53
14. Reddy SVR, Maderdrut JL, Yaksh TL. Spinal cord pharmacology of adrenergic agonist-mediated antinociception. J Pharmacol Exp Ther 1980;213:525–33
15. Brennan TJ, Vandermeulen EP, Gebhart GF. Characterization of a rat model of incisional pain. Pain 1996;64:493–501
16. Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976;17:1031–6
17. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Meth 1994;53:55–63
18. Obata H, Saito S, Koizuka S, Nishikawa K, Goto F. The monoamine-mediated antiallodynic effects of intrathecally administered milnacipran, a serotonin noradrenaline reuptake inhibitor, in a rat model of neuropathic pain. Anesth Analg 2005;100:1406–10
19. Obata H, Kimura M, Nakajima K, Tobe M, Nishikawa K, Saito S. Monoamine-dependent, opioid-independent antihypersensitivity effects of intrathecally administered milnacipran, a serotonin noradrenaline reuptake inhibitor, in a postoperative pain model in rats. J Pharmacol Exp Ther 2010;334:1059–65
20. Peters CM, Hayashida K, Ewan EE, Nakajima K, Obata H, Xu Q, Yaksh TL, Eisenach JC. Lack of analgesic efficacy of spinal ondansetron on thermal and mechanical hypersensitivity following spinal nerve ligation in the rat. Brain Res 2010;1352:83–93
21. Yaksh TL, Pogrel JW, Lee YW, Chaplan SR. Reversal of nerve ligation-induced allodynia by spinal alpha-2 adrenoceptor agonists. J Pharmacol Exp Ther 1995;272:207–14
22. Obata H, Saito S, Sasaki M, Ishizaki K, Goto F. Antiallodynic effect of intrathecally administered 5-HT2 agonists in rats with nerve ligation. Pain 2001;90:173–9

23. Obata H, Conklin D, Eisenach JC. Spinal noradrenaline transporter inhibition by reboxetine and Xen2174 reduces tactile hypersensitivity after surgery in rats. Pain 2005;113:271–6
24. Millan MJ. Descending control of pain. Prog Neurobiol 2002; 66:355–474
25. Hamel E, Robert J-P, Young AR, MacKenzie ET. Pharmacological properties of the receptor(s) involved in the 5-hydroxytryptamine-induced contraction of the feline middle cerebral artery. J Pharmacol Exp Ther 1989;249:879–89
26. Suzuki R, Rahman W, Hunt SP, Dickenson AH. Descending facilitatory control of mechanically evoked responses is enhanced in deep dorsal horn neurones following peripheral nerve injury. Brain Res 2004;1019:68–76
27. Rahman W, Suzuki R, Webber M, Hunt SP, Dickenson AH. Depletion of endogenous spinal 5-HT attenuates the behavioural hypersensitivity to mechanical and cooling stimuli induced by spinal nerve ligation. Pain 2006;123:264–74
28. De Witte JL, Schoenmaekers B, Sessler DI, Deloof T. The analgesic efficacy of tramadol is impaired by concurrent administration of ondansetron. Anesth Analg 2001;92:1319–21
29. Arcioni R, della Rocca M, Romanò S, Romano R, Pietropaoli P, Gasparetto A. Ondansetron inhibits the analgesic effects of tramadol: a possible 5-HT(3) spinal receptor involvement in acute pain in humans. Anesth Analg 2002;94:1553–7
30. Garrido MJ, Valle M, Campanero MA, Calvo R, Trocóniz IF. Modeling of the in vivo antinociceptive interaction between an opioid agonist, (+)-O–desmethyltramadol, and a monoamine reuptake inhibitor, (−)–O-desmethyltramadol, in rats. J Pharmacol Exp Ther 2000;295:352–9
31. Moore RA, McQuay HJ. Single-patient data meta-analysis of 3453 postoperative patients: oral tramadol versus placebo, codeine and combination analgesics. Pain 1997;69:287–94
32. O'Connor AB, Dworkin RH. Treatment of neuropathic pain: an overview of recent guidelines. Am J Med 2009;122:22–32
33. Leppert W. Tramadol as an analgesic for mild to moderate cancer pain. Pharmacol Rep 2009;61:978–92
34. Sindrup SH, Otto M, Finnerup NB, Jensen TS. Antidepressants in the treatment of neuropathic pain. Basic Clin Pharmacol Toxicol 2005;96:399–409
35. Dworkin RH, O'Connor AB, Backonja M, Farrar JT, Finnerup NB, Jensen TS, Kalso EA, Loeser JD, Miaskowski C, Nurmikko TJ, Portenoy RK, Rice AS, Stacey BR, Treede RD, Turk DC, Wallace MS. Pharmacologic management of neuropathic pain: evidence-based recommendations. Pain 2007;132:237–51

PAIN® 153 (2012) 990–997

PAIN®

www.elsevier.com/locate/pain

An increase in spinal cord noradrenaline is a major contributor to the antihyperalgesic effect of antidepressants after peripheral nerve injury in the rat

Kunie Nakajima, Hideaki Obata*, Nobuhisa Iriuchijima, Shigeru Saito

Department of Anesthesiology, Gunma University Graduate School of Medicine, Gunma, Japan

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

ARTICLE INFO

Article history:
Received 2 March 2011
Received in revised form 13 January 2012
Accepted 27 January 2012

Keywords:
Antidepressants
Neuropathic pain
Spinal cord
Noradrenaline

ABSTRACT

Antidepressants are often used for the treatment of neuropathic pain. Clinical studies suggest that the efficacy of serotonin (5-HT) and noradrenaline (NA) reuptake inhibitors (SNRIs) for neuropathic pain is greater than that of selective 5-HT reuptake inhibitors (SSRIs). In the present study, we determined the efficacy and mechanisms involved in the antihyperalgesic effects of milnacipran, an SNRI, compared with paroxetine, an SSRI, and maprotiline, a selective NA reuptake inhibitor, using a rat model of neuropathic pain. Male Sprague-Dawley rats underwent spinal nerve ligation (SNL), and the withdrawal threshold to paw pressure was measured. Intraperitoneal injection of milnacipran (3–30 mg/kg) produced a dose-dependent antihyperalgesic effect. The effect was reversed by intrathecal injection of the α_2-adrenoceptor antagonist idazoxan (30 μg), but not by various 5-HT receptor antagonists. Paroxetine produced an antihyperalgesic effect only at the highest dose tested (10 mg/kg). This effect was reversed by intrathecal injection of both idazoxan and ondansetron (30 μg), a 5-HT3 receptor antagonist. Maprotiline produced an antihyperalgesic effect (10 and 30 mg/kg), and the effect was reversed by intrathecal idazoxan. In microdialysis studies, NA and 5-HT concentrations in the spinal dorsal horn were increased after injection of either milnacipran or paroxetine, and only NA was increased after maprotiline. Furthermore, the NA content in the spinal cord of SNL rats was greater than that in normal animals. These findings suggest that an increase in NA in the spinal cord plays an important role in the antihyperalgesic effects of not only NA reuptake inhibitors but also SSRIs.

1. Introduction

Peripheral nerve injury leads to neuropathic pain, which is associated with various changes in sensory processing from the primary afferent neuron to the spinal cord and to supraspinal and cortical regions. Brainstem-spinal descending noradrenaline (NA) and serotonin (5-HT) systems suppress nociceptive signals from primary afferent neurons to the spinal dorsal horn, and these inhibitory systems may play an important role in neuropathic pain states [24]. Activation of spinal adrenoceptors and 5-HT receptors produces antinociceptive effects in various pain models in rodents (see reviews [8,12,24,37]). In addition, intrathecal administration of adrenoceptor agonists and 5-HT receptor agonists suppresses allodynia in a rat model of neuropathic pain [29,46]. Although the 5-HT pathway is also reported to cause descending facilitation after peripheral nerve injury in rodents [35,42], recruitment of monoaminergic descending inhibitory systems might be a promising treatment for the management of neuropathic pain.

Antidepressants are widely used for the treatment of neuropathic pain. Among antidepressants, tricyclic antidepressants (TCAs) and serotonin NA reuptake inhibitors (SNRIs) are considered first-line drugs for the management of neuropathic pain [7]. Although antidepressants may inhibit neuropathic pain in the spinal cord by blocking NA or 5-HT reuptake, the mechanisms of the antinociceptive effects of TCAs are complex (see reviews [4,6,23,41]). For example, TCAs activate the endogenous opioidergic system as well as GABA-B receptor and adenosine A1 receptors. They also block several ion channels, and most TCAs have an affinity for multiple receptors including *N*-methyl-d-aspartate (NMDA) receptors. In contrast, SNRIs selectively inhibit the reuptake of NA and 5-HT without relevant affinity for any other receptors or ion channels [41]. One representative SNRI is milnacipran [25].

We hypothesized that reuptake of NA and the subsequent increase of NA in the spinal cord had a key role in suppression of neuropathic pain by antidepressants, because the efficacy of selective serotonin reuptake inhibitors (SSRIs) for treating neuropathic pain is less than that of SNRIs and TCAs [11,40,41]. To test this hypothesis, we investigated the mechanisms of the antihyperalgesic effects of milnacipran using a rat model of neuropathic pain, first by defining which receptors are involved in the antihyperalgesic effects

* Corresponding author. Tel.: +81 27 220 8454; fax: +81 27 220 8473.
E-mail address: hobata@showa.gunma-u.ac.jp (H. Obata).

0304-3959/$36.00
doi:10.1016/j.pain.2012.01.029

produced by milnacipran in the spinal cord and then by evaluating the increase in NA and 5-HT levels in the dorsal horn spinal cord after milnacipran injection using microdialysis. We also performed these studies using paroxetine, an SSRI, and maprotiline, a selective NA reuptake inhibitor, to compare the efficacy and mechanisms of the antihyperalgesic effects of milnacipran. Pharmacologically, milnacipran blocks NA and 5-HT reuptake with almost equal affinity [21,25,43], paroxetine has a high selectivity for 5-HT reuptake inhibition, and maprotiline has a high selectivity for NA reuptake inhibition [25]; therefore, the use of these drugs together in the present study allows the determination of the relative roles of noradrenergic and serotonergic signaling in the antihyperalgesic effects of antidepressants.

2. Method

2.1. Animals

The experiments were approved by the Animal Care and Use Committee of the Gunma University Graduate School of Medicine. Male Sprague-Dawley rats (200–250 g) were used in all experiments. Animals were housed under a 12-h light–dark cycle with free access to food and water. At the end of the study, all animals were killed by an overdose of pentobarbital.

Spinal nerve ligation (SNL) was performed as previously described [20]. In brief, animals were anesthetized with inhalational isoflurane (2%) in oxygen, the lateral laminae of the lower lumbar and upper sacral vertebrae were exposed, the right L6 transverse process was removed, and the right L5 and L6 spinal nerves were identified and tightly ligated using a 5-0 silk suture. The wound was then closed. One week after SNL surgery, an intrathecal catheter was inserted for drug administration. A sterilized 32-gauge polyethylene catheter (RecathCo, Allison Park, PA) connected to 8.5-cm Tygon external tubing (Saint-Gobain Performance Plastics, Akron, OH) was inserted while the rat was under isoflurane anesthesia as previously described [47]. The catheter was passed caudally 7.5 cm from the cisterna magnum to the lumbar enlargement. The animals were allowed to recover for 7 days before drug testing.

2.2. Behavioral assessments

The person performing the behavioral test was blinded to drug and dose. Withdrawal threshold to pressure applied to the hind paw, expressed in grams, was measured using an analgesimeter (Ugo Basile, Comerio, Italy) as previously described [36]. The device applies increasing pressure to the hind paw. When the animal withdrew its paw, the pressure was immediately released, and the withdrawal threshold was read on a scale. Animal training for this test was performed for 3 to 5 days before the drug treatment. A cut-off of 250 g was used to avoid tissue injury. We used these animals 2 or 3 times at 4- to 5-day intervals. The drugs and doses were randomly assigned.

2.3. Drugs and drug administration

The first series of experiments was performed to examine the time course of the antihyperalgesic effects and the dose-response effects of intraperitoneally administered milnacipran (0, 3, 10, 30 mg/kg), paroxetine (0, 1, 3, 10 mg/kg), and maprotiline (0, 3, 10, 30 mg/kg). The withdrawal threshold was determined before (before SNL surgery) and at time 0 (before drug injection), then at 30-min intervals after the injection. For intraperitoneal injection, drugs were injected in a 1-mL volume. Milnacipran and maprotiline were dissolved in saline, and paroxetine was dissolved in 25% dimethylsulfoxide solution. The second series of experiments was performed to determine the effects of intrathecal treatment with 1 of the following drugs: an α_2-adrenoceptor antagonist, idazoxan; a 5-HT1/2 receptor antagonist, methysergide; a 5-HT2A/2C receptor antagonist, ketanserin; and a 5-HT3 receptor antagonist, ondansetron. Saline or 30 μg of each antagonist was administered intrathecally 30 minutes before the peak effect of each drug (60 minutes after milnacipran injection, 30 minutes after paroxetine injection, and simultaneously with maprotiline injection), and the threshold was then determined 30 minutes later. The withdrawal thresholds after the injection of these receptor antagonists were compared with those of the saline-treated group. The dose of the antagonist was selected based on the results of a previous study [28] and our preliminary studies. For intrathecal injection, each antagonist was dissolved in saline, and injected in a volume of 5 μL, followed by a 10-μL injection of saline to flush the catheter. Milnacipran was a kind donation from the Asahi Kasei Corporation (Osaka, Japan). Methysergide was purchased from Research Biochemicals International (Natick, MA). Other drugs were purchased from Sigma (St. Louis, MO).

2.4. Microdialysis studies

Microdialysis studies were performed with normal rats as unoperated control and SNL rats as previously described [26]. Anesthesia was induced with urethane (1.2-1.5 g/kg administered intraperitoneally) and then maintained with 0.5% isoflurane in 100% oxygen through a nose cone. The left femoral vein was cannulated for fluid infusion or drug injections. The rectal temperature was maintained at 37° to 38°C with a heating pad placed beneath the animal. The L3 to L5 level of spinal cord was exposed using a thoracolumbar laminectomy, and then the rat was placed in a stereotaxic apparatus. After opening the dura, a dorsal root that enters the spinal cord above the level of the recording sites was lifted using a glass retractor, so that a microdialysis probe could be advanced into the superficial layer of the dorsal horn. The probe was inserted just lateral to the dorsal root and advanced at an angle of 15° to a depth of 1 mm using a micromanipulator (model WR-88; Narishige, Tokyo, Japan). The surface of the spinal cord was covered with mineral oil. Microdialysis probes comprised a 1-mm length of cylindrical dialysis membrane (OD: 0.22 mm, ID: 0.20 mm), and the membrane was attached to a 1-cm silica double-lumen tube (OD: 0.35 mm; Eicom, Kyoto, Japan). The microdialysis probe was perfused with Ringer's solution (147 mmol/L NaCl, 4 mmol/L KCl, and 2.3 mmol/L CaCl2) at a constant flow rate (1 μL/min) using a microsyringe pump (ESP-64; Eicom Co.). After 120 minutes of constant perfusion, 2 consecutive samples were collected to determine basal NA and 5-HT concentrations in the dialysate. SNL rats were used in time course studies. In the pilot study, we observed that the time course of the increase in NA and 5-HT after milnacipran injection was the same between intraperitoneal and intravenous injections (Fig. 4, inset). Therefore, all drugs were injected intravenously in the microdialysis studies because of technical reasons (eg, difficulty of intraperitoneal injection in rats lying prone in the microdialysis apparatus and to permit the stable increase of the blood concentration of the drugs, especially in the cumulative dose-response studies). The maximum effective dose of milnacipran (30 mg/kg), paroxetine (10 mg/kg), maprotiline (10 mg/kg), or saline (1 mL) was administered intravenously through a cannula in the femoral vein. We also performed cumulative dose-response studies with milnacipran to compare the NA and 5-HT increase in SNL and control rats. Milnacipran was administered intravenously at doses of 1, 2, 7, and 20 mg/kg (cumulative doses: 1, 3, 10, and 30 mg/kg) at 30-min intervals. The 15-min perfusate fractions were collected into an auto injector (EAS-20; Eicom Co.), and the NA and 5-HT concentrations were analysed using high-performance liquid chromatography (HPLC) with

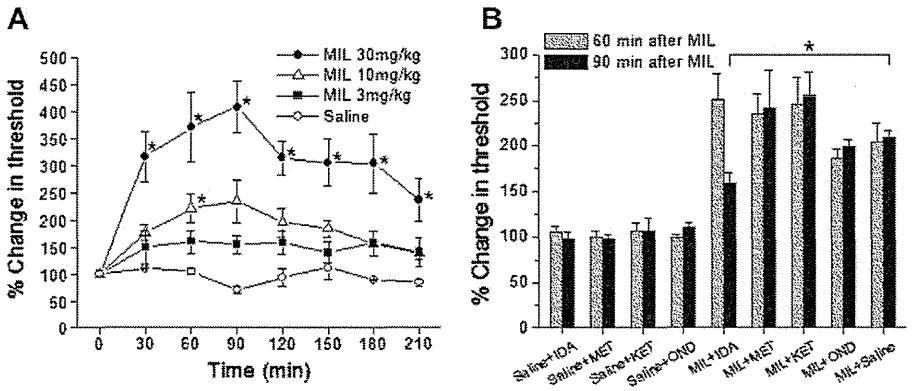

Fig. 1. (A) Time course of the antihyperalgesic effect of intraperitoneal injection of milnacipran (MIL), an SNRI, in rats with SNL. Intraperitoneal injection of milnacipran produced a dose-dependent increase (3–30 mg/kg, $P < .05$ by 2-way ANOVA, n = 6 in each group) in withdrawal threshold compared with saline-treated group. All values represent the mean ± SEM for 6 rats. *$P < .05$ compared with the saline-treated group. (B) Effect of intrathecal injection with α_2-adrenoceptor and 5-HT receptor antagonists on the antihyperalgesic effect of 30 mg/kg of milnacipran. After determining the threshold 60 minutes after milnacipran injection (60 min after MIL), rats received intrathecal administration of 30 μg idazoxan (IDA), an α_2-adrenoceptor antagonist; 30 μg methysergide (MET), a 5-HT1/2 receptor agonist; 30 μg ketanserin (KET), a 5-HT2A/2C receptor antagonist; 30 μg ondansetron (OND), a 5-HT3 receptor antagonist; or saline, and then threshold was determined 30 minutes later (90 min after MIL). All values represent the mean ± SEM for 6 rats. *$P < .05$ between the 2 groups.

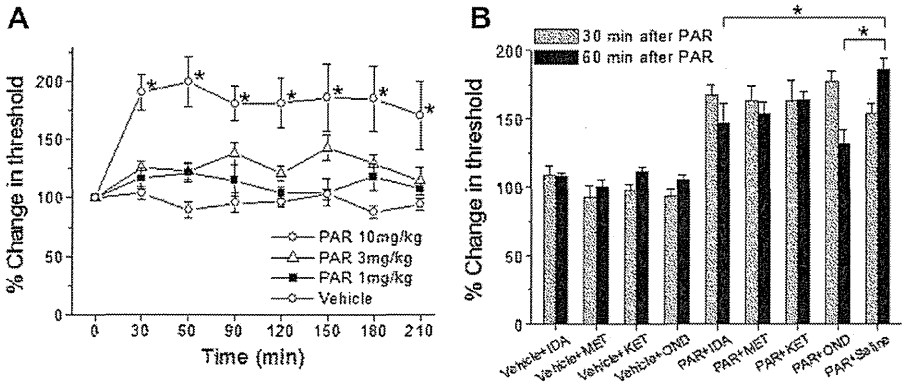

Fig. 2. (A) Time course of the antihyperalgesic effect of intraperitoneal injection of paroxetine (PAR), an SSRI, in rats with SNL. Intraperitoneal injection of paroxetine produced an antihyperalgesic effect at the highest dose (10 mg/kg, $P < .05$ by 2-way ANOVA, n = 6 in each group) in the withdrawal threshold compared with vehicle-treated group. All values represent the mean ± SEM for 6 rats. *$P < .05$ compared with the vehicle-treated group. (B) Effect of intrathecal injection with α_2-adrenoceptor and 5-HT receptor antagonists on the antihyperalgesic effect of 10 mg/kg of paroxetine. After determining the threshold 30 minutes after paroxetine injection (30 minutes after PAR), rats received intrathecal administration of 30 μg idazoxan (IDA), an α_2-adrenoceptor antagonist; 30 μg methysergide (MET), a 5-HT1/2 receptor agonist; 30 μg ketanserin (KET), a 5-HT2A/2C receptor antagonist; 30 μg ondansetron (OND), a 5-HT3 receptor antagonist; or saline, and then threshold was determined 30 minutes later (60 min after PAR). All values represent the mean ± SEM for 6 rats. *$P < .05$ between the 2 groups.

electrochemical detection using an HTEC-500 analyzing system (Eicom Co.). The chromatographic conditions were as follows: The mobile phase comprised 0.1 mol/L ammonium acetate buffer (pH 6.0), 0.05 mol/L sodium sulfonate in methanol (7:3 vol/vol), and 50 mg/L Na2-EDTA, and the column was an EICOMPAC CAX (2.0 mm × 200 mm; Eicom). The working electrode was glassy carbon (WE-3G, Eicom Co.); flow rate, 0.25 ml/min. The detector voltage was set at 0.45 V. The detector temperature was set at 35.0°C. The retention time for NA was 5.4 minutes, and that for 5-HT was 13.1 minutes. The detection limit of this assay in the current study was 30 fg per injection (information from Eicom Co.).

2.5. NA and 5-HT content in the spinal cord

We also measured the NA and 5-HT content in the spinal cord in normal rats as a control and in SNL rats as previously described [32]. The lumbar spinal cords (L4–L6) were dissected out and divided into the left and right halves. The spinal cords were weighed and kept on ice. Each spinal cord was homogenized in 500 μL of 0.2 mol/L perchloric acid containing 0.1 mmol/L Na2-EDTA and isoproteronol (0.02 mg/mL) as an internal standard, and centrifuged at 20,000g at 0°C for 15 minutes. Supernatants were kept at pH 3.0 by adding 1 mol/L sodium acetate, and filtered through a 0.45-μm-pore-sized centrifugal filter (Millipore, Bedford, MA). Samples (10 μL) were injected into an HTEC-500 analyzing system (Eicom) and the concentrations of NA and 5-HT were analysed using HPLC with electrochemical detection. The chromatographic conditions were as follows: The mobile phase comprised 0.1 mol/L phosphate buffer (pH 6.0) containing 5 mg/L Na2-EDTA, 190 mg/l sodium 1-octanesulphate acid, and 17% methanol, and the column was an EICOMPAK SC-5ODS (3.0 mm × 150 mm, Eicom). The detection limit of this assay in the current study was 30 fg per injection. The working electrode was glassy carbon (WE-3G, Eicom) with a flow rate of 0.5 mL/min. The detector voltage was set at 0.75 V. The detector temperature was set at 35.0°C. The retention time for NA was 4.43 minutes, and that for 5-HT was 22.95 minutes. The detection limit of this assay in the current study was 30 fg per injection (information from Eicom).

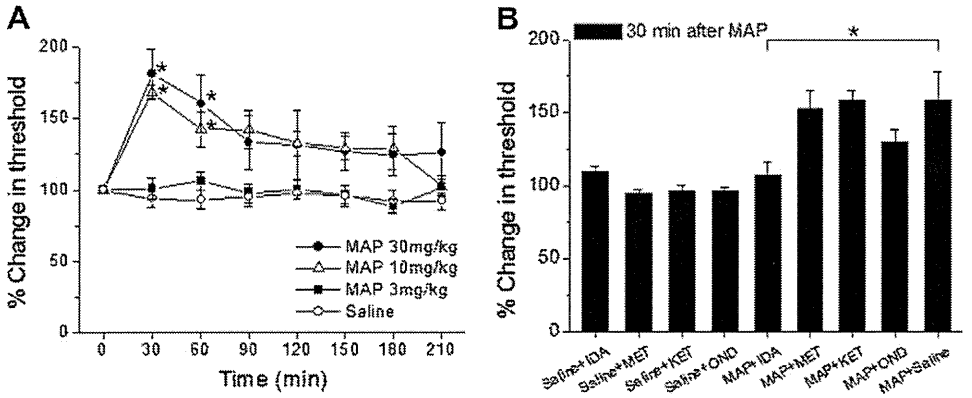

Fig. 3. (A) Time course of the antihyperalgesic effect of intraperitoneal injection of maprotiline (MAP), a selective NA reuptake inhibitor, in rats with SNL. Intraperitoneal injection of maprotiline (10 and 30 mg/kg) produced an antihyperalgesic effect ($P < .05$ by 2-way ANOVA, n = 6 in each group) in withdrawal threshold compared with saline-treated group. All values represent the mean ± SEM for 6 rats. *$P < .05$ compared with the saline-treated group. (B) Effect of intrathecal injection with α_2-adrenoceptor and 5-HT receptor antagonists on the antihyperalgesic effect of 10 mg/kg maprotiline. After determining the threshold, rats received intrathecal administration of 30 μg idazoxan (IDA), an α_2-adrenoceptor antagonist; 30 μg ketanserin (KET), a 5-HT2A/2C receptor antagonist; 30 μg methysergide (MET), a 5-HT1/2 receptor agonist; 30 μg ondansetron (OND), a 5-HT3 receptor antagonist; or saline simultaneously with maprotiline injection, and then the threshold was determined 30 minutes later. All values represent the mean ± SEM for 6 rats. *$P < .05$ between the 2 groups.

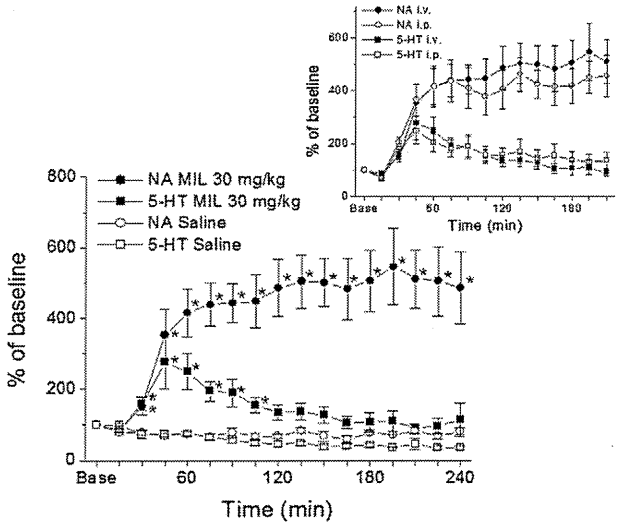

Fig. 4. Microdialysis studies for NA and 5-HT after milnacipran injection in the dorsal horn of the lumbar spinal cord. SNL rats received intravenous saline or milnacipran (MIL, 30 mg/kg). Both NA and 5-HT increased after the injection ($P < .05$ by 2-way ANOVA). A similar increase in NA and 5-HT after intravenous (i.v.) and intraperitoneal (i.p.) injection of 30 mg/kg of milnacipran is shown in the inset. Data are presented over time as percentage change of baseline. All values represent the mean ± SEM for 6 rats. *$P < .05$ compared with the saline-treated group.

2.6. Data analysis and statistics

We selected a sample size of 6, assuming a minimal meaningful difference of 7% and a within-group standard deviation of 4%, based on previous studies. Data from the behavioral studies were expressed as the percentage of the change in threshold (percent change in threshold), where percent change in threshold = 100 × threshold after drug administration/threshold before drug administration after SNL. The data were normally distributed and are presented as mean ± SEM. The time course data were analysed using a 2-way analysis of variance (ANOVA), followed by a Student–Newman–Keuls post hoc test for multiple comparison. Cumulative dose–response curves from microdialysis studies were compared using the software package PharmToolsPro (McCary Group, Emmaus, PA). Other data were analysed by one-way ANOVA. Differences were considered significant at a P value of less than .05.

3. Results

3.1. Antihyperalgesic effects of milnacipran, paroxetine, and maprotiline

Spinal nerve ligation decreased the withdrawal thresholds of the ipsilateral hind paw by approximately 40% (126.6 ± 6.1 g before SNL and 75.7 ± 2.6 g after SNL, n = 72). Intraperitoneal injection of milnacipran (3–30 mg/kg) produced antihyperalgesic effects in a dose-dependent manner ($P < .05$ by 2-way ANOVA), and the peak effect was observed at 90 minutes after injection (Fig. 1A). In normal rats, however, the highest dose of milnacipran (30 mg/kg) did not affect the withdrawal threshold compared with the saline-treated group (n = 6, data not shown), indicating that milnacipran might be effective only in a neuropathic pain state. Intrathecal administration of idazoxan, an α_2-adrenoceptor antagonist, reversed the antihyperalgesic effect (30 mg/kg) when it was injected 30 minutes before the peak antihyperalgesic effect of milnacipran ($P < .05$ by 1-way ANOVA; the threshold decreased by 27% compared with the milnacipran + saline group, Fig. 1B). Intrathecal injection of 5-HT receptor antagonists did not reverse the peak antihyperalgesic effect of milnacipran (Fig. 1B). Intraperitoneal injection of paroxetine produced an antihyperalgesic effect only at a maximum dose of 10 mg/kg ($P < .05$ by 2-way ANOVA), and the peak effect was observed 60 minutes after injection (Fig. 2A). Intrathecal administration of idazoxan and ondansetron, a 5-HT3 receptor antagonist, reversed this antihyperalgesic effect of paroxetine (10 mg/kg) when each antagonist was injected 30 minutes before the peak antihyperalgesic effect ($P < .05$ by 1-way ANOVA; the threshold decreased by 21% and 29%, respectively, compared with the paroxetine + saline group; Fig. 2B). Intraperitoneal injection of maprotiline produced an antihyperalgesic effect at a doses of 10 and 30 mg/kg ($P < .05$ by 2-way ANOVA), and the peak effect was observed 30 minutes after injection (Fig. 3A). There was no difference between the groups treated with10 and 30 mg/kg. Intrathecal administration of idazoxan but not 5-HT receptor antagonists reversed this antihyperalgesic effect of maprotiline (10 mg/kg) on injection 30 minutes before the peak antihyperalgesic effect ($P < .05$ by 1-way ANOVA compared with maprotiline + saline-treated group, the threshold decreased by 36%, Fig. 3B).

The intraperitoneral administration of milnacipran, paroxetine or maprotiline at the doses used in the behavioral studies did not produce any adverse effect. A previous report also showed that the intraperitoneal administration of milnacipran 60 mg/kg did

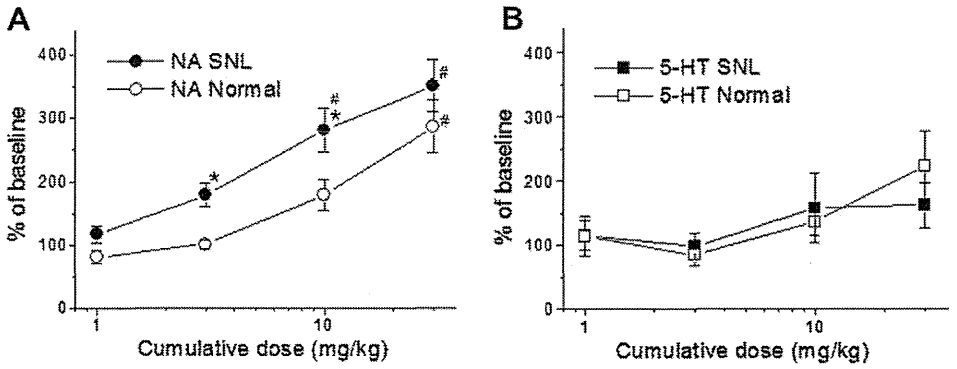

Fig. 5. Microdialysis studies for NA (A) and 5-HT (B) in the dorsal horn of the lumbar spinal cord in normal rats and rats with spinal nerve ligation (SNL) after a cumulative dose injection of milnacipran (1–30 mg/kg). Milnacipran was administered intravenously at doses of 1, 2, 7, or 20 mg/kg. The increase in NA after the cumulative dose injection was greater in SNL rats than in normal rats ($P < .05$). Data are presented as percentage change of baseline. All values represent the mean ± SEM for 6 rats. *$P < .05$ compared with the normal rats; #$P < .05$ compared with the baseline value.

not show any adverse effect [18]. We did not use 30 mg/kg of paroxetine because this dose produced a severe adverse effect (convulsions). Intrathecal administration of each antagonist alone at the same dose used in this study did not alter the withdrawal threshold (Figs. 1B, 2B, and 3B).

3.2. Increase in NA and 5-HT levels in the spinal cord after injection of milnacipran, paroxetine, and maprotiline based on microdialysis

Fig. 4 shows the time course of the percentage change of the NA and 5-HT concentration in the spinal dorsal horn in SNL rats after milnacipran injection. As described in Methods, the time course changes of NA and 5-HT were similar after intravenous and intraperitoneal injection of milnacipran (30 mg/kg; Fig. 4, inset). Therefore, all drugs were injected intravenously in the microdialysis studies. After injection of milnacipran (30 mg/kg), both NA and 5-HT increased ($P < .05$ by 2-way ANOVA). NA concentrations increased within 30 minutes and reached approximately 500% of the baseline value at 2 hours, and the increase continued for more than 6 hours after the injection (data not shown). The concentration of 5-HT also increased, but peaked 45 minutes after the injection and then gradually decreased within 2 hours. In the saline-treated group, NA and 5-HT concentrations in the dialysates did not change over time.

The cumulative dose-response study with milnacipran is shown in Fig. 5. The NA concentration increased dose-dependently in both normal and SNL rats ($P < .05$ by 1-way ANOVA; Fig. 5A). The increase in NA was greater in SNL rats than in normal rats after milnacipran injection at doses from 1 to 30 mg/kg ($P < .05$ compared by PharmToolsPro). The NA concentration in SNL rats was higher than that in normal rats at 3 mg/kg (0.119 ± 0.012 pg/μL in SNL rats, and 0.067 ± 0.005 pg/μL in normal rats, respectively, $P < .05$ by 1-way ANOVA) and 10 mg/kg (0.188 ± 0.023 pg/μL in SNL rats, and 0.120 ± 0.016 pg/μL in normal rats, respectively, $P < .05$ by 1-way ANOVA). The NA concentration in SNL rats increased by approximately 350% after the injection of cumulative doses from 1 to 30 mg/kg. The lesser increase in NA compared with a single injection of 30 mg/kg might be from the result of drug clearance between injections (30-minute intervals). Baseline NA concentration before drug injection in SNL rats was greater than that in normal rats (0.029 ± 0.005 pg/μL in SNL rats, and 0.012 ± 0.001 pg/μL in normal rats, respectively,; $P < .05$ by 1-way ANOVA). Therefore, the total NA content in the dialysates collected during the cumulative dose–response study was greater in SNL rats (9.28 ± 0.97 pg/60 μL) than in normal rats (6.48 ± 0.63 pg/60 μl, $P < .05$ by 1-way ANOVA). In contrast, the 5-HT concentration did not increase after the injection of cumulative doses from 1 to 30 mg/kg, and there was no difference between SNL rats and normal animals (Fig. 5B). Baseline 5-HT concentration before drug injection was similar in SNL rats and normal rats (0.089 ± 0.015 pg/μL in SNL rats, and 0.081 ± 0.012 pg/μL in normal rats, respectively). The total 5-HT content in the dialysates collected during the study was similar in SNL rats (5.33 ± 1.05 pg/60 μL) and in normal rats (5.59 ± 0.79 pg/60 μL).

Fig. 6 shows the time course of the change in NA and 5-HT concentrations in SNL rats after paroxetine injection. After intravenous injection of paroxetine (10 mg/kg), both NA and 5-HT increased in the spinal dorsal horn ($P < .05$ by 2-way ANOVA). 5-HT concentrations were elevated within 30 minutes and reached approximately 600% of the baseline value at 45 minutes, then gradually decreased. In contrast, NA concentrations were elevated at 75 minutes, and increased slowly thereafter for more than 5 hours after drug injection (data not shown). In the vehicle-treated group, NA and 5-HT concentrations in the dialysates did not change throughout the experiment.

Fig. 7 shows the time course of the change in NA and 5-HT concentration in SNL rats after maprotiline injection. After an intravenous injection of maprotiline (10 mg/kg), NA increased within 30 minutes and reached approximately 350% to 400% of the baseline ($P < .05$ by 2-way ANOVA), and the increase continued for more than 6 hours after the injection (data not shown). The concentration of 5-HT did not increase compared with the saline-treated group. In the saline-treated group, the NA and 5-HT concentrations in the dialysates did not change over time.

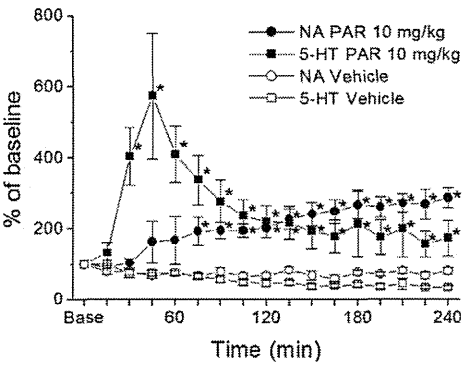

Fig. 6. Microdialysis studies for NA and 5-HT after paroxetine injection in the dorsal horn of the lumbar spinal cord. SNL rats received intravenous saline or paroxetine (PAR, 10 mg/kg). Both NA and 5-HT increased after the injection ($P < .05$ by 2-way ANOVA). Data are presented over time as percentage change of baseline. All values represent the mean ± SEM for 6 rats. *$P < .05$ compared with the vehicle-treated group.

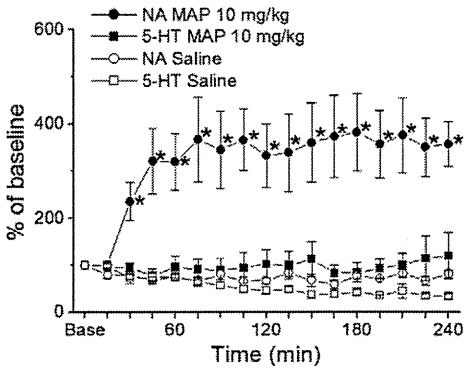

Fig. 7. Microdialysis studies for NA and 5-HT after maprotiline injection in the dorsal horn of the lumbar spinal cord. SNL rats received intravenous saline or maprotiline (MAP, 10 mg/kg). Both NA and 5-HT increased after the injection ($P < .05$ by 2-way ANOVA). Data are presented over time as percentage change of baseline. All values represent the mean ± SEM for 6 rats. *$P < .05$ compared with the saline-treated group.

Intravenous administration of milnacipran, paroxetine, or maprotiline at the doses used in the microdialysis studies did not produce any adverse effects, including respiratory depression, upon injection in awake or anesthetized animals.

3.3. NA and 5-HT contents in the spinal cord in control and neuropathic pain animals

NA and 5-HT contents in homogenized tissue from the spinal cord of normal rats and SNL rats were also determined (Fig. 8). The NA concentration was greater on the ipsilateral side of the spinal cord in SNL rats (320.3 ± 13.0 pg/mg, n = 6) compared with normal rats (228.8 ± 9.1 pg/mg, n = 6, $P < .05$ by 1-way ANOVA). SNL also induced an increase in the 5-HT concentration in both ipsilateral (732.5 ± 25.0 pg/mg, n = 6) and contralateral (762.3 ± 20.0 pg/mg, n = 6) sides of the spinal cord compared with normal rats (565.4 ± 30.0 pg/mg, n = 6, $P < .05$ by 1-way ANOVA).

4. Discussion

In the present study, the intraperitoneal injection of milnacipran, paroxetine, and maprotiline produced antihyperalgesic effects in SNL rats, and the effects were reversed by the spinal injection of an α_2-adrenoceptor antagonist. Both NA and 5-HT increased in the spinal cord after the systemic injection of milnacipran or paroxetine, and only NA increased after maprotiline administration. These findings suggest that increased NA in the spinal cord strongly contributes to the antihyperalgesic effects of antidepressants even SSRIs.

4.1. Antihyperalgesic effects of milnacipran

In the present study, intraperitoneal injection of milnacipran showed an antihyperalgesic effect in a dose-dependent manner in SNL rats. In normal animals, however, milnacipran did not produce an antinociceptive effect. The disconnection between the spinal NA increase and the lack of an antinociceptive effect in the normal animals reflects the relatively low increase in spinal NA or differences between the normal and neuropathic states in the location and function of α_2 adrenoceptors in the spinal cord. In support of the latter possibility, intrathecal injection of the α_2-adrenoceptor agonist clonidine, which mimics the effects of spinally released NA, has increased potency and efficacy in neuropathic pain states in animals [31] and in humans [10]. Several potential causes for this plasticity after nerve injury have been demonstrated in animals, including increased expression of inhibitory α_2-adrenoceptors on calcitonin gene–related peptide–expressing afferents [9], increased G protein-coupling efficiency of spinal α_2-adrenoceptors [3], and increased α_2-adrenoceptor-mediated activation of inhibitory cholinergic interneurons [16,27]. Consistent with the increased NA level and noradrenergic axon sprouting in the spinal dorsal horn after SNL [14], the present study also demonstrated that milnacipran induced a greater increase in spinal NA in SNL rats compared with normal animals based on the cumulative dose-response obtained with microdialysis studies. These findings suggest that peripheral nerve injury enhances the efficacy and release of NA in the spinal cord and that milnacipran uses these plastic changes to induce more powerful analgesia by NA after nerve injury than in the control condition.

Microdialysis studies showed an increase in NA more than 5 hours after intraperitoneal injection of milnacipran (30 mg/kg). The antihyperalgesic effect of milnacipran was only reversed by intrathecal injection of an α_2-adrenoceptor antagonist idazoxan. This indicates that the NA increase in the spinal dorsal horn is involved mainly in the antihyperalgesic effect of milnacipran in SNL rats. The intrathecal injection of idazoxan, however, did not completely reverse the antihyperalgesic effect of milnacipran. It is possible that the 5-HT increase in the spinal cord played a role in the antihyperalgesic effect of milnacipran, although 5-HT receptor antagonists did not reverse the effect of milnacipran, In the present study, the antagonists were injected 60 minutes after the milnacipran injection when the antihyperalgesic effect was already established and the 5-HT level decreased. Therefore, it is likely that the 5-HT increase in the spinal cord contributes, at least in part, to the induction phase of the antihyperalgesic effect of milnacipran. Recent reports demonstrated the involvement of β_2-adrenoceptors in the antihyperalgesic effects of several antidepressants in a murine neuropathic pain model [48,49], which may provide another possible mechanism for the observed effect.

A previous study demonstrated that intrathecal administration, but not intraperitonal administration of milnacipran (30 mg/kg) inhibited mechanical allodynia in rats with SNL [28]. In the present study, however, the intraperitoneal injection of milnacipran produced a dose-dependent antihyperalgesic effect. The oral administration of 200 and 300 mg/kg of milnacipran has been reported to produce antiallodynic effects in SNL rats [18]. Therefore, the observed discrepancy between the 2 studies might be related to the dosage.

4.2. Antihyperalgesic effects of paroxetine and maprotiline

In the present study, intraperitoneal injection of paroxetine also reduced mechanical hyperalgesia, but only at the highest dose

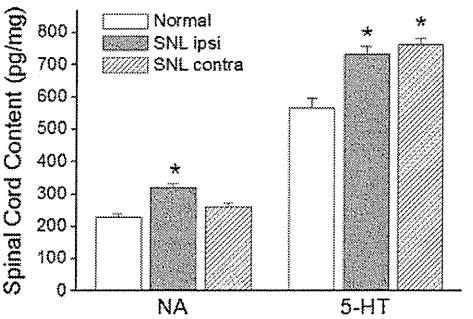

Fig. 8. The contents (pg/mg wet tissue) of NA and 5-HT in the lateral half of the lumbar spinal cord were measured in normal and SNL rats. In SNL rats, the ipsilateral (ipsi) and contralateral (contra) halves of the lumbar spinal cord were measured. All values represent the mean ± SEM for 6 rats. *$P < 0.05$ compared with the normal rats.

(10 mg/kg). Descending serotonergic pathways have been implicated in both inhibitory and facilitatory modulation of spinal neuronal activity. Several 5-HT receptor subtypes, including the 5-HT1A, 5-HT1B, 5-HT2A, 5-HT2C, and 5-HT3 receptors, contribute to the antinociceptive action of 5-HT in the spinal cord [24]. However, there is some controversy because the effects observed are highly dependent on the animal model and behavioral testing paradigm used as well as the dose of selective agents administered. A previous study reported that 5-HT1A receptors in the spinal cord are involved the antinociceptive effect of systemic venlafaxine, an SSRI, in the late phase of the formalin test in rats [5]. Another study demonstrated that spinal 5-HT2A/2C receptors contributed to the antiallodynic effect of the systemic administration of the SSRI fluvoxamine in mice with peripheral nerve injury [17]. In the present study, the antihyperalgesic effect of paroxetine was reversed by the intrathecal injection of the 5-HT3 receptor antagonist ondansetron. In contrast, the 5-HT2A/2C antagonists ketanserin and methysergide, which have affinity for both 5-HT1 and 5-HT2 receptor subtypes, did not reverse the antihyperalgesic effect of paroxetine. This result is contrary to previous reports that neuropathic pain is associated with an enhanced descending facilitation mediated by 5-HT acting at spinal 5-HT3 receptors [35,42]. This discrepancy might be explained by dual effects of 5-HT3 receptor activation in the spinal cord dorsal horn. Several previous studies demonstrated that the dominant effect of 5-HT3 receptor activation in the spinal cord is antinociceptive by promoting γ-amino butyric acid (GABA)ergic inhibitory synaptic transmission [1,13,19]. Interestingly, intrathecal administration of the selective 5-HT3 receptor agonist chlorophenylbiquanide (m-CPBQ) in rats with SNL reverses established mechanical allodynia in a GABA-A dependent manner [30], suggesting that spinal 5-HT3 receptor activation in the neuropathic pain state still possesses inhibitory effects. Although it is not clear from the present study if similar endogenous spinal 5-HT3 receptor activation occurs after paroxetine injection, our data strongly suggest that an increase in 5-HT in the spinal cord contributes to the antihyperalgesic effect of paroxetine.

Previous studies in rats demonstrated that spinal antinociception by 5-HT was reversed by the depletion of endogenous NA [2,39]. Another report suggests that NA is taken up via a 5-HT transporter, and 5-HT releases NA in response to neuronal activity [44]. Consistent with these reports, NA in the spinal cord increased after paroxetine injection in our microdialysis study. The antihyperalgesic effect of paroxetine was also reversed by intrathecal injection of idazoxan. These results suggest that increased NA in the spinal cord plays an important role in the antihyperalgesic effect of paroxetine.

The important role of NA increase in the antihyperalgesic effect of antidepressants is further supported by the results of maprotiline. We demonstrated that intraperitoneal administration of maprotiline (10 and 30 mg/kg) produced an antihyperalgesic effect, which was reversed only by intrathecal injection of idazoxan. The microdialysis study showed that maprotiline increased NA, but not 5-HT, in the spinal cord. In the present study, however, the antihyperalgesic of milnacipran was more potent than that of maprotiline. Consistent with clinical studies [11,41], our data suggest that the dual reuptake inhibition of NA and 5-HT, and not selective NA or 5-HT inhibition, is necessary to strengthen the inhibitory effects of antidepressant for neuropathic pain. Taken together, our results suggest that NA reuptake inhibition is more important than that of 5-HT in inhibition of neuropathic pain, although an increase in 5-HT plays some role in the effect of antidepressants.

4.3. Roles of NA and 5-HT in the spinal cord in neuropathic pain

There is some evidence that patients with neuropathic pain have a reduced ability to physiologically recruit descending inhibitory pathways [45]. Several approved treatments for neuropathic pain in patients, including the α_2-adrenoceptor agonists clonidine [10] and gabapentin [15], as well as SNRIs as suggested in the current report, interact with or mimic the activation of bulbospinal noradrenergic pathways to produce analgesia and may potentially overcome or compensate for decreased descending inhibitory pathway function. Consistent with a recent report [14], NA content in the spinal cord was increased in SNL rats compared with normal animals in the present study. Taken together with results from behavioral and microdialysis studies, our findings strongly suggest that activation of the descending noradrenergic inhibitory pathway and a subsequent increase of NA in the spinal dorsal horn in the neuropathic pain state contribute to the antihyperalgesic effects of antidepresants.

The density of serotonergic terminals and 5-HT content are dynamically regulated after nerve injury. Following dorsal rhizotomy, sprouting of serotonergic terminals was observed in segmental regions of the spinal cord corresponding to deafferented terminals [33,34]. After chronic constriction injury to the sciatic nerve, bilateral increases in 5-HT content were observed between 7 and 14 days postoperatively [38]. In a recent study, spinal levels of 5-HT and its metabolite transiently decreased in the dorsal ipsilateral lumbar spinal cord of SNL rats 7 days postoperatively, but recovered to baseline levels within 28 days [22]. In the present study, 5-HT levels in the spinal cord increased bilaterally in SNL rats. It is unclear why both increases and decreases in descending serotonergic system have been described after nerve injury, but this may be due to differences in the degree or type of peripheral nerve injury. Further studies are necessary to examine the role of 5-HT in the spinal dorsal horn in different types of nerve injuries.

Conflict of interest statement

The authors declare no conflicts of interest in publishing these data.

Acknowledgements

This work was supported by a Grant-In-Aid for Scientific Research (grant number 21791435, KN; 20591823, HO; 20791063, NI; 20791063) from the Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan. The authors thank Dr. Devang Thakor, PhD, for advice on the English correction.

References

[1] Alhaider AA, Lei SZ, Wilcox GL. Spinal 5-HT3 receptor-mediated antinociception: possible release of GABA. J Neurosci 1991;11:1881–8.
[2] Archer T, Jonsson G, Minor BG, Post C. Noradrenergic-serotonergic interactions and nociception in the rat. Eur J Pharmacol 1986;120:295–307.
[3] Bantel C, Eisenach JC, Duflo F, Tobin JR, Childers SR. Spinal nerve ligation increases alpha2-adrenergic receptor G-protein coupling in the spinal cord. Brain Res 2005;1038:76–82.
[4] Berton O, Nestler EJ. New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci 2006;7:137–51.
[5] Bonnefont J, Chapuy E, Clottes E, Alloui A, Eschalier A. Spinal 5-HT1A receptors differentially influence nociceptive processing according to the nature of the noxious stimulus in rats: effect of WAY-100635 on the antinociceptive activities of paracetamol, venlafaxine and 5-HT. Pain 2005;114:482–90.
[6] Dharmshaktu P, Tayal V, Kalra BS. Efficacy of antidepressants as analgesics: a review. J Clin Pharmacol 2012;52:6–17.
[7] Dworkin RH, O'Connor AB, Backonja M, Farrar JT, Finnerup NB, Jensen TS, Kalso EA, Loeser JD, Miaskowski C, Nurmikko TJ, Portenoy RK, Rice AS, Stacey BR, Treede RD, Turk DC, Wallace MS. Pharmacologic management of neuropathic pain: evidence-based recommendations. Pain 2007;132:237–51.
[8] Eide PK, Hole K. The role of 5-hydroxytryptamine (5-HT) receptor subtypes and plasticity in the 5-HT systems in the regulation of nociceptive sensitivity. Cephalalgia 1993;13:75–85.
[9] Eisenach JC, Zhang Y, Duflo F. Alpha2-adrenoceptors inhibit the intracellular $Ca2^+$ response to electrical stimulation in normal and injured sensory neurons, with increased inhibition of calcitonin gene-related peptide expressing neurons after injury. Neuroscience 2005;131:189–97.

[10] The Epidural Clonidine Study Group, Eisenach JC, DuPen S, Dubois M, Miguel R, Allin D. Epidural clonidine analgesia for intractable cancer pain. Pain 1995;61:391–9.
[11] Finnerup NB, Otto M, McQuay HJ, Jensen TS, Sindrup SH. Algorithm for neuropathic pain treatment: an evidence based proposal. Pain 2005;118:289–305.
[12] Fürst S. Transmitters involved in antinociception in the spinal cord. Brain Res Bull 1999;48:129–41.
[13] Giordano J. Analgesic profile of centrally administered 2-methylserotonin against acute pain in rats. Eur J Pharmacol 1991;199:233–6.
[14] Hayashida K, Clayton BA, Johnson JE, Eisenach JC. Brain derived nerve growth factor induces spinal noradrenergic fiber sprouting and enhances clonidine analgesia following nerve injury in rats. Pain 2008;136:348–55.
[15] Hayashida K, Obata H, Nakajima K, Eisenach JC. Gabapentin acts within the locus coeruleus to alleviate neuropathic pain. Anesthesiology 2008;109:1077–84.
[16] Hayashida K, Eisenach JC. Spinal alpha 2-adrenoceptor-mediated analgesia in neuropathic pain reflects brain-derived nerve growth factor and changes in spinal cholinergic neuronal function. Anesthesiology 2010;113:406–12.
[17] Honda M, Uchida K, Tanabe M, Ono H. Fluvoxamine, a selective serotonin reuptake inhibitor, exerts its antiallodynic effects on neuropathic pain in mice via 5-HT2A/2C receptors. Neuropharmacology 2006;51:866–72.
[18] Iyengar S, Webster AA, Hemrick-Luecke SK, Xu JY, Simmons RM. Efficacy of duloxetine, a potent and balanced serotonin-norepinephrine reuptake inhibitor in persistent pain models in rats. J Pharmacol Exp Ther 2004;311:576–84.
[19] Kawamata T, Omote K, Toriyabe M, Yamamoto H, Namiki A. The activation of 5-HT(3) receptors evokes GABA release in the spinal cord. Brain Res 2003;978:250–5.
[20] Kim SH, Chung JM. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 1992;50:355–63.
[21] Koch S, Hemrick-Luecke SK, Thompson LK, Evans DC, Threlkeld PG, Nelson DL, Perry KW, Bymaster FP. Comparison of effects of dual transporter inhibitors on monoamine transporters and extracellular levels in rats. Neuropharmacology 2003;45:935–44.
[22] Liu FY, Qu XX, Ding X, Cai J, Jiang H, Wan Y, Han JS, Xing GG. Decrease in the descending inhibitory 5-HT system in rats with spinal nerve ligation. Brain Res 2010;1330:45–60.
[23] Micó JA, Ardid D, Berrocoso E, Eschalier A. Antidepressants and pain. Trends Pharmacol Sci 2006;27:348–54.
[24] Millan MJ. Descending control of pain. Prog Neurobiol 2002;66:355–474.
[25] Mochizuki D, Tsujita R, Yamada S, Kawasaki K, Otsuka Y, Hashimoto S, Hattori T, Kitamura Y, Miki N. Neurochemical and behavioural characterization of milnacipran, a serotonin and noradrenaline reuptake inhibitor in rats. Psychopharmacology (Berl) 2002;162:323–32.
[26] Obata H, Kimura M, Nakajima K, Tobe M, Nishikawa K, Saito S. Monoamine-dependent, opioid-independent antihypersensitivity effects of intrathecally administered milnacipran, a serotonin noradrenaline reuptake inhibitor, in a postoperative pain model in rats. J Pharmacol Exp Ther 2010;334:1059–65.
[27] Obata H, Li X, Eisenach JC. Alpha2-Adrenoceptor activation by clonidine enhances stimulation-evoked acetylcholine release from spinal cord tissue after nerve ligation in rats. Anesthesiology 2005;102:657–62.
[28] Obata H, Saito S, Koizuka S, Nishikawa K, Goto F. The monoamine-mediated antiallodynic effects of intrathecally administered milnacipran, a serotonin noradrenaline reuptake inhibitor, in a rat model of neuropathic pain. Anesth Analg 2005;100:1406–10.
[29] Obata H, Saito S, Sasaki M, Ishizaki K, Goto F. Antiallodynic effect of intrathecally administered 5-HT_2 agonists in rats with nerve ligation. Pain 2001;90:173–9.
[30] Okazaki R, Namba H, Yoshida H, Okai H, Miura T, Kawamura M. The antiallodynic effect of Neurotropin is mediated via activation of descending pain inhibitory systems in rats with spinal nerve ligation. Anesth Analg 2008;107:1064–9.
[31] Paqueron X, Conklin D, Eisenach JC. Plasticity in action of intrathecal clonidine to mechanical but not thermal nociception after peripheral nerve injury. Anesthesiology 2003;99:199–204.
[32] Peters CM, Hayashida K, Ewan EE, Nakajima K, Obata H, Xu Q, Yaksh TL, Eisenach JC. Lack of analgesic efficacy of spinal ondansetron on thermal and mechanical hypersensitivity following spinal nerve ligation in the rat. Brain Res 2010;1352:83–93.
[33] Polistina DC, Murray M, Goldberger ME. Plasticity of dorsal root and descending serotoninergic projections after partial deafferentation of the adult rat spinal cord. Comp Neurol 1990;299:349–63.
[34] Ramer LM, McPhail LT, Borisoff JF, Soril LJ, Kaan TK, Lee JH, Saunders JW, Hwi LP, Ramer MS. Endogenous TrkB ligands suppress functional mechanosensory plasticity in the deafferented spinal cord. J Neurosci 2007;27:5812–22.
[35] Rahman W, Suzuki R, Webber M, Hunt SP, Dickenson AH. Depletion of endogenous spinal 5-HT attenuates the behavioural hypersensitivity to mechanical and cooling stimuli induced by spinal nerve ligation. Pain 2006;123:264–74.
[36] Randall LO, Selitto JJ. A method for measurement of analgesic activity on inflamed tissue. Arch Int Pharmacodyn Ther 1957;111:409–19.
[37] Pertovaara A. Noradrenergic pain modulation. Prog Neurobiol 2006;80:53–83.
[38] Satoh O, Omote K. Roles of monoaminergic, glycinergic and GABAergic inhibitory systems in the spinal cord in rats with peripheral mononeuropathy. Brain Res 1996;728:27–36.
[39] Sawynok J, Reid A. Interactions of descending serotonergic systems with other neurotransmitters in the modulation of nociception. Behav Brain Res 1996;73:63–8.
[40] Sindrup SH, Jensen TS. Efficacy of pharmacological treatments of neuropathic pain: an update and effect related to mechanism of drug action. Pain 1999;83:389–400.
[41] Sindrup SH, Otto M, Finnerup NB, Jensen TS. Antidepressants in the treatment of neuropathic pain. Basic Clin Pharmacol Toxicol 2005;96:399–409.
[42] Suzuki R, Rahman W, Hunt SP, Dickenson AH. Descending facilitatory control of mechanically evoked responses is enhanced in deep dorsal horn neurones following peripheral nerve injury. Brain Res 2004;1019:68–76.
[43] Vaishnavi SN, Nemeroff CB, Plott SJ, Rao SG, Kranzler J, Owens MJ. Milnacipran: a comparative analysis of human monoamine uptake and transporter binding affinity. Biol Psychiatry 2004;55:320–2.
[44] Vizi ES, Zsilla G, Caron MG, Kiss JP. Uptake and release of norepinephrine by serotonergic terminals in norepinephrine transporter knock-out mice: implications for the action of selective serotonin reuptake inhibitors. J Neurosci 2004;24:7888–94.
[45] Witting N, Svensson P, Jensen TS. Differential recruitment of endogenous pain inhibitory systems in neuropathic pain patients. Pain 2003;103:75–81.
[46] Yaksh TL, Pogrel JW, Lee YW, Chaplan SR. Reversal of nerve ligation-induced allodynia by spinal alpha-2 adrenoceptor agonists. J Pharmacol Exp Ther 1995;272:207–14.
[47] Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976;17:1031–6.
[48] Yalcin I, Choucair-Jaafar N, Benbouzid M, Tessier LH, Muller A, Hein L, Freund-Mercier MJ, Barrot M. Beta(2)-adrenoceptors are critical for antidepressant treatment of neuropathic pain. Ann Neurol 2009;65:218–25.
[49] Yalcin I, Tessier LH, Petit-Demoulière N, Doridot S, Hein L, Freund-Mercier MJ, Barrot M. Beta2-adrenoceptors are essential for desipramine, venlafaxine or reboxetine action in neuropathic pain. Neurobiol Dis 2009;33:386–94.

Neuroradiology (2012) 54:1127–1131
DOI 10.1007/s00234-012-1007-y

INTERVENTIONAL NEURORADIOLOGY

Location of major vessels in prone-positioned patients undergoing percutaneous lumbar sympathectomy

Shiro Koizuka · Shigeru Saito · Souta Masuoka · Kunie Nakajima · Yoshinori Koyama

Received: 7 September 2011 / Accepted: 6 January 2012 / Published online: 15 February 2012

Abstract
Introduction The topographic relationship between major vessels and the sympathectomy target is not identical across patients and may not be clear, especially in patients in the prone position. The aim of this study was to provide anatomic data regarding the location of the major vessels (i.e., vena cava and aorta) based on computed tomography (CT) images obtained during lumbar sympathectomy under CT fluoroscopic guidance.
Methods Thirty-six patients with peripheral arterial occlusive disease or chronic pain syndrome were treated using fluoroscopic CT-guided percutaneous lumbar sympathectomy between April 2006 and March 2010. We analyzed the shortest distances between the sympathectomy target and the major vessels, and the relationship between the location of the major vessels and the vertebral anterior line using CT images obtained during the procedure.
Results At the L3 level, the shortest distances from the right side target to the inferior vena cava were significantly shorter than the other distances ($P<0.05$). In 11 of 36 patients (30.6%), the IVC was located dorsal to the vertebral anterior line at the L3 level.
Conclusion Needle insertion for right side sympathectomy at the L3 level may present a higher risk of major vessel puncture than sympathectomy at other sites. CT guidance is recommended for lumbar sympathectomy to reduce the risk of vascular puncture.

Keywords Lumbar sympathectomy · Major vessel · CT fluoroscopy · Anatomy

S. Koizuka (✉) · S. Saito · S. Masuoka · K. Nakajima
Department of Anesthesiology, Gunma University Hospital,
3-39-15 Showa-machi,
Maebashi 371-8511, Japan
e-mail: skoizuka@med.gunma-u.ac.jp

Y. Koyama
Department of Diagnostic Radiology and Nuclear Medicine,
Gunma University Hospital,
Maebashi, Japan

Introduction

Lumbar sympathectomy is an effective treatment for severe peripheral vascular disease that is resistant to other types of treatment and no longer suitable for arterial reconstruction [1, 2]. In addition, lumbar sympathectomy may also provide effective relief for some chronic lower limb pain, especially that associated with sympathetic activity [1, 2]. Lumbar sympathectomy is conventionally performed using laparoscopy or X-ray fluoroscopy. While percutaneous methods are less invasive than a laparotomy, percutaneous methods under classic X-ray fluoroscopy can potentially cause injury to organs and major vessels that are not visualized [1]. Puncture of the kidney or ureter may cause renal dysfunction [3], and puncture of a major vessel may cause critical hemorrhage. In addition, it is difficult to perform a lumbar sympathectomy in patients with a deformed lumbar spine or anatomic variability [5–9]. Recent advances in real-time computed tomography (CT) fluoroscopic guidance have increased the accuracy and safety of several types of nerve blocks [5, 6], especially lumbar sympathectomy [5–7]. In our previous study [6], we presented the anatomical details of the location of the kidney using CT images, and recommended the use of CT guidance for lumbar sympathectomy. In addition, we have experienced cases in which CT images revealed that the major vessels were very close to the sympathectomy target (Fig. 1). The topographic relationship between major vessels and the sympathectomy target is

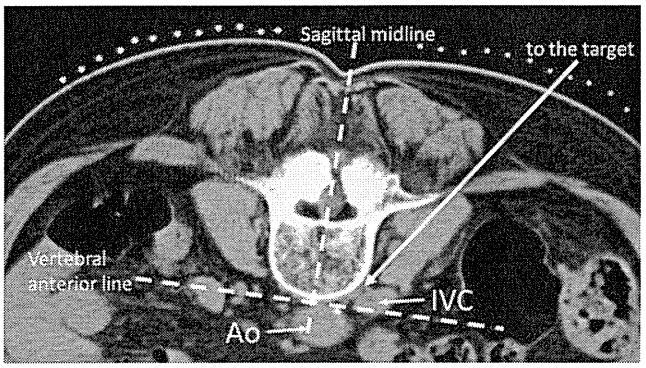

Fig. 1 Computed tomography image for planning the route for percutaneous lumbar sympathectomy. The *long arrow* indicates the safest and shortest route to the target. *Ao* aorta, *IVC* inferior vena cava

not identical across patients and not always clear, especially in patients in the prone position. The aim of the present study was to provide anatomic data regarding the location of major vessels (i.e., vena cava and aorta) from CT images obtained during lumbar sympathectomy under CT fluoroscopic guidance.

Patients and methods

Patients

After obtaining approval from our institutional review board, written informed consent was obtained from the patients. Thirty-six patients [23 men, 13 women; 24–89 years of age (mean, 63.2 years)] with peripheral arterial occlusive disease or chronic pain syndrome were treated with fluoroscopic CT-guided percutaneous lumbar sympathectomy between April 2006 and March 2010. There were ten patients with atherosclerosis obliterans, nine with collagen disease with peripheral vascular symptoms, eight with thromboangitis obliterans, seven with complex regional pain syndrome, and two with low back and limb pain.

Fluoroscopic CT-guided percutaneous lumbar sympathectomy technique

The patient was placed on the CT table in the prone position. A marking device constructed using radiograph opaque wires was attached to the patient's lower back. CT images (SOMATOM volume Zoom; Siemens, Erlangen, Germany) were obtained at the L2 and L3 levels, and the safest and shortest route to the target (between the anterior angle of the psoas muscle and the anterolateral plane of the vertebral body [5–7] (Figs. 1 and 2) was determined to avoid injury to the kidney, inferior vena cava (IVC), aorta (Ao), and ureter on the CT images. The insertion angle of the needle was adjusted using the red guiding laser emitted from the

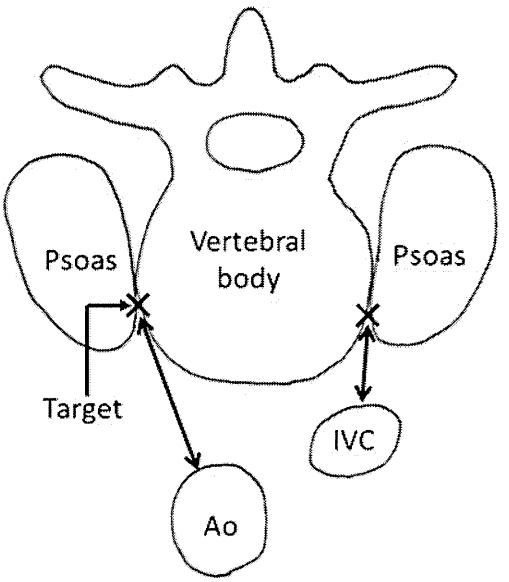

Fig. 2 The shortest distances between target and major vessels were measured. The measurement target point was determined to be the most dorsal point (*cross sign*) in the triangle-shaped compartment area. The right side target to the inferior vena cava (*IVC*); The left side target to aorta (*Ao*)

CT gantry, which conformed to the scanning section. A 22-gauge 140-mm needle (Hakko, Tokyo, Japan) was advanced to the target following a predetermined route under real-time CT fluoroscopy. After confirming the proper spread of the agent using contrast medium and effect (the skin temperature rise in the lower extremities) of test block using local anesthetics, the neurolytic agent (7% phenol) was injected to chemically degenerate the sympathetic trunk each at the L2 and L3 level.

Image analysis methods

We analyzed CT images that were obtained during fluoroscopic CT-guided percutaneous lumbar sympathectomy. CT images, including the cranio-caudal midpoint of the vertebral column, were captured at the L2 and L3 spinal levels.

Measurement 1

The anatomic distances were measured as follows (Figs. 1 and 2). The measurement target point was determined to be the most dorsal point in the triangle-shaped compartment area (between the anterior angle of the psoas muscle and the anterolateral plane of the vertebral body). The distance L2–IVC was the shortest distance from the right side target to the IVC at the L2 level; distance L2–Ao was the shortest distance from the left side target to the Ao at the L2 level; distance L3–IVC was the shortest distance from the right side target to the IVC at the L3 level; and distance L3–Ao

was the shortest distance from the left side target to the Ao at the L3 level (Fig. 2).

Measurement 2

The vertebral anterior line is considered a landmark for needle advancement during lumbar sympathectomy in the lateral view of X-ray fluoroscopy [1, 2]. When a major vessel is located dorsal to the vertebral anterior line, the risk of vascular puncture is considered to be high. The anatomic distances in such cases were measured at the L2 and L3 levels as follows. The distance r–Ao was the distance from the most dorsal margin of the Ao to the vertebral anterior line perpendicular to the sagittal midline (connecting line between the spinous process and midpoint of the vertebral body); and distance r–IVC was the distance from the most dorsal margin of the IVC to the vertebral anterior line (Fig. 3).

Statistical analysis

All data are expressed as means±SD. Comparison between groups was performed using Student's *t* test. Tukey's test was used to test for differences between groups. Differences with a value of $P<0.05$ were considered significant.

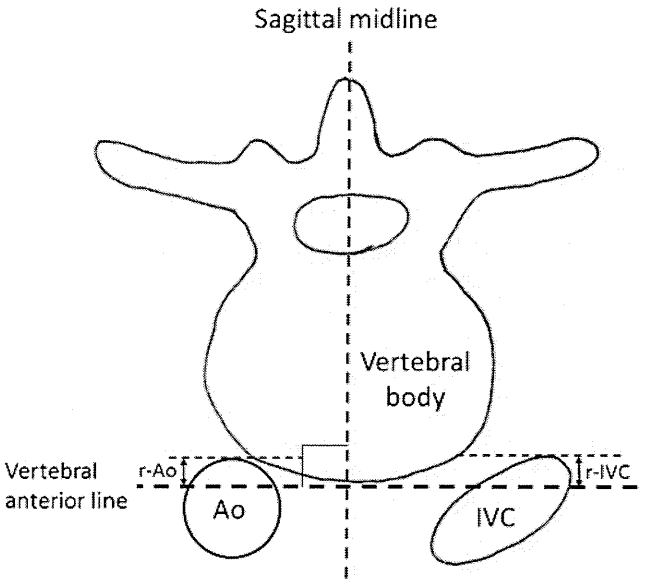

Fig. 3 The location of major vessels dorsal to the vertebral anterior line was examined. Distance *r–Ao* is the distance from the most dorsal margin of the Ao to the vertebral anterior line perpendicular to the sagittal midline (connecting the line between the spinous process and the midpoint of the vertebral body), *r–IVC* is the distance from most dorsal margin of the IVC to the vertebral anterior line. *Ao* aorta, *IVC* inferior vena cava

Results

Apparent beneficial effects, such as elevated skin temperature or reduced pain intensity, were observed in all patients. No complications were observed in any of the patients. Because we could confirm a skin temperature rise in the lower extremities in all patients immediately after the procedure, the needle tips were considered to be correctly placed, although we could not identify the sympathetic ganglion in the CT images.

The patient characteristics are summarized in Table 1. Height and weight were significantly greater in the men than in the women ($P<0.05$).

The shortest distances between target and major vessel

At the L3 level, the shortest distances from the right side target to the IVC were significantly shorter than the other distances ($P<0.05$, Table 2). There were no significant differences in any distances between men and women. The distributions of the distances are shown in Fig. 4.

Cases in which a major vessel located dorsal to the vertebral anterior line

In 11 of the 36 cases (30.6%), the IVC located dorsal to the vertebral anterior line at the L3 level (Table 3). In the cases with a major vessel located dorsal to the vertebral anterior line, distance r–Ao was 2.8±0.7 mm ($n=4$) at the L2 level and 3.6±3.1 mm ($n=2$) at the L3 level, and distance r–IVC was 6.3±2.51 mm ($n=3$) at the L2 level and 4.5±2.1 mm ($n=11$) at the L3 level. There were no significant differences among the groups.

Discussion

Percutaneous neurolytic lumbar sympathectomy can lead to complications, such as lumbar nerve neuralgia; genitofemoral neuralgia; subarachnoid injection; and perforation of the aorta, IVC, kidney, and ureter [1–7]. Furthermore, the incidence rate of procedure-related complications may increase in patients with anatomic abnormalities. The

Table 1 Patient characteristics

	Age (years)	Height (cm)	Weight (kg)
Men ($n=23$)	62.1±14.6*	168.1±5.5*	58.4±7.3*
women ($n=13$)	65.1±8.98	151.1±7.5	45.6±10.0
Total ($n=36$)	63.2±14.2	161.5±11.7	53.8±11.8

Data values are means±SD

*$P<0.05$ compared with the women group

Table 2 The shortest distances between target and major vessel

	L2–IVC (mm)	L2–Ao (mm)	L3–IVC (mm)	L3–Ao (mm)
Men (n=23)	10.8±4.0	12.3±5.1	7.6±2.7*	13.4±4.7
Women (n=13)	9.8±3.8	11.3±4.8	6.3±3.3*	11.6±4.8
Total (n=36)	10.4±3.9	12.0±4.9	7.1±3.0*	12.8±4.7

Data values are means±SD

Ao aorta, *IVC* inferior vena cava, *L2–IVC* the shortest distance from the right side target to the IVC at the L2 level, *L2–Ao* the shortest distance from the left side target to the Ao at the L2 level, *L3–IVC* the shortest distance from the right side target to the IVC at the L3 level, *L3–Ao* the shortest distance from the left side target to the Ao at the L3 level

*P<0.05 compared with L2–IVC, L2–Ao, and L3–Ao

Table 3 The cases with location of a major vessel dorsal from vertebral anterior line

	Inferior vena cava	Aorta
L2 level	8.3% (n=3)	11.1% (n=4)
L3 level	30.6% (n=11)	5.6% (n=2)

complications of nerve block are generally classified into two categories: (1) needle insertion into organs or vessels and (2) spread of the neurolytic agent to an inappropriate site. Because classical X-ray fluoroscopy cannot visualize lumbar nerve, aorta, vena cava, kidney, ureter, and sympathectomy, X-ray fluoroscopy might present a higher risk of such complications than CT fluoroscopic guidance. In our previous study [6], we demonstrated the potential risk of kidney puncture at the L2 level and recommended using CT images to perform lumbar sympathectomy. Based on our experience with the procedure, we take special care not to puncture major vessels, especially the IVC, as well as the kidney. Puncture of the IVC with a 22-G needle might not cause serious hemorrhage, except in special cases with severe anticoagulant status. Many patients who are candidates for sympathectomy, however, have atherosclerosis and are under anticoagulant therapy.

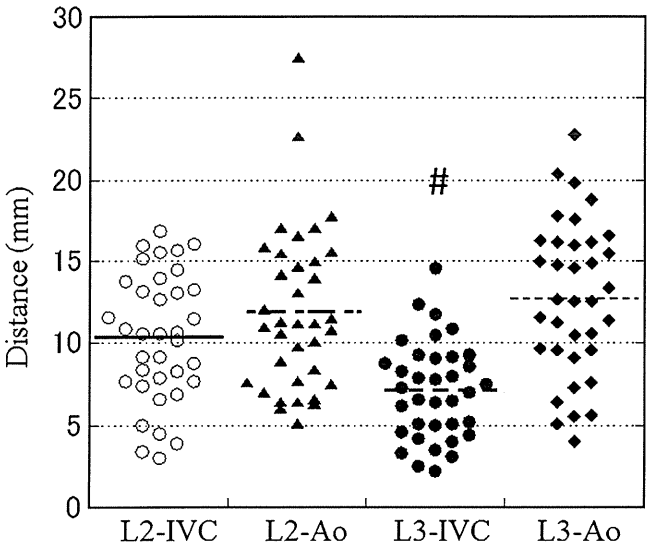

Fig. 4 Distribution of the shortest distances between the target and major vessels at the L2 and L3 levels. #P<0.01 compared with L2–IVC, L2–Ao, and L3–AO. *L2–IVC* the shortest distance from the right side target to the IVC at the L2 level, *L2–Ao* the shortest distance from the left side target to the Ao at the L2 level, *L3–IVC* the shortest distance from the right side target to the IVC at the L3 level, *L3–Ao* the shortest distance from the left side target to the Ao at the L3 level. *Ao* aorta, *IVC* inferior vena cava

The topographic relationship between major vessels and the sympathectomy target is not identical across all patients. The relation has not been previously clarified, especially in patients placed in the prone position. Our present findings provide anatomic and demographic detail relating the location of major vessels.

First, we measured the shortest distances between the sympathectomy target and the major vessels. At the L3 level, the shortest distances from the right side target to the IVC were significantly shorter than the other distances. This finding indicates that needle insertion for right-side sympathectomy at the L3 level may carry a higher risk for major vessel puncture than sympathectomy at other sites. In addition, the large variation in distribution of the location of the major vessels resulted in a large variation in the distances between the vessels and the sympathectomy target.

Next, we examined the relationship between the location of major vessels and the vertebral anterior line. In some cases, a major vessel was located dorsal to the vertebral anterior line. In 11 cases (30.6%), the IVC was located dorsal to the vertebral anterior line at the L3 level. This finding indicates that the potential risk of major vascular puncture is not low, especially with a right-side sympathectomy at the L3 level. Therefore, physicians should take special care when performing a sympathectomy under X-ray fluoroscopy.

Ohno et al. [11] reported the transdiscal approach for lumbar sympathectomy to avoid genitofemoral neuritis resulting from the spread of the neurolytic agent into the psoas muscle. Although this technique is considered to be a safe approach for lumbar sympathectomy under X-ray fluoroscopic guidance, there is a potential risk for discitis, disc degeneration, and vascular puncture. Furthermore, this technique might not be suitable for patients with anatomic deformations.

Based on our preliminary findings (data not shown because these findings are not novel) indicating variability in the location of major vessels not related to patient characteristics [6], CT imaging is considered to be an effective