Spinal \(\gamma\)-Aminobutyric Acid Interneuron Plasticity Is Involved in the Reduced Analgesic Effects of Morphine on Neuropathic Pain

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Abstract: Systemic administration of morphine increases serotonin (5-HT) in the spinal dorsal horn (SDH), which attenuates the analgesic effects of morphine on neuropathic pain through spinal 5-HT3 receptors. We hypothesized that dysfunction of the descending serotonergic system, including the periaqueductal gray (PAG), contributes to attenuate the efficacy of morphine on neuropathic pain through spinal 5-HT3 receptors and GABA neurons. Morphine (100 ng) injected into the PAG produced analgesic effects in normal rats, but not in spinal nerve ligation (SNL) rats. In vivo microdialysis showed that PAG morphine increased the SDH 5-HT concentration in both groups. Intrathecal injection of the 5-HT3 receptor antagonist ondansetron and the GABA\(\alpha\) receptor antagonist bicuculline attenuated the analgesic effects of PAG morphine in normal rats, but increased the effects in SNL rats. The increased analgesic effect of PAG morphine induced by bicuculline was reversed by pretreatment with the tropomyosin receptor kinase B (TrkB) antagonist K252a. Activation of spinal 5-HT3 receptors by 2-methyl-5-HT increased the GABA concentration in both groups. Morphine activates GABAergic interneurons in the SDH by activating descending serotonergic neurons. Functional changes in GABA\(\alpha\) receptors from inhibitory to facilitatory through the activation of TrkB receptors may contribute to the attenuated efficacy of morphine against neuropathic pain.

Perspective: Although morphine provides strong analgesia against acute pain, it has limited efficacy against neuropathic pain. This article demonstrates that functional changes in GABA\(\alpha\) receptors in the spinal dorsal horn after nerve injury might strongly contribute to the attenuation of opioid-induced analgesia for neuropathic pain.

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Key Words: Morphine, neuropathic pain, serotonin, descending pain modulation, \(\gamma\)-aminobutyric acid.

Morphine administration exerts strong analgesic effects against acute pain, but has diminished efficacy against neuropathic pain. Rats with peripheral nerve injury, a neuropathic pain model, are less sensitive to the analgesic effects of morphine than normal animals. The analgesic effects of opioids are mainly produced by direct post- and pre-synaptic inhibition of nociceptive transmission in the dorsal horn of the spinal cord and activation of the descending inhibitory pathway, including the periaqueductal gray (PAG) and rostral ventromedial medulla (RVM) systems. The PAG contains \(\mu\)-opioid receptors and its role in modulating nociceptive inputs has been widely studied. The PAG projects glutamatergic neurons to the RVM and the RVM projects serotonergic...
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neurons to the spinal cord. Therefore, the PAG is an important source of descending serotoninergic inhibitory pathways. Previous studies demonstrated that systemic administration of morphine increases the levels of serotonin (5-hydroxytryptamine: 5-HT) in the spinal cord, which facilitates morphine-induced analgesia in the normal rat, but attenuates morphine-induced analgesia through spinal 5-HT3 receptors in a rat neuropathic pain model. In the spinal dorsal horn, excitatory 5-HT3 receptors are expressed in the presynaptic terminals of primary afferents and in the somatodendritic regions of projection neurons, as well as inhibitory and excitatory interneurons and presynaptic terminals of inhibitory γ-aminobutyric acidergic (GABA) neurons. The efficacy of morphine to relieve pain might depend on the equilibrium of inhibitory and facilitatory pathway activation through spinal 5-HT3 receptors. GABAergic neuron activity in the spinal dorsal horn is attenuated in the neuropathic pain model as a result of neuronal apoptosis and dysfunction. and a decrease in glutamic acid decarboxylase. Coull et al reported that microglial secretion of brain-derived neurotrophic factor (BDNF) alters the function of GABA_{	ext{A}} receptors in the spinal dorsal horn, thereby contributing to allodynia through TrkB receptors in a neuropathic pain model. Thus, in the present study, we hypothesized that in the neuropathic pain setting, 5-HT3 receptor activation in the spinal dorsal horn enhances pain due to dysfunction of the inhibitory GABAergic system and we investigated the plasticity of descending inhibitory systems from the PAG to the spinal cord by comparing the analgesic effects of morphine between normal rats and a rat model of neuropathic pain caused by spinal nerve ligation (SNL).

Methods

This study adhered to the applicable ARRIVE guidelines.

Animals

The study protocol was approved by the Animal Care and Use Committee of the Gunma University Graduate School of Medicine. Male Sprague-Dawley rats (200 g) obtained from SLC (Shizuoka, Japan) were used in all experiments. The rats were group-housed (n = 6/cage) with soft bedding under a 12-h light-dark cycle (lights on at 6AM), with food and water available ad libitum. A total of 249 rats were included in our study, with 19 rats excluded due to malposition or removal of the PAG cannula (n = 13) or a withdrawal threshold exceeding 250 g (n = 6) at the time of post SNL surgery.

Spinal Nerve Ligation and PAG Cannulation

SNL was performed as described previously. After anesthetizing the rats with 2% isoflurane in 100% oxygen, the right L6 transverse process was removed. The right L5 spinal nerve was tightly ligated with a 5-0 silk suture and the nerve was cut just distal to the ligature. Two weeks later, PAG cannulation was performed in a subset of the animals. The animals were anesthetized with 2% isoflurane and injected subcutaneously with ketoprofen (2 mg/kg) and butorphanol (2 mg/kg). Topical lidocaine (1%, 0.2 ml) was applied around the ear bar fixation site and at the skin incision site before placing the animals securely in a stereotaxic apparatus (IP-2 NARISHIGE, JAPAN). A sterile stainless-steel guide cannula (CXG-8, Eicom Co., Kyoto, Japan or C315G, Plastics One, Torrington, CT, USA) was implanted into the right PAG (1.2 mm anterior and 0.6 mm lateral to the interaural line, and 5.5 mm ventral from the surface of the dura mater) according to a rat brain atlas. Animals were allowed to recover for at least 1 week after surgery. After the experiment, methylene blue (1 ul) was injected into the PAG, and animals were killed by intraperitoneal injection of pentobarbital (150 mg/kg). The brain was dissected out and sectioned, and cannula placement in the PAG was verified visually.

Behavioral Testing

The experimenter performing the behavioral testing was blinded to the drug administered to the animals. The mechanical withdrawal threshold to increasing amounts of pressure (expressed in grams, maximum pressure 250 g to avoid tissue injury) applied to a hind paw was measured using an Analgesy-meter (37215, Ugo Basile, Comerio, Italy), as described previously. The applied pressure was released immediately upon paw withdrawal. The thermal withdrawal threshold was measured using a plantar test analgesia meter (390G, IITC Inc Life Science, Woodland Hills, CA), as described previously. Rats were placed in individual plastic boxes (10 x 20 x 24 cm) on the glass surface of the testing apparatus that was maintained at 30°C during all testing, and were allowed to acclimate for 30 minutes. The delay time of the paw withdrawal was recorded as the paw withdrawal latency. Thermal stimuli were repeated twice at 5-minute intervals. To avoid tissue injury, the cut-off latency for this test was 30 seconds. The animals were acclimated to the device for 3 days before baseline values were recorded. In addition, the experimenter handled the rats during the recovery period to acclimate them to handling for 3 days prior to performing the baseline testing.

Drugs

Morphine was dissolved in saline and administered to normal rats and SNL rats through the PAG guide cannula (1, 10, 100 ng/0.5 μl). After anesthetizing the animal by inhalation of isoflurane in oxygen, the PAG injection was performed using a microsyringe pump (ESP-64; Eicom Co.) at a rate of 0.25 μl/min. Anesthesia was maintained for 2 minutes during morphine administration and 1 minute each before and after administration. Antagonist studies were performed using the 5-HT3 receptor antagonist ondansetron. The dose of ondansetron (3 μg) was selected according to a previous
study. To investigate the relation between the altered mechanisms of GABA_{A} receptors in the spinal dorsal horn and BDNF, we used a BDNF antagonist, tropomyosin receptor kinase-B (TrkB; K252a). K252a was administered intrathecally for 5 consecutive days before the morphine injection. The dose of bicuculine (0.03 μg/5 μl) was selected according to a previous study. To investigate the relation between the altered mechanisms of GABA_{A} receptors in the spinal dorsal horn and BDNF, we used a BDNF antagonist, tropomyosin receptor kinase-B (TrkB; K252a). K252a was administered intrathecally for 5 consecutive days before the morphine injection. The dose of bicuculine (0.03 μg/5 μl) was selected according to a previous study. 20,39 Anesthesia with isoflurane was maintained for 2 minutes for the intrathecal administration. All rats that were administered the drug started moving within 5 minutes after the end of the anesthesia and were fully awake at the time of the first behavioral test, which was performed 15 or 30 minutes later. Morphine, ondansetron, and bicuculine were purchased from Millipore Sigma (St. Louis, MO). K252a was obtained from Wako Chemical Industry (Osaka, Japan).

**Microdialysis Studies**

Microdialysis studies to measure noradrenaline/5-HT and GABA/glutamate levels in the spinal dorsal horn of normal rats and SNL rats were performed according to a previously described protocol. 20,39 The rats were anesthetized with 3% isoflurane in 100% oxygen using a nose cone (a surgical depth of anesthesia was maintained with 1.5% isoflurane in 100% oxygen). A cannula was placed in the left femoral vein for fluid infusion. A heating pad underneath the rat was used to maintain the rectal temperature at 37°C to 38°C. A thoracolumbar laminectomy was performed to expose the right spinal cord at the Th12-L1 level.

The rat was then placed in a stereotaxic apparatus and a 30-gauge needle was used to puncture the dura. A microdialysis probe (outer diameter = 0.22 mm, inner diameter = 0.20 mm, length = 1 mm; A-18-01; Eicom Co.) was inserted just lateral to the dorsal root and a micromanipulator (model WR-88; Narishige, Tokyo, Japan) was used to advance the probe to a depth of 1 mm at an angle of 15° to 30°. 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.). In the noradrenaline/5-HT study, after 120 minutes of constant perfusion, 2 consecutive samples were collected to determine the basal noradrenaline and 5-HT concentrations in the dialysate (baseline). PAG administration of saline (0.5 μl) or morphine (100 ng/0.5 μl) was performed using a microsyringe pump (ESP-64; Eicom Co.), and 15-minute perfusate fractions were collected into an autoinjector (EAS-20; Eicom Co.). Samples (15 μl) were automatically injected and the noradrenaline and 5-HT concentrations were analyzed by high-performance liquid chromatography with electrochemical detection using an HTEC-500 analyzing system (Eicom Co.). The sample was then separated on the column (2.0 mm × 200 mm, EICOMPAC CAX; Eicom Co.) using a mobile phase consisting 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.

In the GABA/glutamate study, 1 h after inserting the microdialysis probe and perfusing it with Ringer’s solution, Ringer’s solution containing 2-m-5-HT (100 μmol/L; Millipore Sigma Co.) was perfused for 2 h before measuring GABA and glutamate levels in the spinal dorsal horn in normal and SNL rats. The dose of 2-m-5-HT was selected on the basis of our preliminary study. Samples were derivatized with 2-mercaptoethanol and o-phthalaldehyde (4 mmol/L) in 0.1 mol/L carbonate buffer (pH = 9.5). The phthalaldehyde derivatives were then separated on a column (3.0 mm × 75 mm, FA-3ODS, EICOMPAC Co.) at 40°C using a mobile phase comprising 100 mmol/L phosphate buffer (pH = 6.0), methanol, and acetonitrile (80:7:13 vol/vol) containing 5 mg/mL EDTA-2Na at a flow rate of 0.5 mL/min. At 13 minutes after commencing the analysis, the mobile phase was changed using an ELS-500 switching valve to 100 mmol/L phosphate buffer (pH = 6.0) and acetonitrile (1:1 vol/vol) containing 5 mg/mL EDTA-2Na at a flow rate of 0.5 mL/min.

**Statistical Analysis**

Data are presented as mean ± standard deviation (SD). Behavioral data and microdialysis data were analyzed by 2-way repeated-measures analyses of variance (ANOVA) with group and time as independent variables. Student’s t-test with Bonferroni’s correction was used for multiple comparisons. To compare the withdrawal thresholds or latencies between normal and SNL rats, the area under the time-course curve for the percentages of the maximum possible effect (%MPE) was calculated from individual scores at each time point using the trapezoidal rule over the 240-minute observation period: percentage of the maximum possible effect = (postdrug threshold or latency – predrug threshold or latency) × 100/ (250 g or 30 seconds – predrug threshold or latency). The area under the time-course curve was analyzed by 1-way repeated-measures ANOVA and Student’s t-test with Bonferroni’s correction. P < .05 was considered statistically significant. The statistical analysis was conducted using SigmaPlot 12 (Systat Software Inc, San Jose, CA). We analyzed 230 rats. We performed a power analysis for the primary outcome (mechanical withdrawal threshold in SNL rats) to determine the appropriate sample size, with the assumptions of a mean difference of 50 g in the withdrawal threshold and SD of 30 g in each group, according to a previous study. We found that 6 rats in each group would allow us to detect significant differences with 80% power at a significance level of α = 0.05.
Results

Behavioral Studies

The PAG cannula positions according to a rat brain atlas are shown in Fig. 1. PAG administration of morphine (10 ng, 100 ng) produced analgesic effects against mechanical stimulation in normal rats (P = .04 and .004, respectively; Fig. 2A). There were significant main effects of group (F 3, 270 = 10.141; P < .001) and time (F 5, 270 = 30.875; P < .001), and a group × time interaction (F 15, 270 = 4.695; P < .001). In SNL rats, no analgesic effects of morphine were observed after PAG administration (Fig. 2B). There were significant main effects of time (F 6, 300 = 46.615; P < .001), but no significant main effects of group (F 1, 300 = 2.406; P = .137) or a group × time interaction (F 18, 300 = 1.110; P = .341). Based on the area under the time-course curve, 10 ng and 100 ng morphine had greater effects in normal rats than in SNL rats (P < .001, respectively; Fig. 2C). There were significant main effects of group (F 7, 90 = 15.099; P < .001). On the basis of these results, we selected a morphine dose of 100 ng for the subsequent experiments.

PAG administration of morphine (100 ng) produced analgesic effects against thermal stimulation in normal rats (P < .001, Fig. 3A). There were significant main effects of group (F 1, 95 = 22.066; P < .001) and time (F 5, 95 = 16.421; P < .001), and a group × time interaction (F 15, 95 = 7.491; P < .001). In SNL rats, no analgesic effects of morphine were observed after PAG administration (Fig. 3A). There were significant main effects of time (F 6, 114 = 7.986; P < .001), but no significant main effects of group (F 1, 114 = 2.406; P = .137) or a group × time interaction (F 6, 114 = 1.104; P = .364). Based on the area under the time-course curve, 100 ng morphine had analgesic effects against thermal stimulation in normal rats (P < .001; Fig. 3B), but the findings did not differ significantly from those in SNL rats. There were significant main effects of group (F 3, 28 = 3.048; P = .045).

Microdialysis Studies

The baseline spinal cord dorsal horn 5-HT concentration was not significantly different between normal rats (0.39 ± 0.19 pg/15 μl) and SNL rats (0.27 ± 0.16 pg/15 μl). The baseline spinal cord dorsal horn noradrenaline concentration was not significantly different between groups (0.34 ± 0.27 pg/15 μl, normal; 0.43 ± 0.35 pg/15 μl, SNL). PAG administration of morphine (100 ng) led to an increase in the 5-HT concentration (P = .002 vs saline in normal rats, P < .001 in SNL rats; Fig. 4A). There were significant main effects of group (F 3, 320 = 15.026; P < .001) and time (F 16, 320 = 9.398; P < .001), and a group × time interaction (F 48, 320 = 2.094; P < .001). The 5-HT concentration in the dialysates at 30 minutes was increased compared with the baseline values in both groups: in normal rats, 136.5 ± 17.2% (morphine) vs 75.9 ± 16.8% (saline), and in SNL rats, 123.2 ± 12.6% (morphine) vs 73.7 ± 6.6% (saline). The noradrenaline concentration was not different among groups (P=1.000 vs saline in normal rats, P= 0.873 in SNL rats; P=0.0001 vs saline in SNL rats).
Fig 4B). There were no significant main effects of group ($F_{3, 320} = 2.290; P = .109$), but there were significant effects of time ($F_{16, 320} = 1.803; P = 0.03$), and no significant group $\times$ time interaction ($F_{48, 320} = 0.916; P = .634$).

**Antagonist Studies**

The 5-HT3 receptor antagonist ondansetron was used to examine the role of 5-HT3 receptors in the spinal cord on morphine-induced pain modulation. The dose of ondansetron (3 $\mu$g) was selected according to a previous study. The effects of ondansetron on analgesia induced by PAG-administered morphine differed between normal and SNL rats (Fig 5). Pretreatment with intrathecal ondansetron attenuated the morphine-induced analgesia in the normal group ($P < .001$; Fig. 5A and 5B). In contrast, ondansetron increased the morphine-induced analgesia in the SNL group ($P < .001$; Fig. 5A and 5B). There were significant main effects of

*Figure 3. Effect of periaqueductal gray (PAG) administration of morphine (100 ng) or saline on thermal withdrawal latency in normal and spinal nerve ligation (SNL) rats. The time course (A) and area under the time-course curve (AUC) (B) are shown. Withdrawal latencies are expressed as the mean $\pm$ SD for 10-11 rats in each group. (A)*$P < .05$ compared with the normal saline group at each time-point by Student’s t-test with Bonferroni’s correction after 2-way repeated-measures ANOVA. (B) *$P = .034$ compared with the normal saline group by Student’s t-test with Bonferroni’s correction after 1-way repeated-measures ANOVA.

*Figure 4. Microdialysis from the dorsal horn of the lumbar spinal cord to measure levels of 5-hydroxytryptamine (5-HT: A) and noradrenaline (NA: B) after periaqueductal gray (PAG) administration of morphine. Normal rats ($n = 6$) or spinal nerve ligation (SNL) rats ($n = 6$) were administered saline or morphine (100 ng) into the PAG. Data are presented over time as the percentage of the baseline. *$P < .05$ compared with the normal saline-treated group at each time-point by Student’s t-test with Bonferroni’s correction after 2-way repeated-measures ANOVA. #$P < .05$ compared with the SNL saline-treated group at each time-point by Student’s t-test with Bonferroni’s correction after 2-way repeated-measures ANOVA.
We speculated that the inverted function of the 5-HT3 receptors in the normal and SNL rats was related to dysfunction of the inhibitory GABAergic neurons in the SNL rats. To test our hypothesis, we used the GABA<sub>A</sub> receptor antagonist bicuculline. The dose of bicuculline (0.03 µg/5 µl) was selected according to a previous study. The effects of bicuculline on analgesia induced by PAG-administered morphine differed between normal and SNL rats. Pretreatment with intrathecal bicuculline attenuated the morphine-induced analgesia in the normal group (P < .001; Fig 6A). There were significant main effects of group (F 3, 105 = 15.109; P < .001) and time (F 5, 105 = 12.612; P < .001), and a group × time interaction (F 15, 105 = 8.531; P < .001). In contrast, the bicuculline increased the morphine-induced analgesia in the SNL group (P < .001; Fig 6B). There were significant main effects of group (F 3, 138 = 21.464; P < .001) and time (F 6, 138 = 70.610; P < .001), and a group × time interaction (F 18, 138 = 14.638; P < .001).

To elucidate the relation between the altered mechanisms of GABA<sub>A</sub> receptors in the spinal dorsal horn and BDNF, we used the TrkB antagonist K252a. The dose of bicuculline...
K252a (dissolved in 10% DMSO, 2 \( \mu \)g/10 mL) was selected according to the previous study. In SNL rats, the enhanced morphine antinociception by bicuculline was reversed by 5 daily intrathecal pretreatments of K252a (2 \( \mu \)g/day, \( P < .001 \); Fig. 7A and 7B). There were significant main effects of group (F 1, 50 = 68.949; \( P < .001 \)) and time (F 5, 50 = 28.228; \( P < .001 \)), and a group \( \times \) time interaction (F 5, 50 = 13.291; \( P < .001 \)).

**5-HT3 Receptor-Induced GABA Release**

Further, to examine whether the balance of the GABA and glutamate levels in the dorsal horn of the spinal cord after PAG morphine differed between normal and SNL rats, we performed microdialysis studies. First, we measured GABA and glutamate levels after PAG administration of morphine (100 ng). GABA and glutamate levels, however, did not differ between normal and SNL rats (GABA: \( P = .616 \) vs saline in normal rats, \( P = .827 \) vs saline in SNL rats. Glutamate: \( P = 1.000 \) vs saline both in normal and SNL rats). Therefore, we used the 5-HT3 receptor agonist 2-methylserotonin (2-m-5-HT). Local perfusion of 2-m-5-HT (100 \( \mu \)mol/L) in the spinal dorsal horn increased the GABA concentration (\( P < .001 \) vs saline both in normal and SNL rats, Fig. 8A). There were significant main effects of group (F 3, 120 = 12.770; \( P < .001 \)) and time (F...
Discussion

PAG administration of morphine produced analgesic effects in normal rats, but not SNL rats despite increasing 5-HT levels in the spinal dorsal horn in both groups. Our behavioral studies showed that a descending pathway, including the PAG, and spinal 5-HT3 and GABA_\text{A} receptors, contribute to the analgesic efficacy of morphine in the normal state, but activation of this pathway by morphine has a facilitatory effect on spinal nociceptive processing in SNL rats. Our data using a TrkB antagonist also suggested that post-injury dysfunction of spinal GABA_\text{A} receptors, i.e., change from inhibitory to facilitatory, contributed to this phenomenon in SNL rats. Finally, we revealed that activation of spinal 5-HT3 receptors led to increased GABA levels in the spinal dorsal horn in both normal and SNL rats (Fig 9).

A previous study demonstrated that the analgesic effect of systemically administered morphine is attenuated in SNL rats compared with normal animals.\(^4\) Although the descending serotonergic system and spinal 5-HT3 receptors strongly contribute to the attenuation, the main mechanism remained unclear because systemic administration of morphine acts at a variety of sites to produce analgesia, such as in the periphery.\(^{15}\)

![Figure 9](image-url)
bicuculline produced analgesic effects. On the basis of these results, GABA$_A$ receptors in the spinal dorsal horn contribute to the pain-facilitating effects of morphine in SNL rats. These effects are similar to those of intrathecal administration of the 5-HT3 receptor antagonist ondansetron. Therefore, altered responses to GABA$_A$ receptor activation in the spinal dorsal horn may be involved in these phenomena.

In animal models of neuropathic pain, activated microglia in the spinal dorsal horn release BDNF. Some studies have also reported that primary afferent derived BDNF and potentially spinal neurons also release BDNF and may contribute to some types of neuropathic pain. BDNF changes GABA$_A$ receptors from inhibitory to facilitatory through the potassium-chloride transporter KCC2 via TrkB receptor activation. Therefore, it is possible that dysfunction of the inhibitory GABAergic system in the spinal dorsal horn contributes to attenuate the analgesic effect of morphine against neuropathic pain. We further examined whether these mechanisms were related to attenuation of the analgesic effect of PAG administration of morphine after SNL.

Our study demonstrated that the TrkB antagonist K252a reversed the bicuculline-induced amelioration of the analgesic effects induced by PAG administration of morphine. A previous study reported that 5 daily intrathecal administrations of K252a (2 µg) reverses the effect of TrkB receptor activation. We also considered that changes in the balance of GABA and glutamate release by 5-HT3 receptor activation in the spinal cord after nerve injury contributed to attenuate the analgesic effect of morphine. The microdialysis study, however, revealed that local perfusion of the 5-HT3 antagonist 2-m-5-HT increased GABA levels in the spinal dorsal horn to the same level in both normal and SNL rats, although glutamate levels were not changed in either normal or SNL rats. Taken together, these findings suggest that the altered function of spinal GABA$_A$ receptors from inhibitory to facilitatory through the activation of TrkB receptors is involved in the attenuated analgesic effect of PAG-administered morphine against neuropathic pain.

Opioids have strong analgesic effects against nociceptive pain and are frequently used for pain management during the perioperative period. On the other hand, opioids are less effective for treating neuropathic pain. The International Association for the Study of Pain recommended that strong opioids be used only as a third-line treatment for neuropathic pain. The findings of the present study revealed that functional changes in GABA$_A$ receptors in the spinal dorsal horn after nerve injury might strongly contribute to the attenuation of opioid-induced analgesia for neuropathic pain.

The present study has some limitations. First, a slight anti-allodynic effect of spinal ondansetron (10 µg) was reported in SNL rats. For the experiment using ondansetron, bicuculline, and K252a, we selected a dose that would not change the pain threshold, referring to our previous reports. Second, to reduce the number of rats used in this study, we did not perform the experiment with a group receiving only ondansetron, bicuculline, or K252a, but each of these drugs may change the pain threshold. Third, the effect of a single intrathecal administration of K252a was not confirmed. The rationale for the continuous administration of K252a could not be confirmed, but the administration plan was based on the protocol of previous reports. Previous studies suggested that 5-HT played a minor role in antinociception mediated by the PAG to RVM to spinal cord pathway and RVM GABAergic neurons appeared to play a more prominent role. 5-HT might modulate the antinociceptive effects mediated by the RVM as opposed to driving it in neuropathic pain state. In the present study, we performed behavioral experiments, but electrophysiologic studies are needed to further investigate this issue, especially to confirm the function of GABA$_A$ receptors.

In conclusion, the mechanism underlying the attenuated analgesic effects of opioids on neuropathic pain compared with nociceptive pain might derive from dysfunction of the serotonergic descending pathway from the PAG due to functional changes in GABA$_A$ receptors in the spinal cord.

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