Research article

Spinal dopaminergic involvement in the antihyperalgesic effect of antidepressants in a rat model of neuropathic pain

Mi Chen, Hajime Hoshino, Shigeru Saito, Yang Yang, Hideaki Obata

Department of Anesthesiology, Gamma University Graduate School of Medicine, 3-39-22, Showa, Maebashi, Gunma 371-8511, Japan
Department for Pain Management, Fukushima Medical University Hospital, #1 Hikarigaoka, Fukushima-city, Fukushima 960-1295, Japan
Department of Anesthesiology, Fukushima Medical University, #1 Hikarigaoka, Fukushima-city, Fukushima 960-1295, Japan
Department of Anesthesiology, Affiliated Hospital of Guizhou Medical University, #28 Guiyi Road, Guiyang, Guizhou 550004, China

HIGHLIGHTS

- Systemic injection of 4 antidepressants suppressed hyperalgesia after nerve injury.
- The antihyperalgesia of 4 antidepressants were reversed by intrathecal sulpiride.
- All antidepressants increased the dopamine level in the spinal dorsal horn.

ARTICLE INFO

Article history:
Received 19 January 2017
Revised 20 March 2017
Accepted 10 April 2017
Available online 14 April 2017

Keywords:
Dopamine analgesia
Spinal cord
Neuropathic pain
Antidepressants
Rats

ABSTRACT

Antidepressants such as tricyclic antidepressants, and serotonin noradrenaline reuptake inhibitors are a first-line treatment for neuropathic pain. Here, we aimed to determine the involvement of the spinal dopaminergic system in the antihyperalgesic effects of antidepressants in a rat model of neuropathic pain induced by spinal nerve ligation (SNL). The right L5 spinal nerve of male Sprague-Dawley rats was ligated under inhalation anesthesia to induce hyperalgesia. Behavioral testing was performed by measuring ipsilateral hindpaw withdrawal thresholds after intraperitoneal injection of amitriptyline, duloxetine, milnacipran, and fluoxetine. D2-like receptors were blocked by intrathecal administration of sulpiride. We also determined the concentrations of dopamine in the spinal cord using microdialysis after injection of antidepressants. The dopamine content in the spinal dorsal horn were also measured in normal and SNL rats at 2, 3, 4, and 8 weeks after SNL surgery. Intraperitoneal injection of amitriptyline, duloxetine, milnacipran, and fluoxetine (3–30 mg/kg) produced antihyperalgesic effects, and prevented by intrathecal pre-injection of sulpiride (30 μg). Microdialysis revealed the dopamine levels in the spinal cord were increased after intraperitoneal injection of each antidepressant (10 mg/kg). Furthermore, the dopamine content in homogenized spinal cord tissue were increased at 2 weeks after SNL and then subsequently declined. Our results suggest that the effect of antidepressants against neuropathic pain is related to modulation of not only noradrenaline and serotonin but also dopamine levels in the spinal cord.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Persistent neuropathic pain is common after trauma, chemotherapy, or surgery [1]. The key features of neuropathic pain are increased activation of afferent nociceptors and sensitization of afferent signaling through spinal processing, leading to sensory loss and paradoxical hypersensitivity [2,3]. Brainstem-spinal descending noradrenaline and serotonin (5-hydroxytryptamine: 5-HT) systems suppress nociceptive signaling from primary afferent neurons to the spinal dorsal horn [4], although the 5-HT systems also have a facilitatory effect [5], and these descending inhibitory systems may play an important role in neuropathic pain states. Antidepressants such as tricyclic antidepressants (TCAs), and serotonin NA reuptake inhibitors (SNRIs) are frequently used as first-line drugs for management of neuropathic pain [6], and the reuptake inhibition of NA in the spinal cord, is considered to be the main mechanism underlying the therapeutic effects [7].

Abbreviations: SNL, Spinal nerve ligation; TCA, Tricyclic antidepressant; SNRI, Serotonin noradrenaline reuptake inhibitor.
* Corresponding author at: Center for Pain Management, Fukushima Medical University Hospital, #1 Hikarigaoka, Fukushima-city, Fukushima, 960-1295, Japan.
E-mail address: obata@fmu.ac.jp (H. Obata).
1 These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.neulet.2017.04.017
0304-3940/© 2017 Elsevier B.V. All rights reserved.
Dopamine also plays a critical role in nociceptive transmission in the central nervous system [8]. Dopaminergic innervation of the dorsal horn of the spinal cord projects from the A11 region in the dorsal posterior hypothalamus, and the inhibition of nociceptive transmission at the dorsal horn by dopamine is mediated by D2-like receptors [9,10]. Although TCAs, SNRIs and selective serotonin reuptake inhibitors (SSRIs) have no direct effect on the dopamine transporter, dopaminergic systems might be indirectly involved in the analgesic effect of antidepressants [11]. Nevertheless, the role of dopamine in the spinal cord in the analgesic effects of antidepressants has not been examined.

The first purpose of the present study was to evaluate the antihyperalgesic effects and respective involvement of the spinal dopaminergic system for different types of antidepressants in an animal model of neuropathic pain elicited by spinal nerve ligation (SNL). We also measured the changes of dopamine concentration in the spinal dorsal horn after intraperitoneal injection of antidepressants by in vivo microdialysis. Finally, to examine plastic changes of the descending dopaminergic pain modulation system after nerve injury, we measured the change in the levels of dopamine and its metabolite 3,4-dihydroxy-phenylacetic acid (DOPAC) in the lumbar spinal cord over time after SNL.

2. Materials and methods

2.1. Experimental animals

The experiments were approved by the Animal Care and Use Committee of the Gunma University Graduate School of Medicine. Adult male Sprague-Dawley rats (250 g) were used. The rats were housed in a 12-h light/dark cycle with free access to food and water. Animals were allowed to acclimate and trained for 3 days before experiments.

All surgical procedures were performed under isoflurane anesthesia (2% in oxygen). SNL was performed as described previously [12]. In brief, animals were placed in the prone position and a skin incision was made in the midline lumbar region. The right lumbar 5 vertebral transverse process was removed so that the right L4-L5 spinal nerves were exposed. The right L5 spinal nerve was isolated and tightly ligated with 5-0 silk and cut just distal to the ligature. The wound was then closed.

A chronic intrathecal catheter was implanted for drug administration 7 days after SNL surgery as previously described [13]. A sterilized 32-gauge polyethylene catheter (RecathCo, Allison Park, PA) connected to 8.5 cm of Tygon external tubing (Saint-Gobain Performance Plastics, Akron, OH) was inserted. The catheter was passed caudally 7.5–8.0 cm from the cisterna magnum to the lumbar enlargement. The animals were allocated to individual cages to recover for 7 days before drug testing. Correct catheter placement was confirmed by rapid and reversible hind limb paralysis after a 10-μL intrathecal lidocaine injection (50 mg/mL) at the end of the behavioral testing protocol. All experiments were performed 2–3 weeks after SNL surgery.

2.2. Behavioral testing

An analgesimeter (Ugo Basile, Comerio, Italy) was used to apply mechanical stimuli in increments (to a maximum of 250 g) to the outer mid-plantar surface of the hindpaw until an abrupt hindpaw withdrawal was observed, and the value was recorded in grams [14]. At each time point studied, the hindpaws ipsilateral and contralateral to the nerve injury were measured twice, with repeated measurements each time. The mean value of the paw withdrawal threshold for each limb was calculated for analysis. The animals were randomly assigned to the different drug and dosage groups, and the experiments were blind to the group assignments.

2.3. Drug preparation and delivery

Animals were intraperitoneally administered amitriptyline (a TCA; LKT Laboratories Inc. USA), duloxetine (an SNRI; Wako Pure Chemical Industries Ltd. Osaka, Japan), milnacipran (an SNRI; Asahi Kasei Corporation, Osaka, Japan) or fluoxetine (an SSRI; Sigma, St. Louis, MO) in a volume of 0.5 mL. All drugs were dissolved in a mixture of 50% saline and 50% dimethyl sulfoxide (DMSO), except for milnacipran, which was dissolved in saline and administered at doses of 3, 10, and 30 mg/kg, respectively. The withdrawal threshold was determined at pre-SNL (before SNL surgery), time 0 (before drug injection), and then 15, 30, 60, 120, and 180 min after drug injection. For antagonist studies, 30 μg of sulpiride (a D2-like receptor antagonist; Tocris, Ellisville, MO) was administered intrathecally 15 min before injection of the antidepressants. Sulpiride was dissolved in a mixture of 50% saline and 50% DMSO and injected in a volume of 5 μL, followed by 10 μL of saline to flush the catheter.

2.4. Microdialysis

Microdialysis was performed with normal (i.e., non-injured) rats as previously described [15]. Anesthesia was induced with 3% isoflurane and maintained with 1.5% isoflurane in 100% oxygen through a nose cone. The L3 to L6 level of the right spinal cord was exposed by thoracolumbar laminectomy, and then the rat was placed in a stereotaxic apparatus. After the dura was punctured, the microdialysis probe (outer diameter = 0.22 mm, inner diameter = 0.20 mm, length = 1 mm; A-1-8-01; Eicom Co., Kyoto, Japan) was inserted from just lateral to the dorsal root and advanced 1 mm using a micromanipulator (model WR-88; Narishige, Tokyo, Japan). The microdialysis probe was perfused with Ringer’s solution (147 mM NaCl, 4 mM KCl, and 2.3 mM CaCl2) at a constant flow rate (1 μL/min) using a microsyringe pump (ESP-64; Eicom Co.). After 120 min of constant perfusion, two consecutive samples were collected to determine basal dopamine concentration in the dialysate (baseline), and amitriptyline, duloxetine, milnacipran, fluoxetine (all 10 mg/kg), or vehicle (50% DMSO or saline for milnacipran) was administered intraperitoneally through an indwelling catheter in a volume of 0.5 mL, after which 15-min perfusion fractions were collected into an auto injector (EAS-20; Eicom Co.). The dopamine concentrations in the perfusate were analyzed using high-performance liquid chromatography (HPLC) with electrochemical detection using an HTEC-500 analyzing system (Eicom Co.). The column was an EICOMPAC CAX (2.0 mm × 200 mm; Eicom Co.).

2.5. Analysis of homogenized tissue from lumbar dorsal horn

The dopamine contents in the spinal dorsal horn were also determined in normal and SNL rats at 2, 3, 4, and 8 weeks after SNL surgery. To isolate the dorsal horn of the spinal cord, the portion corresponding to segments L4-L6 was divided into four constituent quadrants: dorsal right, dorsal left, ventral right, and ventral left. The dorsal right (ligation side) portion of the spinal cord was weighed and homogenized in 0.5 mL of 0.2 M perchloric acid containing 0.1 mM Na2-EDTA and isoproterenol (0.02 mg/mL) as an internal standard, and centrifuged at 20,000g at 4°C for 15 min. The supernatants were adjusted to pH 3.0 by adding 1 M sodium acetate and then filtered through a centrifugal filter with a pore size of 0.45 μm (Millipore, Bedford, MA). Samples (10 μL) were injected into an HTEC-500 analyzing system (Eicom Co.), and the concentrations of dopamine and DOPAC were analyzed using HPLC with
3. Results

Table 1 shows the results of the two-way repeated measures ANOVA (group, time, and group × time interaction) for the behavioral studies. Intraperitoneal injection of 30 mg/kg of duloxetine increased withdrawal thresholds compared with intraperitoneal injection of vehicle (Fig. 1A, P < 0.001 by Student’s t-test with the Bonferroni correction). Intrathecal administration of sulpiride (30 μg) attenuated the attenuating effect of 30 mg/kg duloxetine (Fig. 1B, P < 0.001 by Student’s t-test with the Bonferroni correction).

Intraperitoneal injection of 10 and 30 mg/kg of milnacipran increased withdrawal thresholds compared with intraperitoneal injection of saline (Fig. 2A, P = 0.026 and P < 0.001 by Student’s t-test with the Bonferroni correction, respectively). Intrathecal injection of sulpiride decreased withdrawal thresholds of milnacipran (30 mg/kg) compared with the intrathecal injection of vehicle (Fig. 2B, P = 0.001 by Student’s t-test with the Bonferroni correction).

Intraperitoneal injection of 30 mg/kg of amitriptyline increased withdrawal thresholds compared with intraperitoneal injection of vehicle (Fig. 3A, P < 0.001 by Student’s t-test with the Bonferroni correction). Intrathecal injection of sulpiride decreased withdrawal thresholds compared with intrathecal injection of vehicle (Fig. 3B, P < 0.001 by Student’s t-test with the Bonferroni correction).

Intraperitoneal injection of 10 and 30 mg/kg of fluoxetine increased withdrawal thresholds compared with intraperitoneal injection of vehicle (Fig. 4A, P = 0.014 and P < 0.001 by Student’s t-test with the Bonferroni correction, respectively). As the high-
The antihyperalgesic effect of intraperitoneally administered milnacipran (MIL) in SNL rats. *P<0.05 compared with the saline (SAL) group at each time point. (B) The antihyperalgesic effect of intraperitoneally administered milnacipran (30 mg/kg) was reversed completely by intrathecal pretreatment with the dopamine D2 receptor antagonist sulpiride (SUL; 30 μg). *P<0.05 compared with the VEH (i.t.) + MIL 30 mg/kg (i.p.) group at each time point. All values represent the mean ± SEM for 6 rats.

The change in dopamine concentrations in the dorsal horn of the spinal cord in normal rats over time after intraperitoneal injection of each antidepressant was determined by microdialysis. The baseline dopamine concentration before drug injection was 0.37 ± 0.03 pg/15 μL (n = 36). All of the antidepressants (10 mg/kg) increased dopamine levels compared to the control (Fig. 5). Table 2 shows the results of the two-way repeated measures ANOVA (group, time, and group × time interaction) for the microdialysis studies.

![Fig. 2](image1.png)

![Fig. 3](image2.png)

**Table 2** Results of the two-way repeated measures ANOVA for the microdialysis studies.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Source of variation</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>duloxetine</td>
<td>group</td>
<td>F₁,₁₂₀ = 12.92</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>F₁,₁₂₀ = 6.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>group × time interaction</td>
<td>F₁,₁₂₀ = 7.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>milnacipran</td>
<td>group</td>
<td>F₁,₁₂₀ = 9.97</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>F₁,₁₂₀ = 6.69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>group × time interaction</td>
<td>F₁,₁₂₀ = 6.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>amitriptyline</td>
<td>group</td>
<td>F₁,₁₂₀ = 32.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>F₁,₁₂₀ = 20.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>group × time interaction</td>
<td>F₁,₁₂₀ = 21.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>fluoxetine</td>
<td>group</td>
<td>F₁,₁₂₀ = 19.29</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>F₁,₁₂₀ = 8.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>group × time interaction</td>
<td>F₁,₁₂₀ = 9.48</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
The dopamine and DOPAC contents in homogenized tissue from the ipsilateral dorsal spinal cord of normal rats (control) and SNL rats were also determined (Fig. 6). With regard to the dopamine contents, there were significant main effects between groups ($F_{4,25} = 7.01, P < 0.001$). Post hoc testing revealed that an increase in the dopamine concentration in the ipsilateral dorsal spinal cord at 2 weeks after SNL (209.2 ± 41.6 pg/g) compared to normal rats (141.0 ± 23.3 pg/g, $P = 0.003$ by Student’s $t$-test with Dunnett correction), and the dopamine concentration subsequently returned to the baseline level, which was the same as the level in normal rats (Fig. 6A). With regard to the DOPAC content, there were significant main effects between groups ($F_{4,25} = 4.73, P = 0.006$), but no significant effect was detected between normal rats and SNL rats (Fig. 6B). The DOPAC/dopamine ratios, reflecting changes in dopamine turnover, did not differ significantly between normal rats and SNL rats (data not shown).

4. Discussion

In-depth exploration of the potential mechanisms underlying the analgesic effects of antidepressants could provide crucial guidance for management of neuropathic pain. The current study shows that intraperitoneal injection of duloxetine, milnacipran amitriptyline, and fluoxetine induces antihyperalgesic effects in SNL rats, and that intrathecal pretreatment with a D2-like receptor antagonist attenuates these effects. In addition, the concentrations of dopamine in the spinal cord were increased after injection of all of the antidepressants. Our data also suggest the occurrence of plastic changes in the descending dopaminergic system after nerve injury.

SNRIs are recommended as first-line drugs for management of neuropathic pain [6]. In experimental animals, recent evidence has demonstrated that milnacipran elicits antihyperalgesic effects against neuropathic pain through spinal $\alpha$2-adrenoceptors [7].
The analgesic effects of milnacipran at the spinal level have also been observed to occur through inhibition of C-fiber-mediated nociceptive synaptic transmission by activating both the spinal 5-HT and noradrenaline systems [18]. Similar findings have been reported for duloxetine, which inhibits nociceptive signaling from primary afferent neurons to spinal dorsal horn neurons via the spinal 5-HT2A and α2-adrenoceptors [19]. Thus, the underlying antihyperalgesic mechanisms include inhibition of 5-HT and noradrenaline reuptake leading to enhanced descending pain inhibitory systems [20].

TCAs are also used for management of neuropathic pain as first-line drugs [6]. The main mechanism underlying the analgesic effect of TCAs might involve blockade of the reuptake of noradrenaline and 5-HT [17,21]. However, TCAs have multiple effects such as activation of opioid, cholinergic, 5-HT, and N-methyl-D-aspartate receptors and blockade of ion channel activity, thus inhibiting ectopic afferent discharges and the uptake of adenosine; they also block histamine release [22].

Compared to TCAs and SNRIs, less clinical evidence is available to support the use of SSRIs for management of neuropathic pain. In the present study, however, fluoxetine had a dose-dependent antihyperalgesic effect. Although 5-HT is known to be a crucial neurotransmitter in pain regulation, the mechanism by which fluoxetine exerts its antihyperalgesic effect is not clear. Previous reports indicated that the concentration of 5-HT was reduced in the raphe magnus nucleus and midbrain of animals with neuropathic pain [23,24], whereas fluoxetine was shown to increase the 5-HT level in the synaptic cleft and enhance the efficacy of 5-HT transmission [25]; fluoxetine might therefore compensate for compromised 5-HT signaling in neuropathic pain. In addition, the increase in noradrenaline levels mediated by SSRIs might suppress neuropathic pain through spinal α2-adrenoceptors [7].

The dopaminergic system also plays a role in nociceptive transmission. Five distinct types of dopamine receptors have been isolated and divided into two subfamilies, D1-like (D1 and D5) and D2-like (D2, D3 and D4) receptors. Previous studies have shown that dopamine D2-like receptors in the brain contribute to dopamine-induced analgesia for pathological pain such as inflammatory pain [26–28] and neuropathic pain [29]. All dopamine receptor subtypes are expressed in the spinal cord with dominant D2 receptor expression [30]. D2-like receptors are also involved in dopaminergic suppression of nociceptive transmission in the spinal dorsal horn [10,31]. However, few reports have addressed the mechanism underlying the dopaminergic effects of antidepressants against neuropathic pain. In a previous study, we demonstrated that intrathecal injection of 3, 10 and 30 μg of sulpiride dose-
The dopamine (A) and 3,4-dihydroxy-phenylacetic acid (DOPAC; B) contents in the ipsilateral dorsal half of the lumbar spinal cord was measured in uninjured (normal) rats and rats at 2, 3, 4 and 8 weeks after spinal nerve ligation. *P < 0.05 compared with normal rats. All values represent the mean ± SEM for 6 rats.

Figure 6. The dopamine (A) and 3,4-dihydroxy-phenylacetic acid (DOPAC; B) contents in the ipsilateral dorsal half of the lumbar spinal cord was measured in uninjured (normal) rats and rats at 2, 3, 4 and 8 weeks after spinal nerve ligation. *P < 0.05 compared with normal rats. All values represent the mean ± SEM for 6 rats.

increase after injection of antidepressants in the spinal cord is not known. Further studies are needed to determine whether dopamine reuptake is mediated by the noradrenergic transporter or by the dopamine transporter in the spinal cord, as well as to investigate the possibility of a dopamine-noradrenaline interaction (e.g., noradrenaline increase induces dopamine release, and vice versa). Large plastic changes in the central nervous system occur in both the brain and spinal cord in animal models of nerve injury. A previous study demonstrated plastic changes in descending noradrenergic neurons after nerve injury [33], where the density of the descending noradrenergic fibers and noradrenaline content in the ipsilateral lumbar spinal cord were increased 10 days after SNL in rats. In contrast, another report showed a loss of noradrenergic fibers in the ipsilateral lumbar spinal cord 19–21 days after tibial nerve transection in rats [34]. These results suggest that the tone of the descending noradrenergic system dynamically changes over time after nerve injury. Consistent with these findings and our pilot study [16], the dopamine contents in the spinal cord in the present study were increased 2 weeks after SNL, then returned to baseline values within 3 weeks. These findings reveal that peripheral nerve injury stimulates the descending dopaminergic system, leading to changes in the dopamine level in the spinal cord, although our findings did not reveal increased dopamine turnover after SNL.

This study has several limitations. First, we only used male animals and previous studies revealed that sex differences influence analgesic mechanisms. Second, only mechanical withdrawal threshold was measured in the behavioral studies. A reflexive method (withdrawal threshold) may not accurately indicate hyperalgesia after nerve injury. The withdrawal threshold in combination with non-reflexive method (e.g., conditioned place preference) might be a better way to assess the effects of drugs on neuropathic pain. Third, normal (uninjured) rats, but not SNL rats, were used for the microdialysis studies to measure changes in dopamine levels in the spinal cord after injection of the antidepressants. The purpose of the microdialysis studies was to determine changes in the dopamine level in the spinal cord after injection of the antidepressants. Dopamine physiology, however, might differ between normal and injured animals, which could lead to dissociation between behavioral studies and microdialysis studies. Further studies are necessary to determine the role of dopamine in the spinal cord in the inhibitory effect of antidepressants on neuropathic pain.

Acknowledgements

The study was supported by Grants-in-Aid for Scientific Research (KAKENHI) from the Ministry of Education, Culture, Sports, Scientific and Technology of Japan (No. 26670678 to Dr. Obata). We thank Dr. Devang Thakor of Anioplex, LCC, for English editing.

References

[7] K. Nakajima, H. Obata, N. Iriuchijima, S. Saito, An increase in spinal cord noradrenaline is a major contributor to the antihyperalgesic effect of...


学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，
提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。
图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：
图书馆首页  文献云下载  图书馆入口  外文数据库大全  疑难文献辅助工具