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Research article

Antiallodynic effect of tianeptine via modulation of the 5-HT$_7$ receptor of GABAergic interneurons in the spinal cord of neuropathic rats

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HIGHLIGHTS

- Intrathecal tianeptine reduced neuropathic pain.
- 5-HT$_7$ receptor of the GABAergic interneurons contributed to the activity of tianeptine.
- GAD65 colocalized with 5-HT$_7$ receptor in the spinal cord.

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ABSTRACT

Although tianeptine, an atypical antidepressant has been reported to have antinociceptive effects, the mode of action is different from that of tricyclic antidepressants despite structural similarities. We examined the antiallodynic effect of intrathecal tianeptine in neuropathic pain rats and determined the involvement of 5-hydroxytryptamine type 7 (5-HT$_7$) receptor of the GABAergic interneurons in the spinal cord. Neuropathic pain was induced by spinal nerve ligation (SNL). After observation of the effect from intrathecal tianeptine, a 5-HT$_7$ receptor antagonist (SB-269970) was administered intrathecally 10 min before delivery of tianeptine, to determine the contribution of spinal 5-HT$_7$ receptor on the activity of tianeptine. GAD expression and GABA concentrations were assessed. Intrathecal tianeptine dose-dependently attenuated mechanical allodynia in SNL rats. Pre-treatment with intrathecal SB-269970 reversed the antiallodynic effect of tianeptine. Both GAD65 expression and the GABA concentration in the spinal cord were decreased in neuropathic rats but were increased by tianeptine. Additionally, 5-HT$_7$ receptor and GAD65 were co-localized in the spinal cord. Intrathecal tianeptine reduces neuropathic pain. 5-HT$_7$ receptor of the GABAergic interneurons together with GAD65 plays a role in the activity of tianeptine at the spinal cord level.

1. Introduction

Neuropathic pain is a complex, chronic pain state that exerts a substantial impact on quality of life and is more difficult to treat than many other type of chronic pain [1]. Antidepressant drugs can improve neuropathic pain and are considered first-line medications for this type of pain [24]. The mechanism of action of antidepressant analgesic effects involves blocking reuptake of noradrenaline and 5-hydroxytryptamine (5-HT) and both direct and indirect actions on other receptors [22].

One antidepressant, tianeptine, has structural similarities to those of tricyclic antidepressants [30], but its mechanism of action differs from those of other tricyclic antidepressant drugs. In brief, tianeptine is a selective 5-HT enhancer [18], in contrast to classical tricyclic antidepressants. Interestingly, it has been reported that tianeptine have antinociceptive effects in animals [13,27], but the detailed mechanism of action of tianeptine remains unclear.

One the other hand, γ-aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter of the transfer of nociceptive signals from primary afferent fibers to the central nervous system [10]. The loss of GABA inhibitory tone is an important contributing factor to the development of neuropathic pain [10,17]. 5-HT (also known as serotonin) has also been considered to have an important role in the control of pain through a descending inhibitory pathway in the spinal cord [16]. Moreover, 5-HT type 7 (5-HT$_7$) receptors are

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localized in GABAergic interneurons, thereby modulating GABAergic function in the spinal cord [7,29].

Hence, the aim of this study was to examine the effects of intrathecal tianeptine in rats with neuropathic pain induced by spinal nerve ligation (SNL), and to determine the participation of 5-HT_7 receptors in GABAergic interneurons in the spinal cord.

2. Materials and methods

Male, Sprague–Dawley rats weighing 100–120 g were used. The rats were housed four to a cage under a constant temperature of 22–23 °C and a 12-h light/dark cycle. Food was provided with no limitations. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Chonnam National University.

Neuropathic pain was evoked by SNL [14]. Briefly, the left L5 and L6 spinal nerves were isolated during sevoflurane anesthesia and tightly ligated using a 6-0 silk suture. Animals were considered to be in neuropathic pain if they exhibited mechanical allodynia, i.e., a paw-flinching response to the application of a bending force <4 g.

Five days after SNL, a polyethylene tube (PE-10) was inserted into the subarachnoid space of each rat under sevoflurane anesthesia, as described previously [14]. The catheter was inserted through an incision in the atlanto-occipital membrane and was advanced caudally by 8.5 cm to the lumbar enlargement. The external end of the catheter was tunneled subcutaneously to the top of the head and plugged with a piece of steel wire. The skin was closed using a 3-0 silk suture. Rats showing a postsurgical neurological deficit were excluded from the study and euthanized immediately by an overdose of volatile anesthetics.

Paw withdrawal threshold (PWT) in response to mechanical stimulation was measured using the up and down method [14] by applying calibrated von Frey filaments from underneath the cage through openings in the mesh floor to the hind paw. Rats were placed in a separate transparent plexiglass chamber with a wire mesh floor and were acclimated to the test chamber for 30 min. A series of eight von Frey filaments (0.4, 0.7, 1.2, 2.0, 3.6, 5.5, 8.5 and 15 g) were applied vertically to the plantar surface of the hind paw for 5 s while the hair was bent. Brisk withdrawal or paw flinching was considered a positive response. If a positive response was observed, the less stiff filament was used for the next trial, while in the cases of no withdrawal or licking, the stiffer one was used. The PWT cut-off value was determined to be 15 g, the value when no withdrawal or licking response to the application of a 15-g von Frey filament was observed. Rats which did not show allodynia (PWT <4 g) were excluded from this study.

The following drugs were used: tianeptine (JEIL Pharm. Co. Ltd., Seoul, Korea), SB-269970 (Tocris Cookson Ltd., Bristol, Avon, UK), 5,7-DHT (Sigma–Aldrich Co., St. Louis, MO, USA) and desipramine (Sigma). Tianeptine, 5,7-DHT and desipramine were dissolved in saline, while SB-269970 was dissolved in dimethylsulfoxide (DMSO). Intrathecal agents were administered in a solution volume of 10 μl, followed by an additional 10 μl normal saline to flush the catheter using a hand-driven, gear-operated syringe pump. Desipramine was given intraperitoneally at a concentration of 3 ml/kg.

On the day of testing (day 10 after SNL), rats were allowed to acclimate to the testing chamber for 30 min and then randomly allocated into experimental and control groups. Control studies were performed using intrathecal saline or DMSO according to the solvent used for the test drug. Animals were subjected to testing only once in this study, and the investigator was blinded to the drug administered.

First, the effect of intrathecal tianeptine (30, 100, 300, and 1000 μg, $n = 30$) on the neuropathic pain was investigated. Next, to determine the role of the 5-HT_7 receptor on the effect of tianeptine, a 5-HT_7 receptor antagonist (SB-269970, 100 μg) was administered intrathecally 10 min before delivery of intrathecal tianeptine (1000 μg). PWT was evaluated 15, 30, 60, 90, 120, 150 and 180 min after intrathecal administration of tianeptine. The maximum dose of the 5-HT_7 receptor antagonist that did not affect the control response or neurologic function was selected based on pilot experiments.

To examine the role of descending serotonergic pathways ($n = 5$), intrathecal 5,7-DHT (60 μg), known to ablate serotonergic nerve fibers in the spinal cord [25], was administered. In addition, desipramine (30 mg/kg) was injected intraperitoneally 45 min before delivery of 5,7-DHT to prevent non-specific uptake of 5,7-DHT by noradrenergic nerve fibers.

The level of GABA was determined in the spinal cord of naïve, SNL and tianeptine-treated rats ($n = 16$). The left dorsal side of lumbar (L5–L6) spinal cord was quickly dissected from each rat under sevoflurane anesthesia, snap-frozen in liquid nitrogen, and stored at −70 °C. GABA was extracted from spinal cord samples using formic acid and assayed using a validated LC/MS/MS method. Ten microliters of sample were injected onto a Gemini C18, 3.0 μg, 150 mm × 3.0 mm column using a Gemini C18 4.0 mm × 2.0 mm guard cartridge. The mobile phase used was acetonitrile: DW = 30/70 (v/v) with 0.1% formic acid. The chromatographic separation was under isocratic conditions with a flow rate of 250 μl/min. The column temperature was maintained at 40 °C. MS analysis was performed using an API 4000 Q TRAP mass spectrometer equipped with an electrospray ionization source in positive multiple reaction monitoring (MRM) mode. Other parameters of the mass spectrometer were as follows: temperature 350 °C, GS1, GS2: 50, 50 and ion spray voltage 3500 V.

To observe the localization of 5-HT_7 receptor and GAD65, the rats ($n = 3$) were deeply anesthetized with ketamine hydrochloride/xylazine and perfused pericardially with 500 ml 0.9% saline followed by 4% paraformaldehyde. After perfusion, the left dorsal side of lumbar (L5-L6) spinal cord was removed and post fixed in the same fixative for 7 h and then replaced with 20% sucrose for 16 h, followed by 30% sucrose for 1 day. After treatment with the sucrose solutions, the tissues were embedded in OCT compound. Then, 10 μm frozen cross-sections were prepared and examined. All sections were first blocked with 10% normal serum blocking solution (species the same as the secondary antibody) containing 3% (w/v) bovine serum albumin (BSA) and 0.1% Triton X-100 and 0.05% Tween-20 for 2 h at room temperature to avoid non-specific staining. Sections were incubated with either anti-5-HT_7 rabbit polyclonal (1:25; Santa Cruz Biotechnology Inc., Dallas TX, USA) or glutamic acid decarboxylase (GAD) rabbit monoclonal (1:1000; Abcam Inc., Cambridge, MA, USA) primary antibodies. Briefly, sections were incubated with both primary antibodies overnight at 4 °C, followed by a mixture of FITC- and TRITC-conjugated secondary antibodies for 2 h at 4 °C. To test for nonspecific staining by the secondary antibodies, parallel control cultures were processed identically except for removal of the primary antibody. Additional slides were processed in a similar fashion with exclusion of the secondary antibodies to evaluate autofluorescence. The stained sections were examined using a Leica fluorescence microscope (Germany).

GAD65 protein expression was measured in the left dorsal side of lumbar (L5–L6) spinal cord of naïve, SNL and tianeptine-treated ($n = 11$) rats by Western blot analysis. On day 10 after SNL, the spinal cord was quickly removed and stored at −70 °C. The spinal cord was extracted and homogenized in protein extraction solution (PROPREP TM, Intron Biotechnology, Seoul, Korea), resuspended on ice for 30 min, and centrifuged at 13,000 rpm for 10 min. For Western blots, 30 μg protein was loaded and run on a 10% Tris–HCl sodium dodecyl sulfate polyacrylamide gel. Proteins were elec-