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The antiallodynic effect of intrathecal tianeptine is exerted by increased serotonin and norepinephrine in the spinal dorsal horn

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HIGHLIGHTS

- Intrathecal tianeptine reduced mechanical allodynia by spinal nerve ligation.
- The tianeptine effect was reversed by serotonergic and adrenergic receptor antagonists.
- Microdialysis revealed increases in spinal serotonin and norepinephrine by tianeptine.

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ABSTRACT

The purpose of this study was to validate the effects of tianeptine on serotonergic and noradrenergic neurotransmission in a rat model of neuropathic pain. Neuropathic pain was induced by ligating the L5 and L6 spinal nerves in male Sprague–Dawley rats, and mechanical allodynia was assessed using von Frey filaments. The effects of intrathecally administered tianeptine on mechanical allodynia were assessed. Dihydroergocristine or yohimbine, a serotonergic or α-2 adrenergic receptor antagonists, respectively, were intrathecally administered 10 min before tianeptine to investigate its mechanism of action. Additionally microdialysis studies were performed to measure the extracellular levels of serotonin (5-HT) and norepinephrine (NE) in the spinal dorsal horn following tianeptine administration. Intrathecal tianeptine significantly increased the paw withdrawal thresholds in a dose-dependent manner and the antiallodynic effect was antagonized by dihydroergocristine and yohimbine. Microdialysis studies revealed that tianeptine increased the levels of 5-HT and NE in the spinal dorsal horn. These findings suggest that tianeptine may be effective for the management of neuropathic pain and that its analgesic mechanism is exerted by increased levels of 5-HT and NE in the synaptic cleft at the spinal level.

1. Introduction

Tianeptine (S 1574, [3-chloro-6-methyl-5,5-dioxo-6,11-dihydro-(c,f)-dibenzo-(1,2-thiazepine)-11-yl) amino]-7 heptanoic acid, sodium salt) is an atypical antidepressant that exhibits structural similarities to tricyclic antidepressants (TCAs) but with distinct neurochemical properties. Typical antidepressants affect the presynaptic reuptake of serotonin (5-hydroxytriptamine, 5-HT) and norepinephrine (NE), increasing their levels in the synaptic cleft [1]. However, tianeptine reportedly either has the opposite effect on extracellular levels of 5-HT or NE [2,3] or does not elicit any marked alterations [4,5]. Nevertheless, its antidepressant efficacy has been clearly demonstrated in patients with depression [6,7], and its antinociceptive activity has been reported in animal models of acute nociception [8], morphine tolerance [9], and inflammatory pain [10]. In a study by Uzbay et al. [8], the antinociceptive activity of tianeptine was thought to be associated with increased serotonergic activity because its effects were blocked by parachlorophenylalanine. Parachlorophenylalanine is a competitive inhibitor of 5-hydroxy-tryptophan hydroxylase, which is involved in 5-HT synthesis. In our previous report [10], intrathecally administered tianeptine reduced the flinching

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response evoked by formalin injection, and pretreatment with 5-HT, α-1, and α-2 adrenergic receptor antagonists attenuated the effect of tianeptine. Therefore, we assumed that the serotonergic and adrenergic systems may, at least in part, mediate the spinal antinociception of tianeptine. However, we could not formulate a definitive conclusion based on our results, because our observations were contradictory to those of other studies.

The aim of this study was to validate the effects of tianeptine on serotonergic and adrenergic neurotransmission in a rat model of neuropathic pain. First, we examined the effects of intrathecally administered tianeptine on mechanical allodynia in spinal nerve-ligated rats and the effects of pretreatment with pharmacological antagonists of 5-HT and NE receptors on tianeptine analgesia. To further investigate the effects of tianeptine on these neurotransmitters, we measured the extracellular levels of 5-HT and NE in the spinal dorsal horn by microdialysis.

2. Materials and methods

The study was approved by The Institutional Animal Care and Use Committee of Chonnam National University and Gunma University Graduate School of Medicine. The microdialysis study was performed at Gunma University, and all other experiments were performed at Chonnam National University. Male Sprague–Dawley rats weighing 250–300 g were used in the experiments. The rats were housed under an alternating 12-h light/dark cycle in individual cages in a temperature-controlled room (22 °C ± 0.5 °C) with free access to food and water. Neuropathic pain was evoked by L5 and L6 spinal nerve ligation (SNL) according to the method described by Kim and Chung [11]. Rats exhibiting an inability to flex the left hind limb, indicating L4 nerve damage, were excluded from the study. Animals displaying a 50% withdrawal threshold of ≤ 4.0 g by postoperative day 5 were considered to be neuropathic and were intrathecally catheterized with polyethylene-10 tubing for drug administration, using a method described by Yaksh and Rudy [12]. Rats exhibiting any motor or sensory deficits were euthanized immediately with an overdose of sevoflurane. All rats were allowed to recover for at least 5 days postsurgically.

The following drugs were used in this study: tianeptine (JEIL Pharm. Co., Ltd., Seoul, Korea), dihydroergocristine mesylate (Research Biochemical Internationals, Natick, MA, USA), and yohimbine hydrochloride (Sigma–Aldrich Co., St. Louis, MO, USA). The tianeptine was dissolved in 0.9% saline, and the others were dissolved in dimethylsulfoxide (DMSO). Intrathecal administration of these drugs was performed using a hand-driven, gear-operated syringe pump. All drugs were administered as 10-μL volumes of solution followed by an additional 10 μL of 0.9% saline to flush the catheter.

Behavioral testing was performed by an investigator blinded to the experimental drug and dose. To determine the mechanical withdrawal threshold, eight von Frey filaments (Stoelting, Wood Dale, IL, USA) with logarithmically increasing stiffness (0.4, 0.7, 1.2, 2.0, 3.6, 5.5, 8.5, and 15.0 g) were applied perpendicularly to the plantar surface of the rat's paw using an up–down method. A force just sufficient to bend the filament was applied for 5 s, and a positive response was assumed when abrupt withdrawal or licking responses were exhibited. The 50% withdrawal threshold was then calculated by a method described previously [13].

Five to seven days after intrathecal catheterization, the rats were randomly allocated into experimental groups for intrathecal administration of either an experimental drug or vehicle solution. To assess the efficacy of tianeptine, increasing doses of tianeptine (30, 100, 300, and 1,000 μg in 10 μL, $n = 6$) were administered intrathecally. The route and doses of tianeptine were determined based on the previous study [10]. The withdrawal threshold measured prior to SNL was regarded as the preligation baseline threshold. The withdrawal threshold determined just before intrathecal drug delivery was regarded as the postligation control value. After administration of the experimental drugs, the withdrawal threshold was determined at 15, 30, 60, 90, 120, 150, and 180 min. To determine whether the effect of intrathecal tianeptine is mediated via serotonergic and/or adrenergic transmission, dihydroergocristine (serotonin receptor antagonist, 3 μg) and yohimbine (α-2 adrenergic receptor antagonist, 10 μg) were injected intrathecally 10 min prior to the administration of tianeptine. The doses of dihydroergocristine and yohimbine were chosen based on a previous study in which the maximal doses that did not affect the formalin response (control response) were determined [14].

Microdialysis studies were performed 10–14 days after SNL as described previously [15]. The rats were anesthetized with isoflurane in 100% oxygen, and the right femoral vein was cannulated for infusion at a rate of 1 ml/h. The rectal temperature was maintained at 37–38 °C with a heating pad placed under the animal. After creation of a thoracolumbar laminectomy, the L3-to-L5 segment of the spinal cord was exposed, and the surface of the dura was covered with mineral oil. The rat was then placed in a stereotaxic holder. After opening the dura, a microdialysis probe (OD, 0.22 mm; ID, 0.20 mm; length, 1 mm; Eicom Co., Kyoto, Japan) was advanced at an angle of 30° and to a depth of 1 mm using a micromanipulator (model MM-3; Narishige, Tokyo, Japan), such that it could be inserted into the superficial layer of the dorsal horn. The microdialysis probe was perfused with Ringer's solution (147.0 mmol/L NaCl, 4.0 mmol/L KCl, and 2.3 mmol/L $CaCl_2$) at a constant flow rate (1 μL/min) using a microsyringe pump (ESP-64; Eicom Co., Kyoto, Japan). After 120 min of constant perfusion, two consecutive samples were collected to determine the basal 5-HT and NE concentrations in the dialysate, and either saline or tianeptine (1000 μg) was delivered through an intrathecal catheter. Thereafter, the 15-min perfusate fractions were collected into an autoinjector (EAS-20; Eicom Co.). The samples (15 μl) were automatically injected into the HTEC-500 system (Eicom Co.) to analyze the 5-HT and NE concentrations using high-performance liquid chromatography (HPLC) with electrochemical detection. The chromatographic conditions were as follows. The mobile phase comprised 0.1 mol/L ammonium acetate buffer, pH 6.0, methanol (7:3 vol/vol) containing 0.05 mol/L sodium sulfonate, and 50 mg/L EDTA-2Na. The column was an EICOMPAC CAX (2.0 × 200.0 mm; Eicom Co.). The working electrode was glassy carbon (WE-3G, Eicom Co.) with a flow rate of 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 NE was 5.4 min, and that for 5-HT was 13.1 min. The detection limit of this assay was 30 fg per injection (information from Eicom Co.).

Data are shown as means ± SEM. The time–response data are presented as the mechanical withdrawal threshold in grams. The dose–response data are presented as the area under the time course curves for each dose using the trapezoidal rule and were analyzed using one-way analysis of variance (ANOVA) with Bonferroni adjustment for post hoc analysis. The antagonistic effects of tianeptine were compared using an unpaired t test. To calculate the half-maximal effective dose (ED_{50}) and its 95% confidence interval (CI) [16], the withdrawal threshold data from von Frey filament testing were converted to percentages of maximum possible effect (%MPE) according to the following formula: %MPE = ([postdrug threshold − postligation control threshold]/[cutoff threshold − postligation control threshold]) × 100. Microdialysis data were analyzed using repeated-measures ANOVA. Differences were considered statistically significant at a P value < 0.05.