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Antihyperalgesic effect of duloxetine and amitriptyline in rats after peripheral nerve injury: Influence of descending noradrenergic plasticity

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HIGHLIGHTS

- Duloxetine and amitriptyline suppressed hyperalgesia in rats with spinal nerve ligation (SNL).
- Duloxetine and amitriptyline increased spinal noradrenaline and serotonin levels.
- The spinal noradrenaline content in SNL rats 2 weeks after ligation was higher than that in 4 weeks.
- The analgesic efficacy of duloxetine and amitriptyline was similar between two groups.

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ABSTRACT

Antidepressants such as serotonin-noradrenaline reuptake inhibitors (SNRIs) and tricyclic antidepressants (TCAs) are frequently used for the management of neuropathic pain. Noradrenaline (NA) and serotonin (5-HT) increase in the spinal cord by reuptake inhibition is considered to be main mechanism of the therapeutic effect of antidepressants in neuropathic pain. In the present study, we examined the analgesic effects of duloxetine (SNRI) and amitriptyline (TCA) in a rat model of neuropathic pain induced by spinal nerve ligation (SNL). Intraperitoneal administration of duloxetine and amitriptyline dose-dependently (3,10 and 30 mg/kg) suppressed hyperalgesia induced by SNL. In vivo microdialysis in the lumbar spinal dorsal horn revealed that NA and 5-HT concentrations increased after intraperitoneal administration of duloxetine and amitriptyline (10 mg/kg, respectively). We further determined NA and 5-HT contents in homogenized samples from the ipsilateral dorsal spinal cord after SNL. Although the NA content in SNL rats 2 weeks after ligation was higher than that in SNL rats 4 weeks after ligation, the analgesic efficacy of duloxetine and amitriptyline was similar between two groups. The present study suggests that NA/5-HT increase in the spinal cord is crucial in the antihyperalgesic effect of duloxetine and amitriptyline. The plastic change of the descending noradrenergic system does not obviously affect the analgesic efficacy of duloxetine and amitriptyline

1. Introduction

Neuropathic pains are characterized by a partial or complete somatosensory change in the innervation territory corresponding to peripheral or central nervous system pathology. 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 thus play an important role in neuropathic pain states [1]. Antidepressants such as tricyclic antidepressants (TCAs) and serotonin-noradrenaline reuptake inhibitors (SNRIs) are recommended as first-line drugs for the treatment of neuropathic pain [2]. The first purpose of this study was to examine the antihyperalgesic effect of systemic administration of duloxetine, an SNRI, and amitriptyline, a TCA, in a rat model of neuropathic pain produced by spinal nerve ligation (SNL). The change in the NA and 5-HT increase in the spinal dorsal horn over time after injection of these antidepressants was also measured by in vivo microdialysis.

A recent animal study showed that increased NA level in the spinal cord is crucial in the therapeutic effect of antidepressants

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in neuropathic pain [3]. Previous studies demonstrated plastic changes in the descending noradrenergic inhibitory system after nerve injury in rats [4,5]. Therefore, we hypothesized that the efficacy of antidepressants for neuropathic pain varies according to the plasticity of descending monoaminergic systems over time after nerve injury. The second purpose of this study was to compare the efficacy of analgesic effects of antidepressants in rats 2 and 4 weeks after SNL. Our previous study also demonstrated that the NA and dopamine contents in the spinal dorsal horn were increased 2 weeks after SNL and then decreased gradually [6]. However, the change in spinal 5-HT content over time after nerve injury remains unclear. Thus, the third purpose of the present study was to examine NA and 5-HT contents in the dorsal horn of the lumbar spinal cord in homogenized samples at 0 (normal control), 2 and 4 weeks after SNL surgery.

2. Materials & methods

2.1. Experimental animals

Adult male Sprague–Dawley rats were used. The animals were housed with food and water available ad libitum. The animals were allowed to habituate to the housing facilities prior to surgery or behavioral testing. The experiments were approved by the Animal Care and Use Committee of the Gunma University Graduate School of Medicine.

Spinal nerve ligation (SNL) was performed as previously described [7]. In brief, animals were anesthetized with isoflurane in oxygen, and the right L5 spinal nerve was tightly ligated with 5–0 silk and cut just distal to the ligature. The wound was then closed.

2.2. Behavioral assessments

The person performing the behavioral test was blinded to drug and dose. The withdrawal threshold to pressure applied to the hind paw, expressed in grams, was measured using an analgesimeter (Ugo Basile, Comerio, Italy) as previously described [8]. The device applied 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 three times before the drug treatment. A cut-off of 250 g was used to avoid tissue injury.

2.3. Drugs and their administration

Behavioral studies were performed to examine effects of intraperitoneal administration of duloxetine (0,3,10 and 30 mg/kg) and amitriptyline (0,3,10 and 30 mg/kg) for hyperalgesia in rats 2 and 4 weeks after SNL surgery. The withdrawal threshold was determined before (before SNL surgery) and at 0 (before drug injection), 15, 30, 60, 120 and 180 min after the injection. Side effects, such as sedation or agitation, were carefully observed. Motor function was assessed in terms of the placing reflex and righting reflex. The doses of duloxetine and amitriptyline were selected based on previous studies [9,10]. Duloxetine was dissolved by mixing with 50% dimethylsulfoxide and saline (vehicle). Amitriptyline was dissolved in saline. Drugs were administrated intraperitoneally in a volume of 0.5 ml. Duloxetine was purchased from Wako Pure Chemical Industries (Osaka, Japan) and amitriptyline was purchased from LKT Laboratories, Inc. (St. Paul, MN, USA).

The area under the time-course curve (AUC) was calculated from the individual scores at each time point using the trapezoidal rule from 0 to 180 min of the observation period. The antihyperalgesic effects of duloxetine and amitriptyline at 2 and 4 weeks after SNL were compared using dose-response AUCs.

2.4. Microdialysis

To determine the change of NA and 5-HT levels in the spinal cord dorsal horn over time after antidepressant injection, microdialysis studies were performed using normal (i.e., uninjured) rats as previously described [11]. Anesthesia was induced with 3% isoflurane and maintained with 1.5% isoflurane in 100% oxygen through a nose cone. The left femoral vein was cannulated for fluid infusion. The rectal temperature was maintained at 37 °C to 38 °C with a heating pad placed beneath the animal. The L3–L6 level of the right spinal cord was exposed by thoracolumbar laminectomy, and the rat was then placed in a stereotaxic apparatus. The microdialysis probe (outer diameter = 0.22 mm, inner diameter = 0.20 mm, length = 1 mm; A-I-8-01; Eicom Co., Kyoto, Japan) was inserted from just lateral to the dorsal root and advanced to a depth of 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 $CaCl_2$) 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 NA and 5-HT concentrations in the dialysate. Duloxetine (10 mg/kg), amitriptyline (10 mg/kg), vehicle (0.5 ml), or saline (0.5 ml) was administered intraperitoneally through an indwelling catheter and 15-min 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.).

2.5. Analysis of homogenized tissue

We measured the NA and 5-HT contents in the spinal dorsal horn in normal rats and rats at 2 and 4 weeks after SNL as previously described [6]. 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 500 μL of 0.2 M perchloric acid containing 0.1 mM EDTA- Na_2 and isoproterenol (0.02 mg/mL) as an internal standard, and centrifuged at 20,000 g and 0 °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 noradrenaline and 5-HT were analyzed using HPLC 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 EDTA- Na_2, 190 mg/L sodium 1-octanesulfate acid, and 17% methanol, and the column was an EICOMPAK SC-5ODS (3.0 × 150 mm, Eicom Co.).

2.6. Data analysis and statistics

We selected a minimal sample size of six based on our previous study [6]. The data are presented as the mean ± SEM. The effects of the drug treatments on withdrawal thresholds in the behavioral studies and on spinal cord NA and 5-HT levels in the microdialysis studies were analyzed using two-way repeated-measures analyses of variance (ANOVA), followed by Student's *t*-test with Bonferroni correction for dose-response analysis. The change in the NA and 5-HT contents in the spinal cord over time after SNL was evalu-