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Blockade of 5-HT$_7$ receptors reduces tactile allodynia in the rat

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ABSTRACT

This study assessed the role of systemic and spinal 5-HT$_7$ receptors on rats submitted to spinal nerve injury. In addition, the 5-HT$_7$ receptors level in dorsal root ganglion and spinal cord was also determined. Tactile allodynia was induced by L5/L6 spinal nerve ligation. Systemic (0.01–10 mg/kg) or spinal (0.3–30 μg) administration of the selective 5-HT$_7$ receptor antagonist SB-269970 but not vehicle reduced in a dose-dependent manner established tactile allodynia. This effect was maintained for about 6 h. SB-269970 was more potent and effective by the spinal administration route than through systemic injection. Spinal nerve ligation reduced expression of 5-HT$_7$ receptors in the ipsilateral but not contralateral dorsal root ganglia. Moreover, 5-HT$_7$ receptor levels were lower in the ipsilateral dorsal spinal cord of neuropathic rats compared to naïve and sham rats. No changes in the receptor levels were observed in the contralateral dorsal spinal cord and in both regions of the ventral spinal cord. Data suggest that spinal 5-HT$_7$ receptors play a pronociceptive role in neuropathic rats. Results also indicate that spinal nerve injury leads to a reduced 5-HT$_7$ receptors level in pain processing-related areas which may result from its nociceptive role in this model. Data suggest that selective 5-HT$_7$ receptor antagonists may function as analgesics in nerve injury pain states.

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1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) released from descending pain modulation pathways (rostral ventromedial medulla, RVM) to the dorsal horn is crucial to spinal nociception processing (Millan, 2002; Suzuki et al., 2004). 5-HT can exert facilitatory or inhibitory influences onto dorsal horn neurons depending on the spinal 5-HT receptor subtype activated and apparently on the type of pain (Wei et al., 2010). It has been recently suggested that the balance between inhibitory and facilitatory influences of 5-HT shifts toward pronociception after nerve injury *via* enhanced activation of pronociceptive 5-HT receptor subtypes, including the 5-HT$_3$ receptor (Wei et al., 2010). For instance, depletion of endogenous spinal 5-HT reduces mechanical allodynia in several models of nerve injury (Oatway et al., 2004; Rahman et al., 2006; Wei et al., 2010) suggesting that descending 5-HT from the RVM plays an important role in enhanced descending pain facilitation during persistent pain states (tissue and nerve injury) and supporting the conclusion that 5-HT is at least partially required for development and maintenance of persistent pain states. In support of this, tryptophan hydroxylase-2 protein, the rate-limiting enzyme in the synthesis of neuronal 5-HT, in the RVM, is up-regulated after nerve injury and its blockade attenuates behavioral hypersensitivity induced by nerve injury (Wei et al., 2010).

The behavioral nociceptive responses mediated by descending 5-HT projections are dependent on the activation of diverse 5-HT receptor subtypes. All 5-HT receptor subtypes (5-HT$_{1-7}$) are expressed in the spinal dorsal horn (Pierce et al., 1996a, 1996b; Wu et al., 2001; Doly et al., 2004; Liu et al., 2005) and exert a modulatory effect on spinal nociceptive responses. Previous studies have shown that activation of the spinal 5-HT$_{1A/1B}$ (Aira et al., 2010) or 5-HT$_{1B/1D}$ (Kayser et al., 2002) receptors attenuate field potentials evoked by electrical activation of C fibres or pain-related behavior in a rat model of trigeminal neuropathic pain, respectively. Moreover, behavioral studies have reported that activation of spinal 5-HT$_{2A}$ (Pichon et al., 2010), 5-HT$_{2C}$ (Obata et al., 2004; Nakai et al., 2010; Aira et al., 2010) and 5-HT$_3$ (Aira et al., 2010) receptors leads to inhibition of neuropathic pain in rats and mice. Contrariwise, there is evidence that activation of spinal 5-HT$_{2A}$ (Thibault et al., 2008; Van Steenwinckel et al., 2008; Aira et al., 2010), 5-HT$_{2B}$ (Aira et al., 2010) and 5-HT$_3$ (Oatway et al., 2004; Chen et al., 2009) receptors increases pain-related behavior in models of neuropathic pain. The role of 5-HT$_4$, 5-HT$_5$ and 5-HT$_6$ receptors in rats submitted to nerve injury has not been studied. In the case of 5-HT$_7$ receptors, a recent study found that systemic administration of 5-HT$_7$ receptor agonists reduced mechanical hypersensitivity in nerve-injured mice suggesting that 5-HT$_7$ receptors play an antinociceptive role (Brenchat et al., 2010). However, previous evidence from our laboratory (Rocha-González et al., 2005) as well as the transduction mechanism of the 5-HT$_7$ receptors

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(Boess and Martin, 1994; Vanhoenacker et al., 2000) suggests that spinal 5-HT_7 receptors may have a pronociceptive rather than an antinociceptive role. Based on these considerations, we assessed the spinal and systemic administration of the selective 5-HT_7 receptor antagonist SB-269970 in rats submitted to spinal nerve injury. The 5-HT_7 receptors level in dorsal root ganglion and dorsal horn spinal cord in neuropathic rats was also assessed.

2. Materials and methods

2.1. Animals

Female Wistar rats aged 6–7 weeks (weight range, 140–160 g) from our own breeding facilities were used in this study. Female rats were used based on the fact that previous studies from our laboratory have found no differences in tactile allodynia between female and male rats (Caram-Salas et al., 2007). Animals were housed in cages on a standard 12 h/12 h light/dark cycle and had free access to food and drinking water before experiments. All experiments are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985), Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983) and were approved by our local Ethics Committee. In addition, all efforts were done to minimize pain and suffering in the animals and the number of rats used was the minimal required to obtain significant statistical power.

2.2. Spinal nerve ligation-induced neuropathic pain model and measurement of tactile allodynia

Rats were prepared according to the method of Kim and Chung (1992). Briefly, animals were anesthetized with a mixture of ketamine (45 mg/kg, i.p.) and xylazine (12 mg/kg, i.p.). After surgical preparation and exposure of the dorsal vertebral column, the left L5 and L6 spinal nerves were exposed and tightly ligated with 6-0 silk suture distal to the dorsal root ganglion. In the sham group, the surgical procedure was identical to that described above, except that the spinal nerves were not ligated. Rats were allowed to recover from surgery for 14 days before testing pain-related behavior and animals exhibiting motor deficiency (such as paw dragging) were discarded from the study. Tactile allodynia was determined according to a previously reported method (Chaplan et al., 1994). On the 14th day after spinal nerve ligation or sham surgery, each rat was placed in a clear plastic, wire mesh-bottomed cage and allowed to acclimatize for 30–40 min. Von Frey filaments (Stoelting, Wood Dale, IL, USA) were used to measure the 50% paw withdrawal threshold using the up-down method of Dixon (1980). A series of filaments, starting with one that had a buckling weight of 2 g, were applied in consecutive sequence to the plantar surface of the left hind paw with a pressure causing the filament to buckle. Lifting of the paw indicated a positive response and prompted the use of the next weaker filament, whereas absence of paw withdrawal after 5 s indicated a negative response and prompted the use of the next filament of increasing weight. This paradigm continued until four more measurements were made after the initial change of the behavioral response or until five consecutive negative (assigned a score of 15 g) or four consecutive positive (assigned a score of 0.25 g) responses had occurred. The resulting scores were used to calculate the 50% response threshold by using the formula: 50% g threshold $= 10^{(Xf+\kappa\delta)}/10{,}000$, where Xf = value (in log units) of the final von Frey filament used, κ = the value from table published by Dixon (1980) for the pattern of positive and/or negative responses, and δ = the mean difference (in log units) between stimulus strengths. Allodynia was considered to be present when paw withdrawal thresholds were <4 g (Chaplan et al., 1994).

2.3. Spinal surgery

Nine days after the first surgery (spinal nerve ligation), rats were again anesthetized with a ketamine (45 mg/kg, i.p.)/xylazine (12 mg/kg, i.p.) mixture and placed in a stereotaxic head holder in order to expose the atlantooccipital membrane (Yaksh and Rudy, 1976). After piercing the membrane, a PE-10 catheter (7.5 cm) was passed intrathecally to the level of the thoracolumbar junction and the wound was sutured. Rats were allowed to recover from surgery for 5 days in individualized cages before use. Animals showing any signs of motor impairment were discarded from the study and euthanized with a CO_2 chamber.

2.4. Western blot analysis

Fourteen days after surgery, the rats were sacrificed by decapitation. The spinal cord segments L1–S1 as well as ipsilateral and contralateral dorsal root ganglia (L4–L6) were excised, placed on ice-cold isotonic saline solution and cleaned from surrounding tissue. The ventral horns were gently marked unilaterally by a scalpel incision to enable the ipsilateral (injured) and contralateral (uninjured) sides to be identified. Excised tissues were dropped into liquid nitrogen for 1 min and then stored in a freezer. Tissues were homogenized in ice-cold lysis buffer (in mM: 150 NaCl, 50 Tris–HCl, 5 EDTA), pH 7.4 during 30 min at 4 °C. The protease inhibitors PMSF (1 mM), aprotinin (10 g/mL), leupeptin (10 g/mL), pepstatin A (10 g/mL) and 0.1% Triton X-100 (Sigma, St. Louis, MO) were added to the lysis buffer just before usage. After that, they were centrifuged and the supernatant fraction was used to measure protein concentration by Bradford´s method (500-0001, Bio-Rad, Hercules, CA). Protein (120 μg) was resolved by 10% SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to PVDF membranes. Membranes were blocked with 5% non-fat milk in phosphate-buffered saline at pH 7.4 (in mM; 137 NaCl, 2.7 KCl, 10 Na_2HPO_4 and 2 KH_2PO_4) and they were incubated with a rabbit antibody raised against 5-HT_7 receptor (NB100-56352, 1:100; Novus Biologicals, Littleton, CO) or mouse anti-actin (MAB1501R, 1:300; Millipore, Billerica, MA) antibody. Horseradish peroxidase-conjugated secondary antibodies (SC-2350, 1:1000; Santa Cruz Biotechnology Inc, Santa Cruz, CA and 115-035-003, 1:3000; Jackson ImmunoResearch Laboratories Inc, West Grove, PA, respectively) were applied for detecting the primary antibody signal using an enhanced chemiluminescence detection system according to the manufacturer´s instructions (ECL plus, RPN2132, GE Healthcare, Piscataway, NJ).

2.5. Drugs

(2R)-1-[(3-Hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl) ethyl]pyrrolidine hydrochloride (SB-269970) was purchased from Tocris (Ellisvile, MO, USA) and it was dissolved in sterile 0.9% saline solution.

2.6. Study design

Independent groups of sham and neuropathic rats were used for each experimental condition. The effect of SB-269970 was studied after the systemic or spinal administration 14 days after spinal nerve ligation. To ensure that all spinal nerve ligated rats used in the study showed tactile allodynia, we measured the 50% paw withdrawal threshold in response to mechanical stimuli before drug administration. For systemic administration, rats received an i.p. injection of saline or increasing doses of the selective 5-HT_7 receptor antagonist SB-269970 (0.01–10 mg/kg). For spinal drug administration, rats received an intrathecal (i.t.) injection of vehicle (sterile 0.9% saline, 10 μl) or increasing doses of SB-269970 (0.03–30 μg/rat, 10 μl). The

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