Pharmacology, Biochemistry and Behavior 99 (2011) 591–597

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

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

Evelyn Amaya-Castellanos [a], Jorge B. Pineda-Farias [a], Gabriela Castañeda-Corral [a], Guadalupe C. Vidal-Cantú [a], Janet Murbartián [a], Héctor I. Rocha-González [b], Vinicio Granados-Soto [a,*]

[a] Departamento de Farmacobiología, Centro de Investigación y de Estudios Avanzados (Cinvestav), Sede Sur, México, D.F., Mexico
[b] Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina, Instituto Politécnico Nacional, México, D.F., Mexico

ARTICLE INFO

Article history:
Received 28 March 2011
Received in revised form 27 May 2011
Accepted 7 June 2011
Available online 15 June 2011

Keywords:
Serotonin
5-HT$_7$ receptors
SB-269970
Neuropathic pain
Spinal processing

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.

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

* Corresponding author at: Departamento de Farmacobiología, Cinvestav, Sede Sur, Calzada de los Tenorios 235, Colonia Granjas Coapa, 14330 México, D.F., Mexico. Tel.: +52 55 5483 2868; fax: +52 55 5483 2863.
E-mail address: vgranados@prodigy.net.mx (V. Granados-Soto).

0091-3057/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2011.06.005

(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

antiallodynic effect was evaluated for the following 8 h in both conditions.

The scheduling of doses and drug administration for systemic and spinal administration was based on a previously reported study (Rocha-González et al., 2005) and on pilot experiments in the current model. Rats in all groups were observed regarding behavioral or motor function changes induced by the treatments. This was assessed, but not quantified, by testing the animals' ability to stand and walk in a normal posture, as proposed elsewhere (Chen and Pan, 2001).

To determine whether spinal nerve ligation modifies the 5-HT_7 receptors level at dorsal root ganglia and dorsal horn spinal cord, naïve, sham and spinal nerve ligated rats were sacrificed 14 days after L5–L6 spinal nerve ligation and the spinal cord and dorsal root ganglia were excised to carry out the western blot analysis.

2.7. Data analysis and statistics

Behavioral results are given as the mean ± S.E.M. for at least six animals per group. Curves were constructed plotting the threshold for paw withdrawal as a function of time. An increase of 50% withdrawal threshold was considered as antiallodynic effect. Area under the 50% withdrawal threshold-time curve (AUC) was calculated by the trapezoidal method. Percentage of maximum possible effect (%MPE) was calculated with the following equation.

$$\%MPE = ([AUCdrug–AUCsaline] / [AUCsham–AUCsaline]) \times 100$$

Then, the log dose–response curves were constructed for each administration route tested and the experimental points were fitted to a linear function in Sigma plot 11.0 (Systat Software, Inc, Chicago, IL). Effective dose to produce 50% of the maximum possible antiallodynic effect (ED_{50}) and 95% confidence intervals were calculated as is described by Tallarida (2000). For the molecular study, the bands were quantified by scanning densitometry using Labworks 4.5 software (Lablogics, Inc, Mission Viejo, CA) for image acquisition and analysis.

One-way analysis of variance (ANOVA), followed by the Tukey's test, was used to compare differences between treatments in behavioral and molecular experiments. Differences were considered statistically significant when $P<0.05$.

3. Results

3.1. Time course of the antiallodynic effect of SB-269970

After spinal nerve ligation, rats receiving the vehicle showed a clear-cut tactile allodynia in the ipsilateral hind paw, which was evident by a significant decrease in the 50% withdrawal paw threshold response as compared to the sham group (Fig. 1). As expected, sham surgery did not change the 50% withdrawal threshold response on the ipsilateral side. Fig. 1 shows the antiallodynic effects of SB-269970 by systemic and spinal routes of administration. Intraperitoneal administration of SB-269970 (10 mg/kg) produced a significant increase in the 50% withdrawal response in the ipsilateral hind paw. The maximal antiallodynic effect was reached in about 3 h, declining gradually to basal values in approximately 8 h (Fig. 1A). In contrast, intrathecal injection of SB-269970 (30 μg, i.t.) reached a maximum antiallodynic effect in 2 h. This effect was maintained during the next 5 h and then declined slightly at 8 h (Fig. 1B). Twenty-four hours later the antiallodynic effect produced by the spinal injection of SB-269970 had disappeared (data not shown).

3.2. Systemic and spinal effect of SB-269970

Intraperitoneal injection of SB-269970 (0.01–10 mg/kg) significantly ($P<0.05$) reduced tactile allodynia induced by ligation of L5/L6 spinal nerves in a dose-dependent manner. The maximal antiallodynic effect was reached with 1 mg/kg of the 5-HT_7 receptor antagonist and greater doses did not produce a greater antiallodynic effect (Fig. 2A). The maximal effect observed (56.1 ± 8.3%, ED_{50} value 0.45 ± 0.2 mg/kg) with the systemic administration of SB-269970 was reached at about 3 h. Likewise, intrathecal administration of SB-269970 (0.03–30 μg) dose-dependently reduced mechanical allodynia ($P<0.05$) induced by ligation of L5/L6 spinal nerves (Fig. 2B). At spinal level, the 5-HT_7 receptor antagonist had an $ED_{50} = 1.31 \pm 0.37$ μg and a maximal effect of 74.2 ± 1.4% at the greatest dose tested. SB-269970 was more potent and effective by the spinal administration route than through systemic injection (Table 1). No side effects were detected at the doses reported (data not shown).

3.3. Western blot

Based on the results obtained with SB-269970 at 14 days, it was considered of interest to determine the 5-HT_7 receptors level in the dorsal root ganglia and the spinal cord of neuropathic, sham and naïve rats. Western blot analysis, using an antibody previously validated (Mahé et al., 2005), revealed a band of about 50 kDa corresponding to the molecular weight expected for the 5-HT_7 protein in both tissues (Fig. 3). Neuropathic rats showed lower amounts of the 5-HT_7 receptor protein in the ipsilateral ($P<0.05$), but not contralateral, dorsal root ganglion (Fig. 3). Accordingly, the level of 5-HT_7 receptors was lower in the ipsilateral dorsal spinal cord region of the neuropathic rats compared to naïve and sham rats (Fig. 4A). The amount of 5-HT_7 receptor protein in the contralateral dorsal spinal

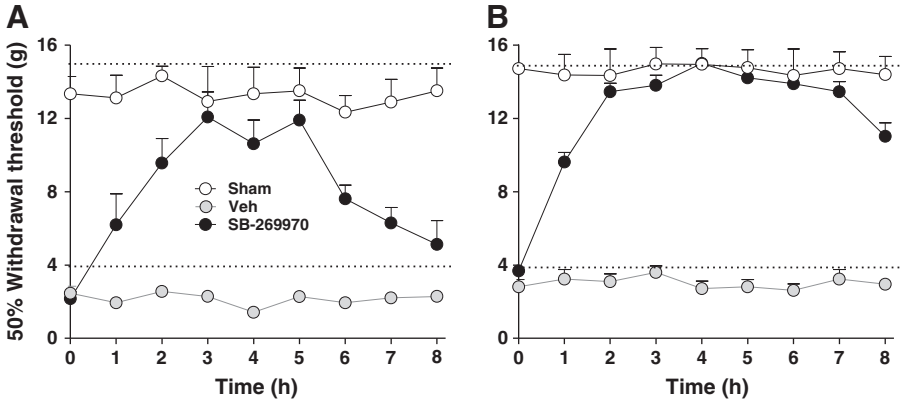

Fig. 1. Time course of the antiallodynic effect of SB-269970 observed after systemic (A, 10 mg/kg, i.p.) or spinal (B, 30 μg, i.t.) administration in rats submitted to L5/L6 spinal nerve ligation. Withdrawal threshold was assessed 14 days after surgery. In both cases, data are presented as mean for at least 6 animals ± S.E.M. Abbreviations: Vehicle (Veh), intrathecal (i.t.) and intraperitoneal (i.p.) administration.

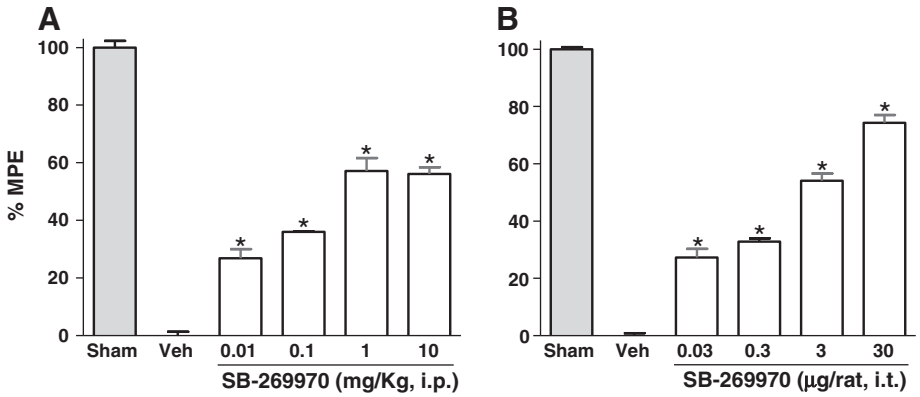

Fig. 2. Antiallodynic effect of systemic (A) or spinal (B) administration of SB-269970 in rats submitted to L5/L6 spinal nerve ligation. Rats were treated with saline or increasing doses of SB-269970 before starting threshold evaluations. Data are expressed as the percent of maximum possible effect (%MPE). Bars are the mean ± S.E.M. for at least 6 animals. Note that SB-269970 increased the %MPE in neuropathic rats. *Significantly different from the saline group as determined by one-way ANOVA, followed by the Tukey's test. Abbreviations: Vehicle (Veh), intrathecal (i.t.) and intraperitoneal (i.p.) administration.

cord (Fig. 4B) as well as in both regions of the ventral spinal cord (Fig. 4C–D) was similar in neuropathic, sham and naïve rats.

4. Discussion

In this study we assessed the antiallodynic effect of SB-269970 in rats submitted to L5/L6 spinal nerve ligation. Systemic administration of the 5-HT_7 receptor antagonist SB-269970 significantly reduced spinal nerve injury-induced tactile allodynia in rats. In addition, the antiallodynic effect of SB-269970 was observed after intrathecal administration. The spinal effect was considerably greater and more sustained than that induced by systemic administration. Since SB-269970 is a selective 5-HT_7 receptor antagonist (Hagan et al., 2000; Lovell et al., 2000; Thomas et al., 2000), our data suggest that 5-HT_7 receptors play a pronociceptive role in neuropathic pain. Our results agree with recent observations showing that SB-269970 is able to reduce long-term secondary hyperalgesia and allodynia induced by formalin (Godínez-Chaparro et al., 2011). In the present study, using the same 5-HT_7 receptor antagonist, we show that blockade of 5-HT_7 receptors reduce tactile allodynia in rats with nerve injury. In marked contrast, a previous study has shown that spinal administration of the 5-HT_7 receptor antagonist SB-269970 (10 µg) does not produce antinociception (Dogrul et al., 2009) in spinal nerve ligated rats. Differences could be due to gender (male *versus* female) or strain (Sprague–Dawley *versus* Wistar). In addition, it has been reported that 5-HT_7 receptor agonists (AS-19 and E-57431) reduce while selective 5-HT_7 receptor antagonists (SB-269970 and SB-258719) increase mechanical and thermal hypersensitivity (Brenchat et al., 2010). In this case, differences could be due to the model (L5/L6 spinal nerve injury *versus* partial sciatic nerve ligation), species (rats versus mice) and primary administration route (systemic and spinal *versus* systemic). However, more studies will be needed in order to clarify the role of 5-HT_7 receptors in neuropathic pain processing. In support of our data, there is evidence that 5-HT_7 receptors are predominantly expressed on presynaptic peptidergic C and Aδ fibers in the rat (Pierce et al., 1996a, 1996b; Wu et al., 2001; Meuser et al., 2002; Doly et al., 2005; Rocha-González et al., 2005) and localized on lamina I and II, the region of input from primary afferent nociceptors (Doly et al., 2005). Moreover, activation of 5-HT_7 receptors leads to an increase of cyclic AMP (Boess and Martin, 1994; Vanhoenacker et al., 2000) and the neuronal marker c-fos (Meuser et al., 2002) as well as depolarization and neuronal excitability of small and medium size cells (Cardenas et al., 1999). 5-HT_7 receptors are also found in local peptidergic interneurons (Doly et al., 2005), astrocytes (Hirst et al., 1998) and microglia (Mahé et al., 2005). On these cells, 5-HT may have a pronociceptive effect by enhancing the release of substance P, CGRP, interleukin-6 or potentiating TRPV1 actions *via* 5-HT_7 receptor activation (Lieb et al., 2005; Doly et al., 2005; Ohta et al., 2006; Wang et al., 2010). Taken together, electrophysiological, immunohistochemical, molecular biology, and behavioral data suggest a pronociceptive role for 5-HT_7 receptors in the spinal cord.

The role of 5-HT_7 receptors in pain processing is complex as activation of these receptors may inhibit and/or facilitate nociceptive transmission depending on the kind of pain and administration site. It seems that peripheral activation of 5-HT_7 receptors leads to nociception in models of tissue injury (Meuser et al., 2002; Rocha-González et al., 2005). Likewise, our group has reported that activation of spinal 5-HT_7 receptors increases formalin-induced nociception and that effect is blocked by the selective 5-HT_7 receptor antagonist SB-269970 but not the selective 5-HT_{1A} receptor antagonist WAY-100635 (Rocha-González et al., 2005). Thus, data suggest that peripheral and spinal activation of 5-HT_7 receptors leads to an increase in formalin-induced nociception. However, recent studies reported that systemic tramadol- or morphine-induced antinociception in the tail-flick test is blocked by spinal SB-269970 suggesting that systemic administration of tramadol and morphine produces antinociception by activation of spinal 5-HT_7 receptors (Dogrul and Seyrek, 2006; Dogrul et al., 2009; Yanarates et al., 2010). Notwithstanding, it is unlikely that activation of 5-HT_7 receptors could directly inhibit primary afferents or nociceptive dorsal horn neurons as these receptors are positively coupled to adenylyl cyclase and their stimulation is excitatory (see above). Moreover, there is evidence that 5-HT depletion in the RVM has no effect on descending pain inhibition induced by intra-RVM injection of [D-Ala^2, NMe-Phe^4, Gly-ol^5]-enkephalin (DAMGO) or morphine (Wei et al., 2010) as previously suggested (Gao et al., 1998; Gao and Mason, 2000). The complexity in the effects of 5-HT_7 receptors may arise when systemically administered morphine or 5-HT_7 agonists access multiple sites within the pain transmission system (periphery, spinally, and supraspinally). In order to avoid this, more studies using a local site of administration (at the periphery, spinal cord, RVM or thalamus) are warranted.

Table 1
Dose necessary to produce 50% of the maximum possible effect (ED_{50}) and maximal antiallodynic effect obtained from systemic and spinal administration of the 5-HT_7 receptor antagonist SB-269970 in rats submitted to spinal nerve ligation.

Administration route	Efficacy	ED_{50}	r^2
Systemic (i.p.)	56.1 ± 8.3%	0.45 ± 0.2 mg/kg 95% CI (0.21–1.57)	0.95
Spinal (i.t.)	74.2 ± 1.4%	1.31 ± 0.37 µg 95% CI (0.7–2.4)	0.97

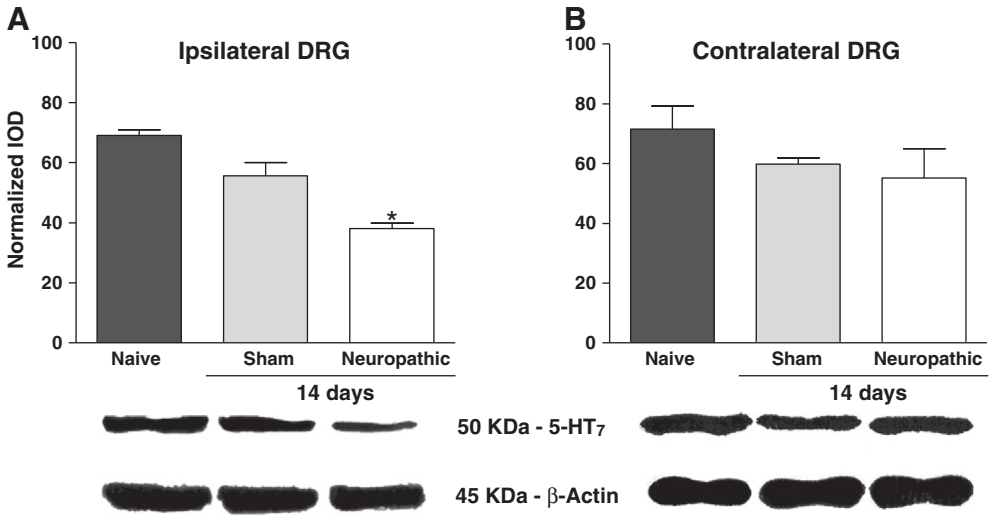

Fig. 3. Western blot analysis of dorsal root ganglia (L4–L6) obtained from ipsilateral (A) and contralateral (B) sides of naïve, sham and neuropathic rats. Data were normalized against β-actin and are expressed as the mean ± S.E.M of two independent experiments which is a tissue mix of three rats. Note that the expression of 5-HT_7 receptors was reduced in neuropathic rats. *Significantly different from the naïve or sham group ($P<0.05$), as determined by one-way ANOVA, followed by the Tukey´s test. Insets in A and B show a representative blot obtained with 5-HT_7 and β-actin primary antibodies, which revealed bands around of 50- and 43-kDa, respectively. Abbreviations: Integrated optical density (IOD).

The level of 5-HT_7 receptors was observed in naïve and sham operated rats. The levels were similar in ipsilateral and contralateral dorsal root ganglia as well as in dorsal and ventral sections of the spinal cord. Our results agree with previous observations showing that 5-HT_7 mRNA is present in DRG (Cardenas et al., 1999; Liu et al., 2005; Ohta et al., 2006) although these results have been disputed (Chen et al., 1998; Nicholson et al., 2003). Besides inducing tactile allodynia, spinal nerve injury significantly reduced levels of the 5-HT_7

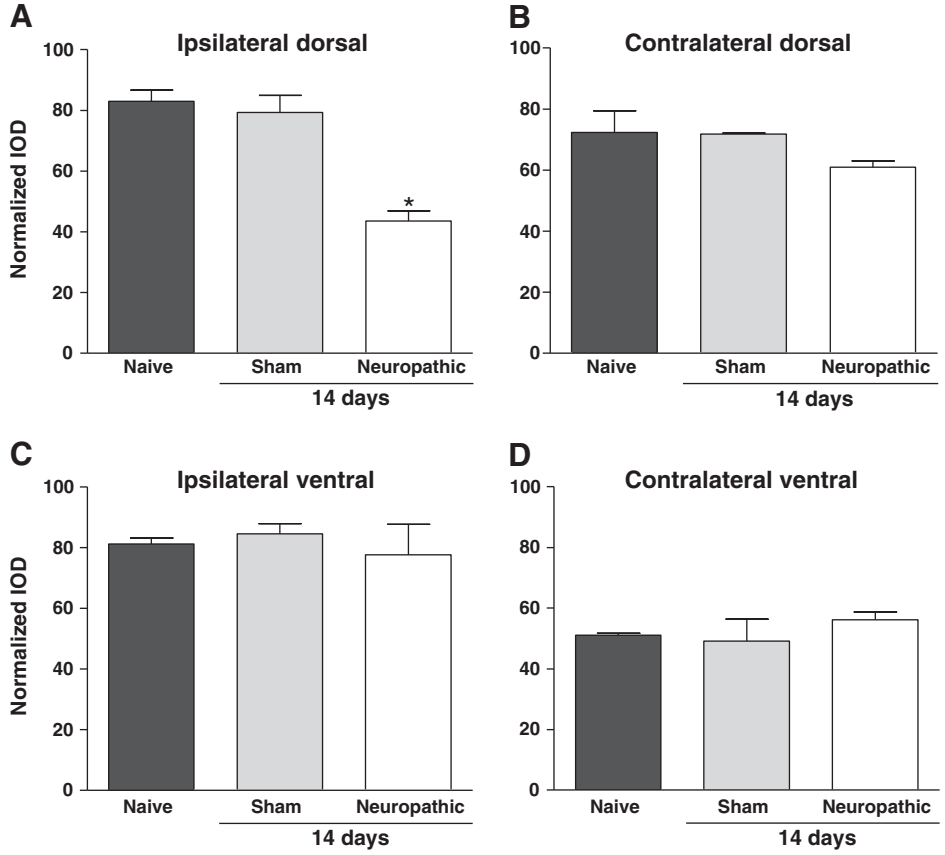

Fig. 4. Western blot analysis of ipsilateral (A) and contralateral (B) dorsal spinal cord and ipsilateral (C) and contralateral (D) ventral spinal cord of naïve, sham and neuropathic rats. Data were normalized against β-actin and are expressed as the mean ± S.E.M of two independent experiments which is a tissue mix of three rats. Note that expression of 5-HT_7 receptors was reduced in ipsilateral, but not contralateral, dorsal spinal cord of neuropathic rats. In addition, no changes were observed in ventral spinal cord either ipsilateral or contralateral. *Significantly different from the naïve or sham group ($P<0.05$), as determined by one-way ANOVA, followed by the Tukey's test. 5-HT_7 primary antibody showed a band of around 50-kDa. Abbreviations: Integrated optical density (IOD).

receptor protein in dorsal root ganglia and dorsal horn spinal cord 14 days after nerve injury. This reduction was observed in the ipsilateral but not contralateral side. Moreover, the reduction was only observed in the dorsal but not ventral sections of the spinal cord suggesting that 5-HT_7 receptors have a significant contribution in the nociceptive sensory process. Our results are in sharp contrast to a recent immunohistochemistry study which found an increased level of 5-HT_7 receptors in the ipsilateral dorsal horn spinal cord of nerve-injured mice (Brenchat et al., 2010). Other studies have found that tissue injury increases the levels of 5-HT_7 mRNA at 4 h and 4 days after bee venom and complete Freund's adjuvant injection, respectively (Wu et al., 2001; Liu et al., 2005; Ohta et al., 2006). Differences could be due to the different models and species used but the ultimate reasons for this discrepancy remain to be examined.

The behavioral nociceptive responses mediated by descending 5-HT are dependent on the activation of diverse spinal 5-HT subtype receptors. 5-HT_7 receptors are expressed in the spinal dorsal horn (Cardenas et al., 1999; Doly et al., 2005; Ohta et al., 2006; this study) and exert a modulatory effect on spinal nociceptive responses. It has been recently proposed that, after tissue and nerve injury, the balance between descending 5-HT inhibitory and facilitatory influences shifts toward facilitation *via* enhanced activation of pronociceptive 5-HT_3 (Dogrul et al., 2009; Wei et al., 2010) and probably 5-HT_7 receptors (this study). However, subtypes of 5-HT receptors and the complex anatomy of the spinal dorsal horn complicate interpretation of the role of 5-HT in pain modulation.

In summary, our results indicate that systemic and spinal administration of the selective 5-HT_7 receptor antagonist SB-269970 reduce tactile allodynia induced by L5/L6 spinal nerve ligation. Thus, our data suggest that 5-HT_7 receptors have a pronociceptive role in this kind of pain. In addition, results suggest that spinal nerve ligation leads to a reduction in the level of 5-HT_7 receptors. Finally, the data suggest that 5-HT_7 receptor antagonists could have a potential as analgesics in neuropathic pain.

Acknowledgments

Authors greatly appreciate the bibliographic assistance of B.Sc. Héctor Vázquez. This work is part of the M.Sc. thesis of Evelyn Amaya-Castellanos. Evelyn Amaya-Castellanos, Jorge B. Pineda-Farias and Gabriela Castañeda-Corral are Conacyt fellows. This study was partially supported by Conacyt grants 59879 (VGS) and 49084 (JM).

References

Aira Z, Buesa I, Salgueiro M, Bilbao J, Aguilera L, Zimmermann M, Azkue JJ. Subtype-specific changes in 5-HT receptor-mediated modulation of C fibre-evoked spinal field potentials are triggered by peripheral nerve injury. Neuroscience 2010;168:831–41.

Boess FG, Martin IL. Molecular biology of 5-HT receptors. Neuropharmacology 1994;33: 275–317.

Brenchat A, Nadal X, Romero L, Ovalle S, Muro A, Sánchez-Arroyos R, Portillo-Salido E, Pujol M, Montero A, Codony X, Burgueño J, Zamanillo D, Hamon M, Maldonado R, Vela JM. Pharmacological activation of 5-HT_7 receptors reduces nerve injury-induced mechanical and thermal hypersensitivity. Pain 2010;149:483–94.

Caram-Salas NL, Reyes-García G, Bartoszyk GD, Araiza-Saldaña CI, Ambriz-Tututi M, Rocha-González HI, Arreola-Espino R, Cruz SL, Granados-Soto V. Subcutaneous, intrathecal and periaqueductal grey administration of asimadoline and ICI-204448 reduces tactile allodynia in the rat. Eur J Pharmacol 2007;573:75–83.

Cardenas CG, Del Mar LP, Vysokanov AV, Arnold PB, Cardenas LM, Surmeier DJ, Scroggs RS. Serotonergic modulation of hyperpolarization-activated current in acutely isolated rat dorsal root ganglion neurons. J Physiol 1999;518:507–23.

Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994;53:55–63.

Chen SR, Pan HL. Spinal endogenous acetylcholine contributes to the analgesic effect of systemic morphine in rats. Anesthesiology 2001;95:525–30.

Chen Y, Oatway MA, Weaver LC. Blockade of the 5-HT_3 receptor for days causes sustained relief from mechanical allodynia following spinal cord injury. J Neurosci Res 2009;87:418–24.

Chen JJ, Vasko MR, Wu X, Staeva TP, Baez M, Zgombick JM, Nelson DL. Multiple subtypes of serotonin receptors are expressed in rat sensory neurons in culture. J Pharmacol Exp Ther 1998;287:1119–27.

Dixon WJ. Efficient analysis of experimental observations. Annu Rev Pharmacol Toxicol 1980;20:441–62.

Dogrul A, Seyrek M. Systemic morphine produce antinociception mediated by spinal 5-HT_7, but not 5-HT_{1A} and 5-HT_2 receptors in the spinal cord. Br J Pharmacol 2006;149:498–505.

Dogrul A, Ossipov MH, Porreca F. Differential mediation of descending pain facilitation and inhibition by spinal 5HT_3 and 5HT_7 receptors. Brain Res 2009;1280:52–9.

Doly S, Fischer J, Brisorgueil MJ, Vergé D, Conrath M. 5-HT_{5A} receptor localization in the rat spinal cord suggests a role in nociception and control of pelvic floor musculature. J Comp Neurol 2004;476:316–29.

Doly S, Fischer J, Brisorgueil MJ, Vergé D, Conrath M. Pre- and postsynaptic localization of the 5-HT_7 receptor in rat dorsal spinal cord: immunocytochemical evidence. J Comp Neurol 2005;490:256–69.

Gao K, Mason P. Serotonergic raphe magnus cells that respond to noxious tail heat are not ON or OFF cells. J Neurophysiol 2000;84:1719–25.

Gao K, Chen DO, Genzen JR, Mason P. Activation of serotonergic neurons in the raphe magnus is not necessary for morphine analgesia. J Neurosci 1998;18:1860–8.

Godínez-Chaparro B, Barragán-Iglesias P, Castañeda-Corral G, Rocha-González HI, Granados-Soto V. Role of peripheral 5-HT_4, 5-HT_6, and 5-HT_7 receptors in development and maintenance of secondary mechanical allodynia and hyperalgesia. Pain 2011;152:687–97.

Hagan JJ, Price GW, Jeffrey P, Deeks NJ, Stean T, Piper D, Smith MI, Upton N, Medhurst AD, Middlemiss DN, Riley GJ, Lovell PJ, Bromidge SM, Thomas DR. Characterization of SB-269970-A, a selective 5-HT_7 receptor antagonist. Br J Pharmacol 2000;130: 539–48.

Hirst WD, Cheung NY, Rattray M, Price GW, Wilkin GP. Cultured astrocytes express messenger RNA for multiple serotonin receptor subtypes, without functional coupling of 5-HT_1 receptor subtypes to adenylyl cyclase. Brain Res Mol Brain Res 1998;61:90–9.

Kayser V, Aubel B, Hamon M, Bourgoin S. The antimigraine 5-$HT_{1B/1D}$ receptor agonists, sumatriptan, zolmitriptan and dihydroergotamine, attenuate pain-related behaviour in a rat model of trigeminal neuropathic pain. Br J Pharmacol 2002;137: 1287–97.

Kim SH, Chung JM. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 1992;50:355–63.

Lieb K, Biersack L, Waschbisch A, Orlikowski S, Akundi RS, Candelario-Jalil E, Hüll M, Fiebich BL. Serotonin via 5-HT_7 receptors activates p38 mitogen-activated protein kinase and protein kinase C epsilon resulting in interleukin-6 synthesis in human U373 MG astrocytoma cells. J Neurochem 2005;93:549–59.

Liu XY, Wu SX, Wang YY, Wang W, Zhou L, Li YQ. Changes of 5-HT receptor subtype mRNAs in rat dorsal root ganglion by bee venom-induced inflammatory pain. Neurosci Lett 2005;375:42–6.

Lovell PJ, Bromidge SM, Dabbs S, Duckworth DM, Forbes IT, Jennings AJ, King FD, Middlemiss DN, Rahman SK, Saunders DV, Collin LL, Hagan JJ, Riley GJ, Thomas DR. A novel, potent, and selective 5-HT_7 antagonist: (R)-3-(2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulfonyl) phenol (SB-269970). J Med Chem 2000;43: 342–5.

Mahé C, Loetscher E, Dev KK, Bobirnac I, Otten U, Schoeffter P. Serotonin 5-HT_7 receptors coupled to induction of interleukin-6 in human microglial MC-3 cells. Neuropharmacology 2005;49:40–7.

Meuser T, Pietruck C, Gabriel A, Xie G-X, Lim K-J, Palmer PP. 5-HT_7 receptors are involved in mediating 5-HT-induced activation of rat primary afferent neurons. Life Sci 2002;71:2279–89.

Millan MJ. Descending control of pain. Prog Neurobiol 2002;66:355–474.

Nakai K, Nakae A, Oba S, Mashimo T, Ueda K. 5-HT_{2C} receptor agonists attenuate pain-related behaviour in a rat model of trigeminal neuropathic pain. Eur J Pain 2010;14: 999–1006.

Nicholson R, Small J, Dixon AK, Spanswick D, Lee K. Serotonin receptor mRNA expression in rat dorsal root ganglion neurons. Neurosci Lett 2003;337:119–22.

Oatway MA, Chen Y, Weaver LC. The 5-HT_3 receptor facilitates at-level mechanical allodynia following spinal cord injury. Pain 2004;110:259–68.

Obata H, Saito S, Sakurazawa S, Sasaki M, Usui T, Goto F. Antiallodynic effects of intrathecally administered 5-HT_{2C} receptor agonists in rats with nerve injury. Pain 2004;108:163–9.

Ohta T, Ikemi Y, Murakami M, Imagawa T, Otsuguro K, Ito S. Potentiation of transient receptor potential V1 functions by the activation of metabotropic 5-HT receptors in rat primary sensory neurons. J Physiol 2006;576:809–22. 809.

Pichon X, Wattiez AS, Becamel C, Ehrlich I, Bockaert J, Eschalier A, Marin P, Courteix C. Disrupting 5-HT_{2A} receptor/PDZ protein interactions reduces hyperalgesia and enhances SSRI efficacy in neuropathic pain. Mol Ther 2010;18:1462–70.

Pierce PA, Xie G-X, Peroutka SJ, Levine JD. Dual effect of the serotonin agonist, sumatriptan, on peripheral neurogenic inflammation. Reg Anesth 1996a;21: 219–25.

Pierce PA, Xie X-G, Levine JD, Peroutka J. 5-hydroxytryptamine receptor subtype messenger RNAs in rat peripheral sensory and sympathetic ganglia: a polymerase chain reaction study. Neuroscience 1996b;70:553–9.

Rahman W, Suzuki R, Webber M, Hunt SP, Dickenson AH. Depletion of endogenous spinal 5-HT attenuates the behavioural hypersensitivity to mechanical and cooling stimuli induced by spinal nerve ligation. Pain 2006;123:264–74.

Rocha-González HI, Meneses A, Carlton SM, Granados-Soto V. Pronociceptive role of peripheral and spinal 5-HT_7 receptors in the formalin test. Pain 2005;117:182–92.

Suzuki R, Rygh LJ, Dickenson AH. Bad news from the brain: descending 5-HT pathways that control spinal pain processing. Trends Pharmacol Sci 2004;25:613–7.

Tallarida RJ. Drug synergism and dose-effect data analysis. 1st Ed. New York: Chapman & Hall/CRC; 2000. p. 1–72.

Thibault K, Van Steenwinckel J, Brisorgueil MJ, Fischer J, Hamon M, Calvino B, Conrath M. Serotonin 5-HT_{2A} receptor involvement and Fos expression at the spinal level in vincristine-induced neuropathy in the rat. Pain 2008;140:305–22.

Thomas DR, Atkinson PJ, Ho M, Bromidge SM, Lovell PJ, Villani AJ, Hagan JJ, Middlemiss DN, Price GW. [^{3}H]-SB-269970-A selective antagonist radioligand for 5-HT$_7$ receptors. Br J Pharmacol 2000;130:409–17.

Van Steenwinckel J, Brisorgueil MJ, Fischer J, Vergé D, Gingrich JA, Bourgoin S, Hamon M, Bernard R, Conrath M. Role of spinal serotonin 5-HT$_{2A}$ receptor in 2′,3′-dideoxycytidine-induced neuropathic pain in the rat and the mouse. Pain 2008;137:66–80.

Vanhoenacker P, Haegeman G, Leysen JE. 5-HT$_7$ receptors: current knowledge and future prospects. Trends Pharmacol Sci 2000;21:70–7.

Wang X, Fang Y, Liang J, Yin Z, Miao J, Luo N. Selective inhibition of 5-HT$_7$ receptor reduces CGRP release in an experimental model for migraine. Headache 2010;50:579–87.

Wei F, Dubner R, Zou S, Ren K, Bai G, Wei D, Guo W. Molecular depletion of descending serotonin unmasks its novel facilitatory role in the development of persistent pain. J Neurosci 2010;30:8624–36.

Wu S-X, Zhu M, Wang W, Wang Y-Y, Li Y-Q, Yew DT. Changes of the expression of 5-HT receptor subtype mRNAs in rat dorsal root ganglion by complete Freund´s adjuvant-induced inflammation. Neurosci Lett 2001;307:183–6.

Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976;17:1031–6.

Yanarates O, Dogrul A, Yildirim V, Sahin A, Sizlan A, Seyrek M, Akgül O, Kozak O, Kurt E, Aypar U. Spinal 5-HT$_7$ receptors play an important role in the antinociceptive and antihyperalgesic effects of tramadol and its metabolite, O-Desmethyltramadol, via activation of descending serotonergic pathways. Anesthesiology 2010;112:696–710.

Zimmermann M. Ethical guidelines for investigations on experimental pain in conscious animals. Pain 1983;16:109–10.