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The role of spinal serotonin receptor and alpha adrenoceptor on the antiallodynic effects induced by intrathecal milnacipran in chronic constriction injury rats

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ABSTRACT

Milnacipran, a reuptake inhibitor of noradrenaline (NA) and serotonin (5-HT), elicits an antiallodynic effect in rats with neuropathic pain; however, the role of NA and 5-HT receptors in the induction of the antiallodynic effect of milnacipran remains unclear. Thus, we examined the effects of prazosin as an α_1 adrenoceptor antagonist, yohimbine as an α_2 adrenoceptor antagonist, metergoline as a 5-HT_1, 5-HT_2 and 5-HT_7 receptor antagonist, cyanopindolol as a 5-$HT_{1A/1B}$ receptor antagonist, ketanserin as a 5-HT_2 receptor antagonist, and ondansetoron as a 5-HT_3 receptor antagonist on the antiallodynic effect of milnacipran in neuropathic rats with chronic constriction injury (CCI). The CCI rats expressed mechanical and thermal allodynia, which was attenuated by intrathecal injection of milnacipran. Yohimbine, but not prazosin, reversed the milnacipran-induced antiallodynic effect. The antiallodynic effect of milnacipran was also reversed by metergoline, ketanserin and ondansetron, while cyanopindolol reversed the antiallodynic effect on mechanical, but not thermal stimulation. Furthermore, c-Fos expression in lamina I/II of the spinal dorsal horn was enhanced by thermal stimulation and the enhanced expression of c-Fos was suppressed by milnacipran. This effect of milnacipran was reversed by yohimbine, metergoline, katanserin and ondansetron, but not prazosin. These results indicate that the effect of milnacipran on mechanical and thermal allodynia and c-Fos expression is elicited through the α_2 adrenoceptor, but not α_1 adrenoceptor, and 5-HT_2 and 5-HT_3 receptors; furthermore, the 5-$HT_{1A/1B}$ receptor is involved in mechanical allodynia, but not thermal allodynia.

1. Introduction

Antidepressants, especially tricyclic antidepressants (TCAs), have been widely used as the first-line drugs for the treatment of neuropathic pain (Micó et al., 2006). The mechanisms underlying the antinociceptive effects of TCAs are associated with various substrates (Sawynok et al., 2001; Sindrup et al., 2005; Attal et al., 2006; Micó et al., 2006). Recently, newer antidepressants, such as a serotonin and noradrenaline reuptake inhibitor (SNRI), have been introduced in the clinical management of persistent pain (Dworkin et al., 2007; Marks et al., 2009; Jefferies, 2010).

The spinal cord is a crucial site of antidepressant-mediated antinociception (Hwang and Wilcox, 1987). Therefore, an increase of NA and 5-HT following administration of SNRI inhibits pain transmission at the spinal cord (Fürst, 1999; Millan, 2002; Ren and Dubner, 2002; Pertovaara, 2006; Yoshimura and Furue, 2006). Milnacipran, an SNRI, elicits an antinociceptive or antiallodynic effect in rodent models with neuropathic pain following intrathecal administration (Obata et al., 2005; King et al., 2006; Suzuki et al., 2008; Ikeda et al., 2009; Takeda et al., 2009) or systemic administration (Yokogawa et al., 2002; Önal et al., 2007; Berrocoso et al., 2011). In addition, the effect of milnacipran on patients with fibromyalgia as well as depression has been clinically evaluated (Clauw et al., 2008; Mease et al., 2009; Branco et al., 2010; Matthey et al., 2013). The antiallodynic effects of SNRIs have been mainly evaluated by examining the change of the threshold of the withdrawal response to mechanical stimulation. On the other hand, pain perception in response to innocuous thermal stimuli (thermal allodynia) has been also reported in patients with spontaneously firing (Woolf and Mannion, 1999); however, there is no available information on the effect of SNRIs on thermal allodynia.

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Furthermore, only a few reports are available on changes in the expression of c-Fos, a marker of neuronal activation, in the spinal cord of rats with neuropathic pain following thermal stimulation (Catheline et al., 1999; Dai et al., 2001).

Intrathecal administration of SNRIs produces an increase of NA and 5-HT in the spinal cord. Thus, it is reasonable that adrenoceptors and 5-HT receptors are involved in the antiallodynic and antinociceptive effects of SNRIs in rodent models with neuropathic pain; however, the involvement of subtypes of these receptors in the antiallodynic effect of SNRIs has been poorly understood. A prior study suggested the involvement of α_1 adrenoreceptors and 5-HT_2 receptors in antidepressant-induced antinociception based on significant antinociceptive effects of simultaneous intraperitoneal administration of antidepressants and either α_1 antagonist or 5-HT_2 antagonist in naive rats (Yokogawa et al., 2002). In addition, the antiallodynic effect of milnacipran was reversed by intrathecal administration of a α_2 adrenoreceptor antagonist or 5-HT receptor antagonists (Obata et al., 2005; Nakajima et al., 2012). The reversed effects by these antagonists on mechanical allodynia have been evaluated by von Frey filament in neuropathic rats with chronic constriction injury (CCI). Thus, there are no available data on the effect of adrenoceptors and 5-HT receptor antagonists on thermal allodynia in CCI rats.

Therefore, we examined whether intrathecal administration of milnacipran has an antiallodynic effect on thermal stimulation as on mechanical stimulation in CCI model rats used in this study. Furthermore, the involvement of subtypes of 5-HT receptors and adrenoceptors in anti-thermal allodynia as well as anti-mechanical allodynia by milnacipran in CCI rats was evaluated using intrathecal administration of receptor antagonists.

2. Materials and methods

2.1. Animals

All protocols used in this study were approved by the Institutional Animal Care and Use Committee of the University of Miyazaki and followed the guidelines for the treatment of animals of the International Association for the Study of Pain (Zimmermann, 1983). The experiments were performed on adult male Sprague-Dawley rats (Charles River Laboratories Japan, Yokohama, Japan) weighing 250–300 g on the day of catheterization. The rats were housed 2 or 3 animals per cage with free access to food and water under a 12/12 h light/dark cycle at 25 °C.

2.2. Intrathecal catheterization

All rats were catheterized intrathecally by modifying the procedure described by Yaksh and Rudy (1976) one week after adaptation to standard housing conditions in the Experimental Animal Center of the University of Miyazaki. The catheters were made from polyethylene tube (PE-10; Becton Dickinson, San Jose, CA) by stretching in a hot water bath (70 °C) to reduce the diameter of the tube. The elongated part of the catheter was inserted caudally into the subarachnoid space through a small slit in the atlanto-occipital membrane to extend 7.3 cm beyond the slit under anesthetic conditions with sodium pentobarbital (50 mg/kg, i.p.). The rostral part of the catheter was sutured to the occipital muscle to immobilize the catheter, and the wound was closed in two layers with a 3-0 silk thread. The catheterized rats were housed in individual cages with free access to food and water before and during the experiments. Rats showing visible signs of tissue inflammation, paralysis or other neurological deficits during a one-week recovery period following catheter implantation were excluded from the study.

2.3. Production of a model animal with chronic constriction injury

The catheterized animals were anesthetized by sodium pentobarbital (50 mg/kg, i.p.), and the left sciatic nerve was exposed at the level of the mid-thigh by separating the biceps femoris muscle with blunt forceps and was isolated from surrounding connective tissue. To produce a model rat with chronic constriction injury (CCI) of the sciatic nerve, two cuffs consisting of a 2–4 mm section of split polyethylene tube (PE-90; Becton Dickinson, San Jose, CA) were applied to the exposed sciatic nerve by a sterilized stainless probe at approximately 0.5 mm intervals, according to the previously described procedure (Mosconi and Kruger, 1996). The muscle layer and skin layer were closed using 3-0 silk threads. The present experiment was conducted by using only rats showing mechanical and thermal withdrawal thresholds of 4 g or less and 46 °C or less, respectively, 4–5 weeks after cuff application. In sham-operated rats, the left sciatic nerve was exposed at the same level, but cuffs were not applied.

2.4. Assessment of behavioral response

To assess mechanical allodynia, the withdrawal threshold of hind paws to mechanical stimulation was determined by a series of von Frey filaments consisting of 18 filaments ranging from 0.008 g to 100 g (Touch-Test Sensory Evaluator; North Coast Medical Inc., Morgan Hill, CA). The von Frey filaments were applied through a mesh floor at the platform of a Plexiglas box that was specifically constructed for von Frey filament testing (Dynamic Planter Anesthesiometer; Ugo Basile, Comerio, Italy). Rats were acclimated to the box for 30 min after transfer to the testing platform. Different von Frey filaments were touched to the planter surface of both hind paws through a mesh floor, and this trial was repeated five times per filament at intervals of a few seconds. The withdrawal threshold of each hind paw was determined by increasing the stimulus strength gradually from a 2 g starting filament until the paw withdrawal response occurred. On the other hand, when rats responded to a 2 g starting filament, the withdrawal threshold was determined by decreasing the stimulus strength gradually until the paw withdrawal response did not occur. The lowest filament in grams that evoked withdrawal responses at least 2 times in 5 applications was considered as the withdrawal threshold (Tal and Bennett, 1994). This test is designed to evaluate the extent of mechanical allodynia in animals with neuropathic pain, such as CCI.

Additionally, to assess thermal allodynia, the withdrawal response of the ipsilateral hind paw in cuff-applied rats was examined by a thermal probe connected to a Pain Thermometer (UDH-104; Unique Medical Co. Ltd, Tokyo, Japan). A 6 mm-wide metal tip constituting the thermal probe of 7 mm in diameter was gently touched to the planter surface of the hind paw, and thermal stimuli were applied to the planter surface through the metal tip at which a fixed temperature was maintained under feedback control. Rats were placed individually in a clear plastic box (14 cm × 17 cm × 22 cm) on an elevated floor with wire mesh, and were acclimated for 30 min. The heated thermal probe through the mesh floor alternatively simulated the plantar surface of each hind paw at intervals of a few tens of seconds. A cut-off time of 30 s was imposed on the stimulus duration to prevent tissue damage and to exclude spontaneous behavior. The withdrawal response was produced by 46 °C of thermal stimulus to the ipsilateral hind paw of cuff-applied animals, while this response was hardly observed in sham-operated animals, indicating that thermal allodynia is induced in the ipsilateral hind paw of cuff-applied animals. Therefore, we applied 46 °C of thermal stimulus to the ipsilateral hind paw to examine the effect of an antidepressant, 5-HT receptor antagonists or alpha adrenoceptor