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Effects of intrathecal administration of newer antidepressants on mechanical allodynia in rat models of neuropathic pain

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ABSTRACT

Antidepressants, especially tricyclic antidepressants (TCAs) are widely used for the treatment of various types of chronic and neuropathic pain. The antinociceptive effects of TCAs are, however, complicated. Therefore, two kinds of newer antidepressants whose functions have been more fully clarified were selected, milnacipran, a serotonin and noradrenaline reuptake inhibitor (SNRI) and paroxetine and fluvoxamine, which are selective serotonin reuptake inhibitors (SSRIs). The antiallodynic effects of intrathecal administration of these newer antidepressants were examined in two rat models of neuropathic pain, chronic constriction injury (CCI) of the sciatic nerve and streptozotocin (STZ)-induced diabetic neuropathy. The antiallodynic effect of these antidepressants was evaluated using the von Frey test. The intrathecal administration of milnacipran had an antiallodynic effect in both CCI and STZ-induced diabetic rats in a dose-dependent manner. On the other hand, the intrathecal administration of either paroxetine or fluvoxamine elicited little antiallodynic effect in CCI rats, while both SSRIs had antiallodynic effects in the STZ-induced diabetic rats in a dose-dependent manner. These results indicate a considerable difference to exist in the development and/or maintenance between these two animal models of neuropathic pain and suggest that each of these three antidepressants may be effective for the treatment of diabetic neuropathic pain.

1. Introduction

Neuropathic pain is defined as a chronic or persistent pain resulting from an injury to the nervous system. The hallmarks of this type pain include an enhanced response to noxious stimuli, thermal and mechanical hyperalgesia and a pain response to previously non-noxious stimuli, known as allodynia (Courteix et al., 1993; Elliott, 1994; Woolf and Doubell, 1994; Calcutt et al., 1996). Neuropathic pain may be due to a pathophysiological alteration of the peripheral and/or central nervous system including spinal dorsal horn neurons (Zimmermann, 2001; Campbell and Meyer, 2006) and is resistant to opiate analgesics.

Antidepressants, especially tricyclic antidepressants (TCAs) are widely used as the first-line drugs for the treatment of neuropathic pain including diabetic neuropathy (Micó et al., 2006; Wong et al., 2007). The mechanisms underlying the antinociceptive effects of TCAs are complicated and are associated with various substrates at the supraspinal, spinal, or peripheral level when they are administered systemically (Sawynok et al., 2001; Micó et al., 2006). Recently, newer antidepressants such as serotonin and noradrenaline reuptake inhibitor (SNRI) and selective serotonin reuptake inhibitor (SSRI) have been introduced and the function of these antidepressants is better understood than TCAs. However, it is unclear whether SNRIs such as milnacipran and SSRIs such as paroxetine or fluvoxamine have similar antinociceptive effects.

The spinal cord is important as an active site of antidepressant-mediated antinociception (Hwang and Wilcox, 1987). The inhibition of pain transmission by the descending systems has been studied by focusing on the neuromodulatory functions of noradrenaline (NA) and serotonin (5-HT) at the synapses between dorsal horn neurons and primary afferents (Yoshimura and Furue, 2006). It is presumed that antidepressants alter nociceptive thresholds and neuropathic pain is, at least in part, inhibited by a blockade of NA and 5-HT reuptake and by holding them at a certain level in the synaptic clefts of the spinal cord. Indeed, SSRIs and SNRIs elicit an antinociceptive or antiallodynic effect on some acute and chronic neuropathic pain in rodent models following intrathecal administration (Obata et al., 2005; King et al., 2006) or

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systemic administration (Yokogawa et al., 2002; Aubel et al., 2004; Iyengar et al., 2004). However, the mechanisms of the analgesic effect of these antidepressants are still unclear, particularly in the diabetic neuropathic pain model.

A single systemic injection of streptozotocin (STZ) induces short-term insulin-deficient diabetes in rodents (Junod et al., 1967) and leads to the development of neuropathic pain characterized by hyperalgesia and allodynia (Lee and McCarty, 1990; Courteix et al., 1993; Calcutt et al., 1996). SNRIs such as duloxetine and milnacipran elicit antiallodynic effects in rats with spinal nerve ligation (Iyengar et al., 2004; Obata et al., 2005) and SSRIs such as paroxetine produced antiallodynic effects in STZ-induced diabetic rats (Aubel et al., 2004). However, the effects of both SNRIs and SSRIs in the same neuropathic model rats have not been extensively examined. The effects of intrathecal administration of newer antidepressants that inhibit reuptake of NA and/or 5-HT would be valuable to evaluate the antiallodynic effect of these drugs at the spinal level. Therefore, the antiallodynic effects of three antidepressants, milnacipran, paroxetine and fluvoxamine, were examined in STZ-induced diabetic rats and in rats with chronic constriction injury (CCI) of the sciatic nerve to compare the effects of intrathecal administration of these drugs in the different animal models of neuropathic pain.

2. Materials and methods

2.1. Animals

All animal protocols were approved by the ethical committee for animal experimentation at University of Miyazaki and followed the guidelines for treatment of animals of the International Association for the Study of Pain (Zimmermann, 1983). The experiments were performed on adult male Sprague–Dawley rats (Charls River Laboratories Japan, Yokohama, Japan) weighing 200–250 g on the day of catheterizing. The rats were housed 2 or 3 animals per cage with free access to food and water and exposed to 12-h cycles of light–dark.

2.2. Intrathecal catheterisation

The antidepressants were administered through a catheter into the subarachnoid space of the rats. 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 University of Miyazaki. The catheters were made from polyethylene tubing (PE-10, Becton Dickinson, San Jose, CA) by stretching in a hot water bath (70 °C) to reduce the diameter of the tubes. The elongated part of the catheter was inserted caudally into the subarachnoid space of rat through a small slit in the atlanto-occipital membrane to extend 7.5 cm beyond the slit under anesthetic conditions with sodium pentobarbital (20 mg/kg, i.p.) and ketamine hydrochloride (100 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 3-0 silk thread. The catheterized rats were housed in individual cages with free access to food and water before and during the experiments. The rats showing visible signs of tissue inflammation, paralysis or other neurological deficits following catheter implantation during a 1-week recovery period were excluded from the study.

2.3. Induction of chronic constriction injury

The catheterized animals were prepared with a unilateral sciatic nerve constriction injury, according to the previously described procedure (Mosconi and Kruger, 1996). Under deep anesthesia with sodium pentobarbital (50 mg/kg, i.p.), the sciatic nerve was exposed by separating the left biceps femoris muscle with blunt forceps and then freed from surrounding connective tissue. Using a sterilized stainless probe, 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 nerve at approximately 0.5 mm intervals. The muscle layer and skin layer were closed using 3-0 silk thread. This experiment was performed using rats in which the withdrawal threshold of the ipsilateral hind paw came down 2 g or less at 2–3 weeks after cuff-implantation.

2.4. Induction of diabetic neuropathy

Diabetic rats were produced by a single injection of STZ (Sigma, St. Louis, MO; 50 mg/kg, i.v.) prepared in 0.1 M sodium citrate buffer (pH 4.4) into the tail vein of the catheterized animals. Intravenous injection of STZ is known to induce insulin-deficient diabetes by ablating pancreatic β cells (Arison et al., 1967; Junod et al., 1967). Three days after the injection of STZ, diabetic rats were confirmed by measuring plasma glucose concentrations in blood samples obtained from the tail vein using a glucose oxidase impregnated strip and reflectance meter (GLUTEST ACE, Arkray Factory, Shiga, Japan) and rats with blood glucose concentrations greater than 350 mg/dl were considered to be diabetic. The plasma glucose concentrations of STZ-treated rats were measured at four time points, 1 day, 3 days, 1 week and 2 weeks following STZ administration and mechanical allodynia was simultaneously assessed at the same time points. The catheterized rats showing diabetes and tactile allodynia 2–4 weeks after STZ injection were used in this experiment.

2.5. Measurement of hind paw withdrawal threshold

In order to assess mechanical allodynia, the withdrawal threshold of hind paws to mechanical stimulation was determined using a series of von Frey filaments (Touch-Test™ Sensory Evaluator, North Coast Medical Inc., Morgan Hill, CA) and was expressed in grams. Eighteen filaments ranging from 0.008 to 100 g were used. The von Frey filaments were applied using a platform constructed specifically for von Frey filament testing and made of a Plexiglas box with a mesh floor (Dynamic Planter Aesthesiometer, UGO BASILE, Italy). The rats were allowed to acclimate to the box for 30 min after transfer to the testing platform. The planter (ventral) surface of both hind paws was touched with different von Frey filaments through mesh floor and the trial was repeated five times per filament at an interval of a few seconds. The withdrawal threshold of each hind paw was determined by increasing the stimulus strength from the 2 g filament until paw withdrawal occurred. A descending series of the filaments were used when rats responded to the starting filament. The lowest filament in grams that evoked withdrawal responses at least two times out of five applications was considered as the withdrawal threshold (Tal and Bennett, 1994). This test is designed to measure the extent of mechanical allodynia seen in neuropathic pain, such as CCI or diabetic model animals. The paw withdrawal threshold in diabetic model rats was determined by an average of the withdrawal thresholds of both hind paws, while that of CCI rats was determined by an average of the withdrawal thresholds ipsilateral to the injured hind paw.

Milnacipran hydrochloride, paroxetine hydrochloride or fluvoxamine maleate was diluted to various concentrations in 10 μl saline and administered intrathecally over a period of 30 s through the catheter, and followed by 10 μl saline at the same rate to flush the catheter. Paw withdrawal thresholds were determined 10 min before and 5 and 30 min and 1, 2, 6 and 24 h after the injection of each antidepressant.