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Antihyperalgesic and antiallodynic effects of mianserin on diabetic neuropathic pain: A study on mechanism of action

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ABSTRACT

This study used various experimental pain methods to investigate the effects of subacute mianserin administration on diabetes-induced neuropathic pain in rats. The effect of mianserin on hyperalgesia occurring in connection with peripheral diabetic neuropathy was examined using the Randall–Selitto (mechanical nociceptive stimulus), Hargreaves (thermal nociceptive stimulus), and cold-plate (4 °C, thermal nociceptive stimulus) tests. The dynamic plantar aesthesiometer, which measures the threshold values for mechanical stimuli, was used for allodynia studies. Thermal allodynia was evaluated with the warm-plate (38 °C) test. At 30 and 45 mg/kg, mianserin effectively improved mechanical and thermal hyperalgesia occurring in connection with diabetic neuropathy. Subacute administration of mianserin also reduced diabetes-associated mechanical and thermal allodynia. The ability of mianserin to reduce diabetic neuropathic pain was comparable to that of pregabalin (10 mg/kg). The antihyperalgesic and antiallodynic effects of mianserin were reversed with α-methyl-*para*-tyrosine methyl ester (AMPT, an inhibitor of catecholamine synthesis), phentolamine (a non-selective α-adrenoceptor antagonist), propranolol (a non-selective β-adrenoceptor antagonist), and naloxone (a non-selective opioid receptor antagonist) administrations. The same effects were not reversed, however, by *para*-chlorophenylalanine methyl ester (PCPA; an inhibitor of serotonin synthesis). These results suggest that the beneficial effect of mianserin on diabetic neuropathic pain is mediated through an increase in catecholamine levels in the synaptic cleft as well as through interactions with both subtypes of adrenoceptors and opioid receptors. Considering that mianserin exhibits simultaneous antidepressant and antinociceptive effects, this drug could provide a good alternative for treating the pain associated with diabetic neuropathy and the mood disorders caused directly by diabetes.

1. Introduction

Diabetic neuropathy is a syndrome that develops only with diabetes mellitus, in the absence of other factors that can cause neuropathy; it affects different components of the nervous system and can involve all types of nerve fibers (Aslam et al., 2014; Boulton et al., 2005). Peripheral diabetic neuropathy (PDN) can cause neuropathic pain that is difficult to treat because of diabetes-induced damage to somatic or autonomous nerves. As the severity of the pain increases, the patient's daily functions are affected and the quality of life reduces (Boulton et al., 2005; Shi et al., 2012).

Strict glycemic control is the best way to slow the progression of neuropathic pain (The Diabetes Control and Complications Trial Research Group, 1993). In addition to glycemic control, drugs such as antidepressants (duloxetine, amitriptyline, imipramine), anticonvulsants (pregabalin, gabapentin), opioids (tramadol), and α-lipoic acid are used for symptomatic treatment of the pain (Boulton et al., 2005; Yamazaki et al., 2008). The drug groups most widely preferred in the clinic for treating neuropathic pain are anticonvulsants and antidepressants (Wong et al., 2007; Yamazaki et al., 2008). Recent studies indicate that, in addition to the central effects mediated by supraspinal and spinal mechanisms, antidepressants have become important alternatives for PDN therapy because of their local peripheral analgesic efficacy (Micó et al., 2006; Sawynok et al., 2001).

Mianserin, a tetracyclic drug, is approved in various countries for treating major depression. Its chemical structure and pharmacological profile differs from those of tricyclic antidepressants. The antidepressant effect of this drug is associated with an increase in noradrenergic neurotransmission due to blockage of presynaptic α_2-adrenoreceptors (Marshall, 1983; Pinder, 1985). Furthermore, mianserin exhibits low affinity for muscarinic cholinergic receptors and high affinity for blocking 5-HT_2 serotonergic, H_1 histaminergic, and α_2-adrenergic receptors (Peroutka and Snyder, 1981;

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Richelson and Nelson, 1984). In addition to its antidepressant activity, mianserin also possess notable antinociceptive effects in some experimental methods (Pakulska and Czarnecka, 2002; Schreiber et al., 1998; Torres et al., 1995).

This study aimed to investigate the possible efficacy of mianserin, an atypical antidepressant, in the treatment of diabetic neuropathic pain, based on the previous papers reporting the ability of antidepressants in the treatment of PDN and notable antinociceptive potential of this drug.

2. Materials and methods

2.1. Animals

Male Wistar rats of the same age (weight, 250–300 g) were used. The animals were kept in well-ventilated rooms at 24 °C ± 1 °C on a 12 h dark:12 h light cycle (lights are on between 8:00 a.m. and 8:00 p.m.) and were fed standard animal feed. The experimental protocol was approved by the Anadolu University Animal Experiments Local Ethics Committee.

2.2. Drugs and treatments

Streptozotocin (STZ), mianserin hydrochloride, pregabalin, *para*-chlorophenylalanine methyl ester hydrochloride (PCPA), α-methyl-*para*-tyrosine methyl ester (AMPT), phentolamine hydrochloride, propranolol hydrochloride and naloxone hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Citric acid and trisodium citrate were purchased from Merck (Darmstadt, Germany).

2.3. Induction of experimental diabetes

Rats assigned to the diabetic experimental group were fasted overnight and were then administered a single 50 mg/kg dose of STZ (prepared in a 0.1 M citrate buffer, pH of 4.5) in the tail vein (Pospisilik et al., 2003). Following STZ injection, waterers containing a 5 mmol/L glucose solution were placed in the rats' cages in order to reduce or prevent hyperinsulinemia and hypoglycemic shock (Skalska et al., 2008). Blood sugar measurements were taken using blood samples obtained 72 h after injection with Glukotrend® (Roche, Basel, Switzerland). Animals with blood glucose levels over 300 mg/dl were considered as diabetic. The control solution was citrate buffer (Can et al., 2011a, 2011b).

Mianserin and pregabalin treatments were initiated 4 weeks after the induction of diabetes to permit development of nociceptive perception deficits in diabetic rats (Yan et al., 2012).

2.4. Experimental groups

To evaluate acute antinociceptive efficacy, rats were divided into 4 groups. Control group: animals received saline solution. Morphine group: animals received a single 10 mg/kg dose of morphine (Silva et al., 2003). MNS-30 group: animals received a single 30 mg/kg dose of mianserin (Drenska et al., 2008). MNS-45 group: animals received a single 45 mg/kg dose of mianserin.

Normoglycemic rats were divided into 3 treatment (subacute administration) groups. Control group: animals received saline solution for 14 d. MNS-30 group: animals received 30 mg/kg mianserin for 14 d. MNS-45 group: animals received 45 mg/kg mianserin for 14 d.

Diabetic rats were divided into 4 treatment (subacute administration) groups. Control (normoglycemic) group: animals were injected with citrate buffer and then received saline solution for 14 d, at 4 weeks after the injection. DM Group: animals were injected with STZ and then received saline solution for 14 d, at 4 weeks after the injection. MNS-30+DM group: animals were injected with STZ and then received 30 mg/kg mianserin for 14 d, at 4 weeks after the injection. MNS-45+DM group: animals were injected with STZ and then received 45 mg/kg mianserin for 14 d, at 4 weeks after the injection. Pregabalin+DM group: animals were injected with STZ and then received 10 mg/kg pregabalin (Yamamoto et al., 2009) for 14 d, at 4 weeks after the injection.

Morphine was administered intraperitoneally (i.p.), while mianserin and pregabalin were administered orally (p.o.). Each experimental group consisted of 7 rats.

2.5. Evaluation of acute pain

2.5.1. Tail-clip test

The tail-clip test is a method used to measure the response to painful mechanical stimuli. For this test, a clip was placed on the rats' tails approximately 5–10 cm from the tip, and the time taken by the animal to turn and bite the clip was measured with a chronometer. The cut-off time was set at 90 s to prevent tissue damage (Can et al., 2011b; Cannon and Hough, 2005).

2.5.2. Hot (55 °C) plate test

The antinociceptive potential of mianserin for thermal noxious stimuli was evaluated using a hot/cold-plate test device (Ugo-basile, 35100, Verase, Italy) consisting of a Plexiglas compartment (20 cm diameter and 25 cm height) on an aluminum plate. The plate was set at 55 °C ± 1.0 °C (Woolfe and MacDonald, 1944). The time of licking the forepaws, or eventually jumping, was recorded as a parameter of nociception. A maximum stimulus time of 40 s was used to prevent tissue damage.

The data obtained from the tail-clip and hot-plate tests were expressed as a percentage of the maximum possible effect (MPE) using the equation:

$$\text{MPE\%} = (\text{postdrug latency} - \text{predrug latency})/(\text{cutoff time} - \text{predrug latency}) \times 100$$

2.6. Evaluation of mechanical hyperalgesia

2.6.1. Randall–Selitto test

Mechanical hyperalgesia was evaluated using the Randall–Selitto device (Ugo-basile, 37215, Verase, Italy) to apply increasing pressure stimuli to the dorsal portion of the rats' back paws. The force (g) at which the rat withdrew its paw was considered as the mechanical nociceptive threshold. The maximum force to be applied was set at 250 g to prevent tissue damage (Bordet et al., 2008).

2.7. Evaluation of mechanical allodynia

2.7.1. Dynamic plantar test

Mechanical allodynia was evaluated using the dynamic plantar aesthesiometer device (Ugo-basile, 37450, Verase, Italy). This device consists of six Plexiglas compartments ($17 \times 69 \times 14\ cm^3$) located on a perforated metal floor, under which, there is a mobile piece that can apply a mechanical stimulus with increasing force by using a metal rod (diameter, 0.5 mm). The test measures the force of the mechanical stimulus required for the subject to lift its paws.

Rats were acclimatized in the Plexiglas compartments for 30 min prior to testing. Once the device was switched on, the metal rod passed through the holes in the metal floor, coming into contact with the sole of the back paw, and increasing pressure (2.5 g/s) was applied. When the animal lifted its paw, the mechanical stimulus stopped automatically, and the withdrawal value was recorded with an accuracy of 0.1 g (Bordet et al., 2008; Villetti