Antinociceptive Effect of Mirtazapine in Rats with Diabetic Neuropathy

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ABSTRACT

Introduction: To evaluate the antinociceptive effect of mirtazapine and the mechanisms mediating this effect in neuropathic pain in rats with diabetes.

Methods: The experiments were performed in Sprague Dawley rats using a hot-plate device. Streptozotocin (STZ) was administered to the rats after taking control measurements. Rats with a blood glucose level of 240 mg/dL or above in the blood specimen obtained from the tail vein 3 days after STZ administration were considered as being diabetic. Three weeks after STZ administration, the hot-plate test was performed. Compared with the control measurements, rats that exhibited >20% decrease in the second hot-plate test measurements were considered to have developed neuropathy. Drugs [mirtazapine, naloxone (opioidergic antagonist), metergoline (serotonergic antagonist), and BRL44408 (adrenergic antagonist)] and drug combinations were administered to

those rats that developed neuropathy. After administrating the drugs or drug combinations, the third hot-plate test was performed.

Results: Mirtazapine at doses of 10 and 15 mg/kg exhibited a significant antinociceptive effect. Naloxone, metergoline, or BRL44408 alone did not cause an antinociceptive effect. However, combinations of these drugs with mirtazapine (15 mg/kg) significantly decreased the antinociceptive effect of mirtazapine.

Conclusion: It is suggested that mirtazapine has a significant antinociceptive effect in diabetic neuropathy and that opioidergic, serotonergic, and adrenergic systems have roles to play in this effect.

Keywords: Nociception, diabetic neuropathy, mirtazapine

INTRODUCTION

Diabetic neuropathy is a chronic complication associated with diabetes that has a significant detrimental effect on the daily activities of patients. It occurs in approximately 50% of patients with diabetes within 20 years of diagnosis (1). Different drugs have been used in the treatment of neuropathy, such as tricyclic antidepressants (amitriptyline), selective serotonin reuptake inhibitors (fluoxetine), antiepileptics (carbamazepine, phenytoin), venlafaxine, amantadine, tramadol, oxycodone, gabapentin, bupropion, and capsaicin (1). Mirtazapine is a new antidepressant that acts in a manner that is different from other antidepressants and is notable for the low frequency of severe side effects when compared with other antidepressants (2).

Mirtazapine blocks the presynaptic α -2 adrenoreceptors in both the central and peripheral nervous systems, while its affinity to α -1 adrenoreceptors is less marked. In addition, it weakly blocks serotonin-1 (5-hydroxytryptamine-1, 5-HT-1) receptors and strongly blocks 5-HT-2 and 5-HT-3 receptors. On the other hand, it has no effect on noradrenaline re-uptake and does not block the subtypes of β -adrenergic receptors to any significant degree (2).

Mirtazapine, with its low side-effect profile, might be considered a good alternative in the treatment of diabetic neuropathy. This study investigated the antinociceptive effect of mirtazapine on diabetic neuropathy in rats and the role of opioidergic, serotonergic, and adrenergic systems in this effect. Naloxone, a competitive opioid μ (mu) receptor antagonist that removes the respiratory depression effect resulting from opioid toxicity, was used to evaluate the role of the opioidergic system (3). Furthermore, a non-selective serotonin receptor antagonist, metergoline (4), and a α -2a adrenergic receptor antagonist, BRL44408 (5), were used to evaluate the roles of the serotonergic and adrenergic systems, respectively.

METHODS

This study was approved by the Local Ethics Board of Animal Research. Sprague Dawley rats weighing 170–225 g were used in the study. They were divided into 11 groups, each containing four males and four females. Rats were kept in a 12-h daylight and 12-h darkness cycle at a room temperature of 20°C and a humidity of 50%–60% and were administered standard pellet feed and water. Rats were brought to the experiment area 1 h prior to the start of experiments to enable them to adjust to the environment.

Sensorimotor Performance

A rotarod test was used to detect the motor activity of rats to be included in the study, with the adequacy of motor activity being defined as being able to stay on the rod for at least 120 s.

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Nociceptive Test (Hot-plate Test)

Rats were placed on a hot plate after the plate temperature had been brought to 52.5°C. The length of time from the placement of the rat on the plate until it shook or rapidly withdrew its posterior leg was measured using a chronometer.

Drugs and Chemical Substances

Streptozotocin (STZ; Sigma-Aldrich, St. Louis, MO, USA) and naloxone (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in physiological saline; mirtazapine (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 10% polyethylene glycol; and metergoline (Sigma-Aldrich, St. Louis, MO, USA) and BRL44408 (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in 10% ethanol. The solutions were immediately prepared prior to administration and were intraperitoneally injected in volumes of 0.3–0.45 mL.

Experimental Procedures

Basal hot-plate test measurements were conducted during the initial stage of the experiment. Mirtazapine was administered before the development of diabetes at doses of 5, 10, and 15 mg/kg. Subsequently, STZ was intraperitoneally injected at a dose of 50 mg/kg to enable the development of diabetes (6,7). The glucose level in the tail vein blood was measured on the third and fifth days using a glucometer (Accu-Chek Active). Rats with a blood glucose level of 240 mg/dL or higher were considered as being diabetic.

After a 3-week waiting period, the second hot-plate test measurements were conducted to check whether hyperalgesia secondary to diabetic neuropathy had developed. The development of hyperalgesia was diagnosed in rats as a 20% decrease in measurements compared with the basal values. Drugs specific to the experimental groups were administered to rats with hyperalgesia (Table 1). Furthermore, hot-plate tests were performed at 30 and 60 min after drug administration.

Statistical Analysis

The Shapiro–Wilk test was used to evaluate the normal distribution. Because the data were normally distributed, the differences between the groups were evaluated using a variance analysis. Dunnett's test was used to compare the nociceptive measurement values of the control group and the other 10 groups. A paired t-test was used for the paired comparison of the in-group values that were found to be significant, with a p value of <0.05 being accepted as significant.

RESULTS

Effects because of STZ Application

Approximately 75% of rats that were administered STZ developed hyperglycemia (diabetes). The body weights of rats with diabetes were significantly reduced compared with their pre-diabetic status ($p<0.05$; Table 2). The hot-plate test values of the group that was administered only STZ were not significantly different from those of the control group (Figure 1). The hot-plate test values obtained 3 weeks after STZ administration were decreased by approximately 20% when compared with the pre-injection values. This decrease was accepted as a sign of the development of hyperalgesia in rats ($p<0.05$; Table 3).

Effects of Mirtazapine and Antagonists

The tests performed on the rotarod equipment demonstrated that the drugs caused no impairment in locomotor activity (Table 4). Mirtazapine (10 and 15 mg/kg) was observed to have a significant antinociceptive effect in rats that had not yet developed diabetes ($p<0.05$; Figure 2). Mirtazapine (15 mg/kg) was also found to have a significant antinoci-

ceptive effect in rats that had developed hyperalgesia because of diabetic neuropathy ($p<0.05$; Figure 3). Naloxone, metergoline, and BRL44408 inhibited the antinociceptive effect of mirtazapine to a significant degree (15 mg/kg); however, the combination of mirtazapine and other drugs still demonstrated an antinociceptive effect when compared with the control group ($p<0.05$; Figure 4). These drugs were observed to have no different effects when applied as a single agent when compared with the control group (Figure 5). Furthermore, polyethylene glycol and ethanol, which were used to dissolve metergoline and BRL44408, respectively, did not produce any different effects when used as a single agent (Figure 6).

DISCUSSION

The antinociceptive effect of mirtazapine in rats with STZ-induced diabetic neuropathy was investigated in this study. Although the diabetes-inducing rate of STZ has not been cited in the literature, cases with no

Table 1. Drugs and doses of the drugs administered to rats with the development of hyperalgesia

Experimental Groups	Drugs and doses	Rats with development of hyperalgesia
Group 1	Control (ps)	4
Group 2	Mirtazapine 5 mg/kg	5
Group 3	Mirtazapine 10 mg/kg	4
Group 4	Mirtazapine 15 mg/kg	6
Group 5	Naloxone 1 mg/kg	4
Group 6	Metergoline 2 mg/kg	5
Group 7	BRL 44408 4 mg/kg	4
Group 8	Mirtazapine 15 mg/kg+Nalokson 1 mg/kg	6
Group 9	Mirtazapine 15 mg/kg+Metergoline 2 mg/kg	5
Group 10	Mirtazapine 15 mg/kg+BRL 44408 4 mg/kg	4
Group 11	Streptozotocin 50 mg/kg	4
ps: physiological saline		

Table 2. Venous blood glucose levels prior to and after (3 days) STZ administration in rats; moreover, the body weights of the animals prior to and 21 days after STZ administration

	Before STZ (Mean and SE)	After STZ (Mean and SE)
Venous blood glucose levels (mg/dL)	115±1.12	384±1.23*
body weights (g)	186±1.24	145±1.35*
* $p<0.05$. STZ: streptozotocin; SE: standard error

Figure 1. Comparison of the nociceptive effect of STZ with the control group through a hot-plate test

*No significant difference was found between the groups

Table 3. Hot-plate measurement values prior to and 3 weeks after STZ administration

Group	Hot-plate values (s) (Mean and SE)
Before STZ	21±3.21
After STZ	17±2.43*

*p<0.05. SE: standard error; STZ: streptozotocin

Table 4. Effects of drugs on sensorimotor performance

Drug (mg/kg)	Percentage of animals that can stand for 2 min on the rotarod	Time spent on the rotarod (s) (Mean and SE)
Control	100	120±1.8*
Mirtazapine 5	100	120±0*
Mirtazapine 10	97	118±1*
Mirtazapine 15	91	117±3*
Naloxone 1	100	120±0*
Metergoline 2	98	119±2.5*
BRL44408 4	94	112±3.1*

*No significant difference was found between the groups. SE: standard error

induction of diabetes have been reported in almost all studies (8,9).

Diabetic neuropathy is one of the most common complications associated with diabetes and can be detrimental to the quality of life of the patient. Thermal, chemical, and mechanical hyperalgesia have all been reported in patients who have developed diabetic neuropathy (10). There are different opinions in the literature regarding the development of thermal hyperalgesia in experimental diabetes induced with STZ; some researchers claim that there is no change in thermal sensitivity in experimental diabetes, while others have reported the development of thermal hyperalgesia (10,11). In this study, thermal hyperalgesia, which was demonstrated by the hot-plate test, was accepted as a sign of diabetic neuropathy. In previous studies, it has been demonstrated that experimental diabetic neuropathy occurs in 2–3 weeks following diabetes induction by STZ (12); the development of diabetic neuropathy was also detected within approximately 3 weeks in this study. Pain measurement is achieved through nociceptive stimulus in animal experiments and may be thermal, mechanical, or chemical in nature. The antinociceptive effects of some antidepressant drugs were evaluated using different pain models in a study by Bomholt et al. (13), who found that amitriptyline, duloxetine, mirtazapine, and citalopram were found to be ineffective in a tail withdrawal test, which is an acute pain model; in contrast, duloxetine and mirtazapine were demonstrated to have a significant antinociceptive effect in a hot-plate test. Therefore, the hot-plate test method was selected for this study.

Mirtazapine demonstrated to have a marked antinociceptive effect in rats in this study, with a dose range of 5–15 mg/kg demonstrating dose-dependent antinociceptive efficacy. In a study of rats by Schreiber et al. (14) using a hot-plate test, mirtazapine demonstrated an antinociceptive effect at a dose of 10 mg/kg; however, it was found to be partially ineffective at a dose of 15 mg/kg. This difference may be attributed to the type of animals used in the studies.

The antinociceptive effect that was induced by mirtazapine was partially antagonized by naloxone [opioidergic system contribution; (14,15)], metergoline (serotonergic system contribution; 14), and BRL44408 [ad-

Figure 2. Effect of mirtazapine (5, 10, and 15 mg/kg) on the nociceptive threshold in a hot-plate test

*p<0.05

Figure 3. Antinociceptive effects of mirtazapine (15 mg/kg) on normal rats and diabetic neuropathy-induced rats with hyperalgesia (D) in a hot-plate test

*p<0.05

Figure 4. Effects of naloxone, metergoline, and BRL44408 on the antinociceptive effects of mirtazapine in rats with diabetes at 30 and 60 min of a hot-plate test

*Mirtazapine, 15 mg/kg, demonstrated an antinociceptive effect when compared to the control group (p<0.05)

***Naloxone, metergoline and BRL44408 inhibited the antinociceptive effect of mirtazapine (15 mg/kg) (p<0.05)

renergic system contribution; (14,16)] in this study. Compatible with our results, Schreiber et al. (14) suggested that opioidergic, serotonergic, and adrenergic systems mediated the antinociceptive effect of mirtazapine. Naloxone has demonstrated antinociceptive efficacy in studies performed in rats (17,18). In this study, when administered as a single agent, naloxone had no antinociceptive effect; however, it significantly decreased the antinociceptive efficacy of mirtazapine (15 mg/kg). This suggests that the analgesic effect of mirtazapine is partially mediated by the opioidergic system. There are two studies in the literature suggesting that antidepressant

Figure 5. Antinociceptive effects of naloxone, metergoline, and BRL44408 in rats with diabetes in a hot-plate test (after 30 and 60 min)

*No significant difference was found between the groups

Figure 6. Comparison of the measurement values of polyethylene glycol and ethyl alcohol in a hot-plate test with the control (ps) group.

*No significant difference was found between the groups

sants have a direct effect on opioid receptors and that these receptors partially mediate the analgesic effects of these antidepressants (19,20). The fact that the antinociceptive effect of clomipramine is blocked by naloxone and naltrexone, both of which are opioid receptor antagonists, was demonstrated as a proof of these suggestions (21). In many previous studies, the mechanisms of the antinociceptive effect of new-generation antidepressants have been investigated (17,18,22), and it was found that most of these drugs increased the effects of small doses (with no analgesic action) of opioids. Similarly, venlafaxine, which is a (powerful) serotonin, (moderate) noradrenaline, and (weak) dopamine reuptake inhibitor, mediates its antinociceptive effect through both opioidergic and adrenergic systems (23). The findings of all these studies and this study underline the role of the opioidergic system in the antinociceptive effect of mirtazapine. Mirtazapine increases serotonergic and adrenergic neurotransmission through the blockage of serotonin receptors of the 5-HT₂ and 5-HT₃ type, as well as of auto and heteroadrenoreceptors (2). The net effect is the stimulation of post-synaptic 5-HT₁ type serotonin receptors. In this study, metergoline demonstrated no antinociceptive effect by itself; however, it was found to decrease the antinociceptive effect of mirtazapine significantly when the two were administered together. Serotonergic neurons end in the posterior horn descending from the brain stem, through the dorsolateral funiculus and up to the medulla spinalis (descending inhibitor pathway), where they play a role in the modulation of pain. This event is reversed by serotonin and adrenergic receptor antagonists (4). Metergoline may block the nociceptive effect of mirtazapine through the mechanism described above.

In this study, the role of the adrenergic system on the mechanism of the antinociceptive effect of mirtazapine was also investigated, in addition to the opioidergic and serotonergic systems. BRL44408, an α -2 adrenergic receptor antagonist, demonstrated no antinociceptive effect by itself, but blocked

significantly the antinociceptive effect of mirtazapine when the two were applied together. This suggests that the adrenergic system also plays a role in the antinociceptive effect of mirtazapine. Schreiber et al. (14) also demonstrated the role of the noradrenergic system in the nociceptive effect of mirtazapine, in addition to the opioidergic and serotonergic systems. Similarly, analgesia induced by tricyclic antidepressants was reported to be removed by an α -2 adrenergic receptor antagonist, RX 821002 (24). Ghelardini et al. (25) reported that the antinociception induced by tricyclic antidepressants was inhibited by reserpine (a drug that decreases the noradrenaline release from adrenergic nerve endings) and yohimbine, an α -2 adrenergic receptor antagonist, in their study in rats using abdominal contraction and hot-plate tests. The results of all these studies and this study suggest that the adrenergic system plays a role in the antinociceptive effect of mirtazapine.

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