The acute effects of mirtazapine on pain related behavior in healthy animals

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ABSTRACT

الأهداف: التحقق من تأثيرعقار الميرتازبين المضاد للاكتئاب، ومدى فعاليته على تخفيف الألم بين مجموعة من الحيوانات التي تتمتع بالصحة الجيدة.

الطريقة: أُجريت هذه الدراسة في قسم الصيدلة التابع لكلية الطب في جامعة أوسمانغازي اسكسهير، اسكسهير، تركيا، وقد استمرت على مدى ثلاثة أشهر وهي مارس، وأبريل، ومايو 2009م. انقسمت هذه الدراسة إلى مرحلتين وفي المرحلة الأولى تمت الاستعانة بفئران ألبينو السويسرية (العدد=64، الوزن= 25–35 غرام) التي تم تقسيمها إلى 8 مجموعات: مجموعة الشاهد وأعطيت محلول الملح فحسب، والمجموعة2 وأُعطيت الميرتازبين (5 ملغ/كلغ)، والمجموعة3 وأُعطيت الميرتازبين (10 ملغ/كلغ)، والمجموعة4 وأُعطيت الميرتازبين (20 ملغ/كلغ)، والمجموعة 5، 6، 7، 8 وأُعطيت الميرتازبين بمقدار 10 ملغ/كلغ مع مركباته: ايستر ميثيل أرجينين– ل نيترو (100 ملغ/كلغ)، وأرجينين–ل (100 ملغ كلغ)، ونالوكسون (1 ملغ/كلغ)، وسيبروهيباتدين (50 ميكروغرام/كلغ). وبعد مرور ساعة واحدة من إعطاء العقار داخل الصفاق خضعت الحيوانات إلى اختبارات الشعور بالألم في الجهاز المركزي والمتمثلة بالتالي: ملقط الذيل، الحركة السريعة للذيل، والطبق الساخن، واختبارات الشعور بالألم في الجهاز المحيطي كاختبار التلوي. أما في المرحلة الثانية من التجربة فقد قمنا بتحليل مستويات أنزيم أكسيد نيتريك سنثاز من خلال الاستعانة بالجرذان من سلالة سبراغو دوللي (العدد=8، الوزن=20±250 غرام)، وتم عزل شرائح الحصين المأخوذة من أدمغتها (0.6 ميكروم)، ومن ثم تم تقسيمها بالمختبر إلى 8 مجموعات أيضاً وهي: مجموعة الشاهد، والميرتازبين $3x10^{-3}$M، $4x10^{-3}$ M ، $5x10^{-3}$ M، والميرتازبين $4x10^{-3}$M ومركباته: ايستر ميثيل أرجينين–ل نيترو، وأرجينين–ل، ونالوكسون، وسيبروهيباتدين $4x10^{-3}$M.

النتائج: أشارت نتائج الدراسة إلى دور عقار الميرتازبين في تخفيف الألم في الجهاز المحيطي وتخفيف الألم الثنائي الطور في الجهاز المركزي، غير أنه لم يكن له تأثير على تخفيف الألم في الجهاز المركزي. ولم يكن هناك فروقاً واضحة في مستويات أنزيم أكسيد نيتريك سنثاز بين المجموعات الثمانية التي تضمنتها الدراسة في مرحلتها الثانية.

خاتمة: أثبتت الدراسة عدم تأثير سبيل النيتريرجيك على نشاط عقار الميرتازبين وقدرته على تخفيف الألم في الجهاز المركزي، في حين كان لسبيل كل من: أوبياتيرجيك وسيروتونرجيك أثراً كبيراً على تخفيف الألم.

Objective: To investigate whether the tetracyclic antidepressant mirtazapine has a pain-suppressing effect in healthy animals.

Methods: In the first step, Swiss albino female mice weighing 25-35 g were used. Eight groups each containing 8 mice were established as follows:- Control (saline), mirtazapine 5 mg/kg, 10 mg/kg, and 20 mg/kg, mirtazapine 10mg/kg and its combinations L-Nitro-L-Arginine Methyl Ester (L-NAME) 100 mg/kg, L-Arginine 100 mg/kg, naloxone 1 mg/kg, and cyproheptadine 50 µg/kg. This study was performed in the Department of Pharmacology, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey during March, April, and May 2009. One hour after the drugs were given intraperitoneally, hot plate, tail clip, tail flick, and writhing tests were used for evaluating antinociceptive effects. In the second step, the brain hippocampus of Sprague Dawley type male rats weighing 250±20 g were isolated and 0.6 µm hippocampus slices were obtained. In vitro groups were established as control, mirtazapine $3x10^{-3}$M, $4x10^{-3}$M, $5x10^{-3}$M, mirtazapine $4x10^{-3}$M and its combinations L-NAME, L-Arginine, naloxone, and cyproheptadine $4x10^{-3}$M.

Results: Mirtazapine did not show central spinal analgesic activity, but had significant peripheral and biphasic central analgesic effects at the supraspinal level. In addition, there were no significant differences between the different groups in nitric oxide synthase levels on the brain slices.

Conclusion: The nitrergic pathway does not have an effect on the central antinociceptive activity of mirtazapine, while opiatergic and serotonergic pathways have a significant role.

Neurosciences 2011; Vol. 16 (3): 217-223

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Received 20th February 2011. Accepted 18th April 2011.

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Many recent studies have shown that antidepressive agents have analgesic effects in addition to their antidepressive effects.[1-4] The same studies have also demonstrated that some antidepressants have a potential analgesic effect, which is totally independent of their antidepressive effects and that it occurs in lower doses. The potential analgesic effects of antidepressants, particularly tricyclic antidepressants (TCAs), have been demonstrated by many trials.[5,6] Therefore, they are indicated for symptomatic treatment of several conditions such as neuropathic pain,[7-9] fibromyalgia,[10,11] headache, and migraine prophylaxis[12] because of their potential analgesic effects. The TCAs have been recognized as first-line treatment for neuropathic pain. Several studies have shown that selective serotonin reuptake inhibitors (SSRIs) also have potential analgesic effects, but this effect is lower compared with the TCAs.[13,14] However, now they have started to replace TCAs in the treatment of chronic pain as they are associated with fewer side effects. Based on this information, the present study examined if mirtazapine, which is considered a prototype of a newer group of antidepressant agents, called the tetracyclic antidepressant agents, has potential analgesic effects. Mirtazapine is a tetracylic antidepressant of the piperazine class, and defined with noradrenergic-specific serotonergic activity. It also potently blocks serotonin 5-HT_2 and 5-HT_3 receptors and leads to activation of 5-HT_{1A} receptors.[15-17] It has also been demonstrated that mirtazapine improves behavioral and neurochemical deficits in rats during subchronic treatment.[18] In that respect, mirtazapine basically has a similar activity profile to that of both typical and atypical antidepressants. It is reported that this may also play a role in the antidepressive activity of mirtazapine. In 1994, an animal model study by Andrews et al[19] for evaluation of impulse control (DRL-72 test) showed that mirtazapine had equivalent efficacy to imipramine, and was associated with less sedation than imipramine. The present study examined the potential analgesic action of mirtazapine using central and peripheral pain behavior tests. The nitrergic, opiatergic, and serotonergic pathways of pain were blocked to clarify the analgesic mechanisms, and an analysis of neuronal nitric oxide synthase (nNOS) was performed in slices of rat brain.

Methods. Sixty-four Swiss Albino mice and 8 Sprague Dawley rats were used throughout the experiment after obtaining permission from the Local Ethics Committee for Animal Experimentation of Eskişehir Osmangazi University, Eskisehir, Turkey. The animals were housed in groups on a 12-hour light/dark schedule with food and water ad libitum. This study was performed in the Department of Pharmacology, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey in March, April, and May 2009. All experiments were performed according to the International Guidelines for the Care and Use of Laboratory Animals.

Disclosure. This study was not supported or founded by any drug company.

Antinociceptive activity in mice. Female Swiss Albino mice weighing 25-35 g were used during this phase of the study. The experiment was carried out under laboratory settings at room temperature of 21^{0}C and alternating light and dark periods of 12 hours each. The mice, which were brought to the experimental setting one-week before the experiment for adaptation purposes, received an identical diet and water. They were assigned into 8 groups (8 mice each group) as follows: Group 1 control group: saline (S) + S. Group 2: mirtazapine (5mg/kg) + S. Group 3: mirtazapine (10mg/kg) + S. Group 4: mirtazapine (20mg/kg) + S. Group 5: mirtazapine (10mg/kg) + L-Nitro-L-Arginine Methyl Ester (L-NAME) (100mg/kg). Group 6: mirtazapine (10mg/kg) + L-Arginine (100mg/kg). Group 7: mirtazapine (10mg/kg) + naloxone (1mg/kg). Group 8: mirtazapine (10mg/kg) + cyproheptadine (50µg/kg). The control group was given an intraperitoneal (i.p.) injection of saline while groups 2, 3, and 4 received mirtazapine 5, 10, and 20mg/kg i.p. Thirty minutes after injection, each group received an i.p. injection of saline. The Tail Clip, Tail Flick, Hot Plate, and Writhing tests were conducted 30 minutes after the second injections. Groups 5, 6, 7, and 8 received i.p. injection of L-Arginine (100mg/kg), L-NAME (100mg/kg), naloxone (1mg/kg), and cyproheptadine (50µg/kg), 30 minutes after i.p. injection of mirtazapine 10mg/kg. The Tail Clip, Tail Flick, Hot Plate, and Writhing tests were conducted 30 minutes after the second injections. Each group was administered the Tail Clip, Tail Flick, and Hot Plate tests prior to the injections for elimination of any individual differences. The results were based on the percent Maximal Possible Effects (%MPE) values.

%MPE = Postdrug-Predrug/Cut off-Predrug X 100

Tail Clip test. Haffner described this in 1929.[20] In the Tail Clip test, a bulldog clip is applied to the base of the tail. The time for the animal's response to the clip (namely, bite) is measured. We used this test to assess the central pain threshold at the spinal level, and the cut off value was considered to be 20 seconds.

Tail Flick test. This was first described by D'Amour et al in 1941,[21] and Tjolsen et al in 1989.[22] In the Tail Flick test, a certain point of the tail is exposed to a radiant heat by a light bulb. There is a photocell circuit under the experiment area. The radiant heat is

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applied approximately 2 cm proximal to the tip. The animal flicks its tail when it feels the pain so that the photocell switches the circuit off. The time from the heat application to the flick response is measured. We used this test to assess the central pain threshold at the spinal level, and the cut off value was considered to be 20 seconds.

Hot Plate test. Although Woolfe and MacDonald first described it in 1944,[23] a modified version of the assay by Eddy and Leinbach in 1953[24] is more commonly used. In the Hot Plate test, the animal is placed on a glass plate, which has a size allowing the animal to move, but keeping it in a constrained area on a surface heated at 55±1^{0}C. The time from placing the mouse on the plate to lift of the hind-paw is measured. The behavior may be in the form of lifting and licking, kicking, or jumping other than lifting the hind-paw. We used this test to assess the central pain threshold at the supraspinal level, and the cut off value was considered to be 30 seconds.

Writhing Test. This was described in the 1950s, and it has been commonly used.[25] In this test, the mice receive an intraperitoneal injection of acetic acid 0.6% (60mg/kg). We used this test to assess the pain threshold at the peripheral level, and 10 minutes after the injection of acetic acid, the number of writhings was recorded for 5 minutes.

Assessment of nitric oxide synthase in the slices of rat brain. The second phase of the study included use of rat hippocampal slices. Male Sprague Dawley rats weighing 250±20 grams were used. Immediately after ether anesthesia, the brain tissue was rapidly excised and placed into ice-cold Krebs solution. The hippocampus was isolated, and sectioned into 0.6 µm slices using a chopper. The slices were washed at 37^{0}C every 15 minutes for one hour, and allowed to stabilize in special chambers in an isolated organ bath. Then, the brain slices were incubated for one hour in the presence of Krebs solution: (mmol/L) NaCl 118.3 mmol/l; KCl 4.7; $MgSO_4.7H_2O$ 1.2; KH_2PO_4 1.2; Glucose 11.1; $NaHCO_3$ 25; $CaCI_2$ 2.5 in the perfusion system. When using 2 agents, the second agent was given 30 minutes after the first one. During the experiment, the incubation system was gassed with a mixture of 95% O_2 and 5% CO_2 at each phase. The resultant perfusates were used in the assessment of nNOS activity. The brain slices were also used for protein measurements after being homogenized.

Nitric oxide synthase assessment. Spectrophotometric measurements were performed in the specimens obtained in accordance with the procedures described in the ELISA Kit for measuring Rat Nitric Oxide Synthase (Cayman Chemical Company, Ann Arbor, Michigan, USA). Spectrophotometric evaluations were made at a wavelength of 540 nm using the Multiscan Ex ELISA Reader (Biotech Lab. Equipment, Chicago, Illinois, USA). The results are in µM.

In vitro groups. 1. Control (S + S). 2. Mirtazapine $3x10^{-3}$ M + S. 3. Mirtazapine $4x10^{-3}$ M + S. 4. Mirtazapine $5x10^{-3}$ M + S. 5. Mirtazapine $4x10^{-3}$ M + L-NAME $4x10^{-3}$ M. 6. Mirtazapine $4x10^{-3}$ M + L-Arginine $4x10^{-3}$ M. 7. Mirtazapine $4x10^{-3}$ M + naloxone $4x10^{-3}$ M. 8. Mirtazapine $4x10^{-3}$ M + cyproheptadine $4x10^{-3}$ M.

Statistical analysis. Data were presented as mean ± SEM for normal distributed variables, and median (25-75 percentiles) for not normally distributed variables. The Kolmogorov-Smirnov test was applied to examine normal distribution. The ANOVA and Kruskal-Wallis One Way ANOVA were used for comparing groups. Data were analyzed using the Statistical Package for Social Sciences for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA). A p-value <0.05 was considered statistically significant.

Results. ***Antinociceptive activity in mice.*** The present study examined if mirtazapine, a tetracyclic antidepressant, has a potential antinociceptive activity by using central (Tail Clip and Tail Flick test, Hot Plate test) and peripheral pain behavior tests (Writhing test). In the Hot Plate test, there was a statistically significant increase in the mirtazapine 5mg/kg and mirtazapine 10mg/kg groups (higher in the 10 mg/kg group) compared with the control group. Although there was a statistically significant increase in the mirtazapine 20 mg/kg group compared with the control group, it was significantly lower than in the mirtazapine 10 mg/kg group (**Figure 1**). In the Hot Plate test, a decrease was observed in the mirtazapine 10mg/kg+L-NAME group and mirtazapine 10mg/kg+L-Arginine group compared with the mirtazapine 10mg/kg group, however, it was statistically not significant. Similarly, compared to the mirtazapine 10mg/kg group, there was a statistically significant decrease in the mirtazapine 10mg/kg+naloxone group and mirtazapine

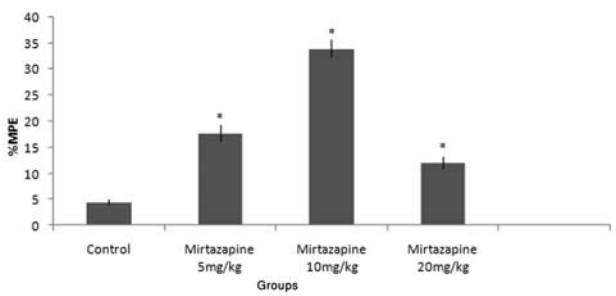

Figure 1 - Evaluated % MPE of mirtazapine 5, 10, and 20 mg/kg with control groups using the hot plate test. *p<0.05 compared with the control group. MPE - maximal possible effects.

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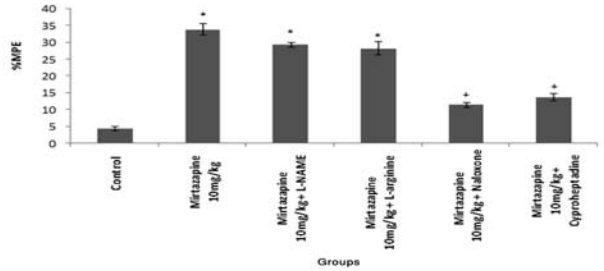

Figure 2 - Evaluated % MPE of mirtazapine 10 mg/kg with control and mirtazapine 10 mg/kg+L-NAME, L-Arginine, naloxone, and cyproheptadine groups using the hot plate test. *p<0.05 compared with the control group, ^{+}p<0.05 compared with the mirtazapine 10 mg/kg group. MPE - maximal possible effects.

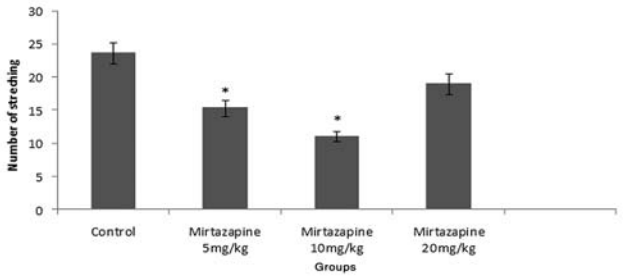

Figure 3 - Evaluated stretching number of mirtazapine 5, 10, and 20 mg/kg with control using the writhing test. *p<0.05 compared with the control group.

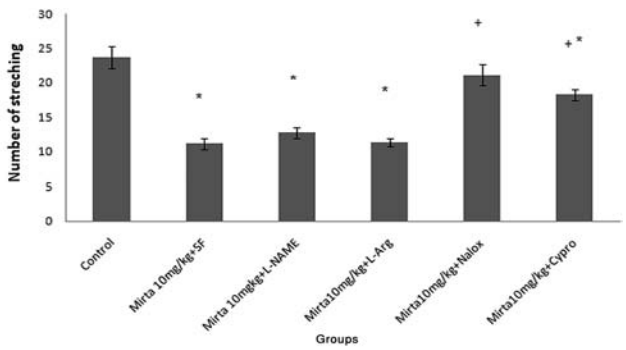

Figure 4 - Evaluated stretching number of mirtazapine 10 mg/kg with control and mirtazapine 10 mg/kg+L-NAME, L-Arginine, naloxone, and cyproheptadine groups using the writhing test. *p<0.05 compared with the control group, ^{+}p<0.05 compared with the mirtazapine 10 mg/kg group. Mirta - mirtazapine, SF - saline, L-Arg - L-Arginine, Cypro - cyproheptadine

10mg/kg+cyproheptadine group (**Figure 2**). In the Writhing test, a statistically significant decrease was observed in the mirtazapine 5 and 10mg/kg groups (higher in the 10mg/kg group) compared with the control group. Although there was also a decrease in the mirtazapine 20mg/kg group compared with the control group, it was statistically not significant (**Figure 3**). When compared with the mirtazapine 10mg/kg group, an increase was observed in the mirtazapine 10mg/kg+L-

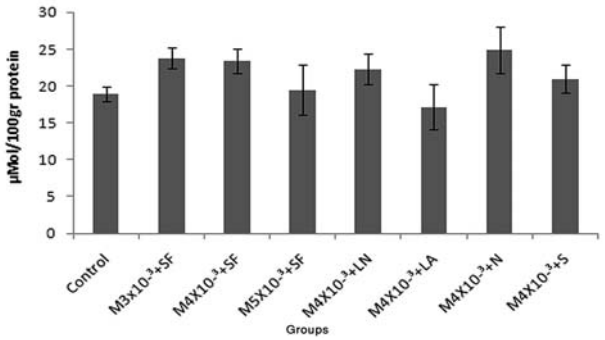

Figure 5 - Assessment of neuronal nitric oxide synthase in the slices of rat hippocampus (p>0.05). M - mirtazapine, SF - saline, LN - L-NAME, LA - L-Arginine, N - naloxone, S - cyproheptadine

NAME 100mg/kg group and mirtazapine 10mg/kg+L-Arginine group, but it was statistically not significant. Similarly, when compared with the mirtazapine 10mg/kg group, a statistically significant increase was found in the mirtazapine 10mg/kg+naloxone group and the mirtazapine 10mg/kg+cyproheptadine group (**Figure 4**).

Assessment of nNOS in the slices of rat hippocampus. There was no statistical difference between groups in terms of nNOS levels of hippocampal slices suggesting that the nitrergic pathway was not involved in this effect (**Figure 5**).

Discussion. In recent years, it has become known that TCA group antidepressants are effective in pain complaints. Many research studies have been conducted in this field. The present study examined if mirtazapine, which is considered a prototype of a newer group of antidepressant agents so called the tetracyclic antidepressant agents, has a potential analgesic effect.

The Tail Clip and Tail Flick pain behavior tests did not show any statistically significant difference between the groups compared with the control group. As already known, the Tail Clip and Tail Flick tests reflect a central pain threshold at the spinal level.[20] In other words, it was concluded that mirtazapine, a tetracyclic antidepressant, had no antinociceptive activity at the spinal level. The results obtained from the Hot Plate test showed that there was a statistically significant increase in the mirtazapine 5, 10, and 20 mg/kg (higher in the 10 mg/kg group) compared with the control group. Although a statistically significant difference was also observed in the mirtazapine 20 mg/kg group compared with the control group, the increase was significantly lower than in the mirtazapine 10mg/kg group. As already known, the Hot Plate test is used for evaluating the pain threshold at the central supraspinal level.[23,24] Consequently, it was found that mirtazapine has significant antinociceptive activity at the supraspinal

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level, and the antinociceptive activity is reduced when the dose is increased, namely, it is a biphasic activity. It is already known that serotonin has analgesic activity at the central level, and algesic activity at the peripheral level.[26] The amount of serotonin is increased when the dose of mirtazapine is increased, which probably results in decreased antinociceptive activity due to the peripheral antinociceptive activity induced by higher amounts of serotonin. Similarly, based on the data obtained from the Hot Plate test, no statistically significant difference was observed in the mirtazapine 10 mg/kg + L-NAME group and the mirtazapine 10 mg/kg + L-Arginine group compared with the mirtazapine 10 mg/kg group. Nitro-L-Arginine Methyl Ester is a nonselective NOS inhibitor. The presence of a significant supraspinal antinociceptive activity in the mirtazapine 10mg/kg group compared with the control group, but absence of any statistically significant difference between the mirtazapine 10mg/kg + L-NAME, and the mirtazapine 10 mg/kg + L-Arginine groups have led to the conclusion that the nitrergic pathway is not involved in the supraspinal antinociceptive mechanisms of mirtazapine. This conclusion was also supported by the absence of any statistical difference in the nNOS measurements performed on the hippocampus slices of rats during the second phase of the study in intragroup and control group comparisons.

In the Hot Plate test, although the mirtazapine 10 mg/kg + naloxone group showed antinociceptive activity compared with the control group, it is statistically not significant. It was also found that in this group, the supraspinal antinociceptive activity induced in the mirtazapine 10mg/kg group was significantly reduced. In other words, mirtazapine showed a significant antinociceptive activity with a dose of 10mg/kg at the supraspinal level, while this activity disappeared to a great extent with combined use of naloxone. Naloxone is a pure antagonist agent, acting on mü, delta, and kappa receptors. It blocks opioid action. Several studies have shown that the opiatergic mechanisms play a major role in the analgesic activity of TCAs.[27-30] Similarly, many studies have demonstrated that the opiatergic system and opiate receptors have a major role in the analgesic mechanism of action of the SSRI group of antidepressants and some atypical antidepressants.[31,32] It can be concluded that opiatergic mechanisms play an important role in the antinociceptive activity of mirtazapine at the supraspinal level, and this antinociceptive activity is inhibited by combined use of naloxone with mirtazapine. In the Hot Plate test, although the mirtazapine 10mg/kg + cyproheptadine group showed supraspinal antinociceptive activity compared with the control group, it is statistically not significant. Also, it was found that the supraspinal antinociceptive activity induced in the mirtazapine 10mg/kg group was significantly reduced in the mirtazapine 10mg/kg + cyproheptadine group. Cyproheptadine is a serotonin receptor blocker, and it was used in the present study to clarify the role of serotonergic mechanisms in the analgesic activity of mirtazapine. The role played by serotonergic mechanisms in the analgesic activity mechanisms of antidepressants has been demonstrated by many studies.[33-35] In the present study, mirtazapine showed significant antinociceptive activity with a dose of 10mg/kg at the supraspinal level, but this activity was blocked to a greater extent with combined use of cyproheptadine as it was in naloxone. These findings suggest that it is likely that the analgesic activity of mirtazapine at the supraspinal level is mainly mediated by serotonergic and opiatergic mechanisms.

In the Writhing test data, the mirtazapine 5 and 10mg/kg groups showed a statistically significant reduction (higher in the 10mg/kg group) compared with the control group. In other words, mirtazapine showed peripheral antinociceptive activity with doses of 5mg/kg and 10mg/kg, but the analgesic activity disappeared when the dose was increased to 20mg/kg. As is already known, the Writhing test is used for evaluating the pain threshold at the peripheral level.[25,36] Consequently, it was found that mirtazapine has a significant antinociceptive activity at the peripheral level, and the analgesic activity is reduced when the dose is increased. It is likely that it is associated with emergence of peripheral algesic activity of serotonin when the dose is increased. According to the data from the Writhing test, the mirtazapine 10mg/kg + L-NAME group, and mirtazapine 10mg/kg + L-Arginine group showed a statistically not significant difference compared with the mirtazapine 10mg/kg group. Presence of a significant peripheral antinociceptive activity in the mirtazapine 10mg/kg group compared with the control group, but absence of any statistically significant difference between the mirtazapine 10mg/kg + L-NAME and mirtazapine 10mg/kg + L-Arginine groups led to the conclusion that the nitrergic pathway is not involved in the peripheral antinociceptive mechanisms of mirtazapine.

Based on the data from the Writhing test, although the mirtazapine 10mg/kg + naloxone group showed antinociceptive activity compared with the control group, it is statistically not significant. It was also found that the peripheral antinociceptive activity in the mirtazapine 10mg/kg + naloxone group was significantly reduced compared with the mirtazapine 10mg/kg group. In other words, mirtazapine showed a significant antinociceptive activity in the 10mg/kg dose at the peripheral level, while this activity disappeared when using naloxone. It can be concluded that opiatergic mechanisms play an important role in the analgesic

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activity of mirtazapine at the peripheral level, and this analgesic activity is blocked by combined use of naloxone. Based on the data from the Writhing test, although the mirtazapine 10mg/kg + cyproheptadine group showed peripheral antinociceptive activity compared with the control group, it is statistically not significant. It was also found that the peripheral antinociceptive activity in the mirtazapine 10mg/kg + cyproheptadine group was significantly reduced compared to the mirtazapine 10mg/kg group. In the present study, mirtazapine showed significant antinociceptive activity with a dose of 10mg/kg at the peripheral level, but using cyproheptadine blocked this activity. In other words, the analgesic activity of mirtazapine at the peripheral level is mediated by serotonergic mechanisms as well as opiatergic mechanisms.

According to the nNOS measurements on the slices of rat brain during the second phase of the present study, there was a statistically not significant difference between the control group and the other groups. Neuronal NOS (nNOS) is found in the central and peripheral neurons.[26,27] During the NOS-mediated transformation of L-Arginine into L-Citrulline, NO is synthesized as a by product. Synthesis of NO depends on the action of nNOS with a calcium-dependent mechanism in the nitrergic neurons. The NOS activity has an unequal regional distribution in the brain, including the hippocampus, striatum, cortex, hypothalamus, midbrain and medulla, from highest to lowest. When the nNOS levels were measured in the perfusates obtained from the hippocampus slices of rats, and were compared with the protein content of homogenates in the specimens of the same tissue, no significant difference was observed in the intragroup and control group comparisons.

In conclusion, mirtazapine, had no antinociceptive activity at the central spinal level, but a significant biphasic analgesic activity at the central supraspinal and peripheral levels in which opioidergic and serotonergic mechanisms played a major role. Mirtazapine did not lead to any change in the central nNOS levels, and the NO and nitrergic pathways are not involved in the central analgesic activity of mirtazapine.

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