

Full Paper

The Different Roles of 5-HT₂ and 5-HT₃ Receptors on Antinociceptive Effect of Paroxetine in Chemical Stimuli in Mice

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Abstract. Serotonin (5-HT) is known to be an important mediator in pain modulation. Some centrally acting agents, like selective serotonin reuptake inhibitors (SSRIs), modulate pain. Activation of the endogenous opioid mechanisms or potentiation of analgesic effect by serotonergic and/or noradrenergic pathways might be involved in antinociception of SSRIs. However, peripheral mechanisms of nociception are not clear. In this study, the antinociceptive effect of paroxetine, its interaction with the opioidergic system and serotonin receptors were tested using the writhing test in mice. Paroxetine (5, 10, 20 mg/kg) induced an antinociceptive effect following i.p. administration in writhing test. For the groups in which the antagonists were tested, the dose of paroxetine that caused a significant and equipotent analgesic effect similar to 0.5 mg/kg morphine was selected. Naloxone significantly antagonized the antinociceptive effects of both paroxetine and morphine in a similar pattern and magnitude. Ketanserin (5-HT₂-receptor antagonist) or ondansetron (5-HT₃-receptor antagonist) alone did not alter the nociceptive action of acetic acid. While the antinociceptive effect of paroxetine was highly potentiated by ketanserin, ondansetron reduced that antinociception. In conclusion, our results indicate that the antinociceptive effect of paroxetine mainly depends on central opioidergic and serotonergic mechanisms. Peripheral serotonergic mechanisms/receptors may contribute to this antinociceptive effect, especially by 5-HT₃-receptor subtypes.

Keywords: paroxetine, nociception, writhing test, 5-HT receptor subtype

Introduction

Pain is an increased sensitivity to harmful stimulations. It occurs through peripheral and / or central mechanisms. Some centrally acting agents, like selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitor (SSRI) drugs, modulate pain. Paroxetine is the most potent inhibitor of serotonin reuptake among SSRIs (1). In clinical practice, paroxetine is used as an adjuvant drug in chronic pain in addition to the treatment of depressive disorders (1, 2). The possible mechanisms for the antinociceptive effect of SSRIs are thought to be central potentiation of the endogenous opioid system or activation of an analgesic effect mediated by serotonergic and/or noradrenergic pathways or combination of these mechanisms (3, 4). However, the peripheral

mechanisms of SSRIs on nociception are not clear.

5-HT is an endogenous bioactive substance that plays a role in the inflammatory chemical milieu as a major chemical mediator and is released from platelets, mast cells, and basophils in injured or inflamed tissues (5 – 8). These cells release 5-HT and other chemical mediators, which might be involved in cascades of inflammatory and hyperalgesic processes (6 – 8).

5-HT is an important mediator in pain modulation with its distinct receptor subtypes located at different levels of the nervous system and shows controversial effects. When intrathecally administered, 5-HT has an antinociceptive effect in acute pain models (9, 10). 5-HT is recognized to be important in inhibition of nociceptive transmission at the spinal level. In the periphery, 5-HT has been shown to produce an algesic response as a component of the inflammatory process (11, 12). Peripherally applied 5-HT has been shown to evoke pain

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in humans (13). A number of 5-HT receptor classes have been identified in peripheral neuronal and non-neuronal tissues (8). 5-HT₃ receptors are located at ends of afferent neurons. In mast cells and mucosal epithelial cells of intestine, 5-HT₂ receptors are present (14–16). Some authors showed that 5-HT₂ and 5-HT₃ receptors are present in mouse intestine (7, 15–18). The roles of 5-HT receptor subtypes (especially 5-HT₂ and 5-HT₃) involved in 5-HT-induced hyperalgesia or analgesia at the peripheral sides are controversial (6–8, 12, 19).

When choosing stimulus parameters in nociception tests, it is essential to control some parameters such as intensity, duration, and the surface area of stimulation. Additionally, it must be reminded that the test to be used must represent the level of antinociception and the phases of pain. We previously showed an antinociceptive effect of paroxetine in the hot plate test (20) which mainly represents the “phasic pain” in terms of short-duration stimuli. The hot plate test with its short stimulation properties show the action on somatic rather than visceral sites. Using an irritant and nociceptive chemical stimulation with a long duration stimuli writhing test represents “tonic pain”. The chemical stimulus induced by acetic acid provokes a continuous and unavoidable pain causing abdominal contractions, movement of whole body, twisting of dorsoabdominal muscles, and a reduction in motor activity which are evidences of visceral but not somatic pain (21, 22).

Both the hot plate and writhing test are commonly used pain tests and are suitable for the evaluation of antinociceptive drugs’ action sites which are at the supraspinal level. Although they are not specific tests, we tried to assess paroxetine’s antinociceptive action with the writhing test, which is more relevant for the clinical situation.

Materials and Methods

Animals

BALB/c mice (30–40 g) of either sex were used in experiments. Eight or ten animals were used in each study group, and half of the animals were male. All of the animals were housed in cages with free access to food and water and they were placed in a quiet and temperature-humidity controlled room ($22 \pm 2^\circ\text{C}$ and $60 \pm 5\%$, respectively) in which a 12:12-h light-dark cycle was maintained. Experiments were conducted between 9:00 and 17:00 h to minimize the diurnal variation. The experimental protocol was approved by the Local Ethics Committee of the School of Medicine, Karadeniz Technical University (2002/48).

Study groups

The animals were divided into 12 groups on the basis of the drugs used (Table 1).

Writhing test

The abdominal constrictor test (23, 24) was performed by the intraperitoneal (i.p.) injection of 3% acetic acid in a volume of 0.1 ml/10 g body weight. The number of stretching movements (arching of back, development of tension in the abdominal muscles, elongation of the body, and extention of the forelimbs) were observed. Stretching movements were started 3 min after acetic acid injection. After a latent period of 3 min, these movements (contractions) were observed and recorded for 10 min. At the end of this observation period, all the animals took a posture of immobilization possibly to relieve abdominal pain, which can negatively affect the criteria of pain selected in this study. The writhing test induced by acetic acid was performed 60 min after the intraperitoneal injection of paroxetine, morphine, or saline (control group) which is within the efficient therapeutic duration range of morphine. Saline at the same volume as the drugs was injected to the animals in Group 1. Naloxone (5 mg/kg), ondansetron (0.1 mg/kg), and ketanserin (0.3 mg/kg) were injected intraperitoneally 30 min after the injection of paroxetine or morphine in mice.

Antinociceptive activity was expressed as the reduction in the number of abdominal constrictions.

Drugs

Paroxetine hydrochloride (Novartis, Istanbul, Turkey), morphine sulphate (Galen, Istanbul, Turkey), naloxone

Table 1. Study groups, drug names, and drug doses

Study groups	Drug	Dose
Group 1	Saline group	10 ml/kg
Group 2	Paroxetine	5 mg/kg
Group 3	Paroxetine	10 mg/kg
Group 4	Paroxetine	20 mg/kg
Group 5	Morphine	0.5 mg/kg
Group 6	Ketanserin	0.3 mg/kg
Group 7	Naloxone	5 mg/kg
Group 8	Ondansetron	0.1 mg/kg
Group 9	Paroxetine + Naloxone	5 mg/kg + 5 mg/kg
Group 10	Morphine + Naloxone	0.5 mg/kg + 5 mg/kg
Group 11	Paroxetine + Ondansetron	5 mg/kg + 0.1 mg/kg
Group 12	Paroxetine + Ketanserin	5 mg/kg + 0.3 mg/kg

n = 8–10 for each group.

hydrochloride (Abbott, Istanbul, Turkey), ketanserin tartrat (Janssen Pharmaceutica, Basel, Switzerland), and ondansetron hydrochloride (Glaxo-Wellcome, Istanbul, Turkey) were dissolved in distilled water. All drugs were injected intraperitoneally in a dose volume of no more than 10 ml/kg of mouse.

Data analyses

Number of writhes were expressed as the mean \pm S.E.M. Statistical analyses were carried out by the one way ANOVA test followed by the post hoc Dunnett's test. The level of significance was set at $P < 0.05$.

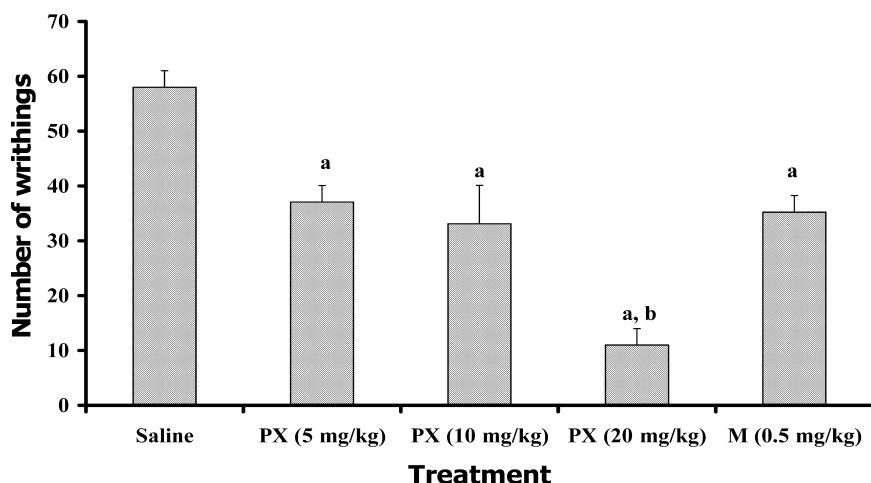


Fig. 1. Antinociceptive effect of paroxetine (5, 10, 20 mg/kg) and morphine (0.5 mg/kg) on chemical stimuli evaluated in the writhing test. Vertical lines show means \pm S.E.M. ($n = 8 - 10$). ^a $P < 0.05$, as compared to saline; ^b $P < 0.05$, as compared to morphine per se. PX: paroxetine, M: morphine.

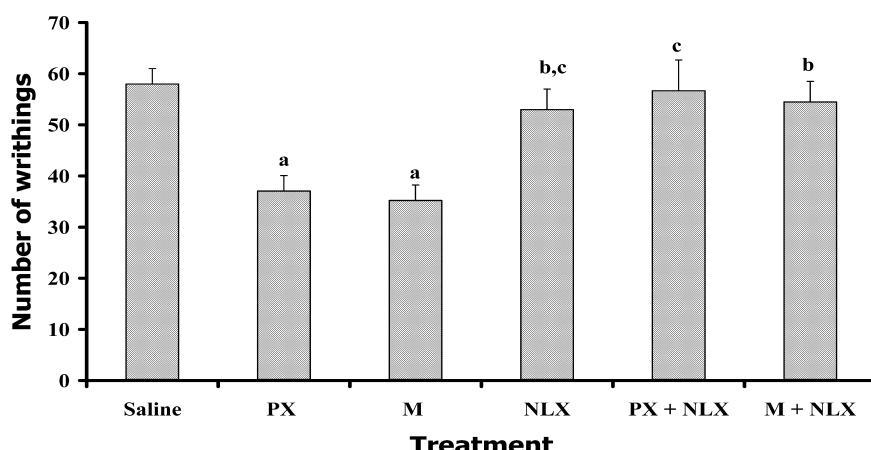


Fig. 2. The inhibitory effect of naloxone (5 mg/kg) on the antinociception induced by paroxetine (5 mg/kg) and morphine (0.5 mg/kg) on chemical stimuli evaluated in the writhing test. Vertical lines show means \pm S.E.M. ($n = 8 - 10$). ^a $P < 0.05$, as compared to saline; ^b $P < 0.05$, as compared to morphine per se; ^c $P < 0.05$, as compared to paroxetine per se. PX: paroxetine, M: morphine, NLX: naloxone.

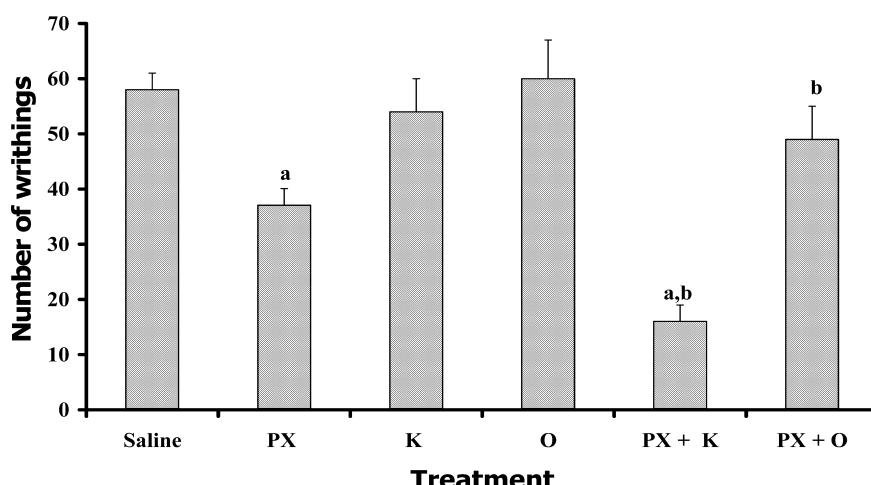


Fig. 3. The contrary effects of ketanserin (0.3 mg/kg) and ondansetron (0.1 mg/kg) on the antinociception induced by paroxetine (5 mg/kg) on chemical stimuli evaluated in the writhing test. Vertical lines show means \pm S.E.M. ($n = 8 - 10$). ^a $P < 0.05$, as compared to saline; ^b $P < 0.05$, as compared to paroxetine per se. PX: paroxetine, K: ketanserin, O: ondansetron.

Results

Intraperitoneally injected morphine (0.5 mg/kg) inhibited the nociceptive effect of acetic acid. Systemic administration of paroxetine (5, 10, and 20 mg/kg) also produced a dose-dependent and significant ($P<0.05$) analgesic effect against acetic acid-induced abdominal constrictions (Fig. 1). The antinociceptive effect of paroxetine at the doses of 5 and 10 mg/kg was equipotent to morphine (0.5 mg/kg). At the dose of 20 mg/kg, paroxetine's effect was greater than morphine's at the dose used in this study calculated on the basis of the decreases in the number of writhes (Fig. 1). In the study groups in which antagonists were used, the dose of paroxetine (5 mg/kg) that caused a significant and equipotent analgesic effect similar to 0.5 mg/kg morphine but did not cause any behavioral and motor activity, was selected.

Naloxone alone did not alter the number of writhes and was not significantly different from the saline group. The antinociceptive effect produced by paroxetine (5 mg/kg) was significantly antagonized by naloxone at the dose of 5 mg/kg (Fig. 2). At the same dose, naloxone also antagonized the antinociceptive effect of morphine (0.5 mg/kg). The patterns and magnitudes of this inhibition due to naloxone were similar both for morphine and paroxetine (Fig. 2).

Ketanserin (a selective 5-HT₂-receptor antagonist, 0.3 mg/kg) or ondansetron (a selective 5-HT₃-receptor antagonist, 0.1 mg/kg) alone did not alter the nociceptive action of acetic acid. While the antinociceptive effect of paroxetine was highly potentiated by ketanserin (0.3 mg/kg), ondansetron (0.1 mg/kg) reduced the antinociceptive effect of paroxetine (Fig. 3).

Discussion

The analgesic effect of SSRIs has been shown both in animal models (3, 25–27) and human cases suffering from different types of chronic (28–31) but not acute pain. Each SSRI drug may have a different mechanism of analgesic effect. Among their possible antinociceptive mechanisms, opioidergic (3, 4, 26) and noradrenergic (3, 32) pathways/receptors are listed in addition to serotonergic ones. In our study, we found that paroxetine caused an antinociceptive effect similar to that of morphine in magnitude in the writhing test. Naloxone, an opioid receptor antagonist, decreased both the antinociceptive effects of morphine and paroxetine, in a comparable manner. This inhibition clearly shows the involvement of opioidergic mechanisms/receptors in the antinociceptive effect of paroxetine. In several studies, authors have reported a central potentiation of the endogenous

opioidergic system that was related to the analgesic effect of SSRIs (3, 4, 20, 32).

The basic mechanism underlying their central, especially antidepressant effect is a selective serotonin reuptake inhibition and an indirect interaction with 5-HT receptors via 5-HT. This inhibitory action was well demonstrated in the central nervous system, but was not very clear in peripheral sites, like isolated vas deferens (33, 34). If it is valid in peripheral tissues, an increase in serotonergic activity would be expected. Indeed, 5-HT is known as a nociceptive mediator when peripherally applied (8). These controversial findings suggest a limited interaction for SSRIs (paroxetine in this case) with 5-HT in their algesic action process. Central mechanisms probably precede the peripheral mechanism(s) of action in the antinociception obtained by systemically (i.p.) applied paroxetine.

5-HT, when peripherally applied, is a potent proinflammatory and noxious agent, which causes hyperalgesia both in human and rodents (6). 5-HT, released from platelets, mast cells, and basophils in injured or inflamed tissues may play a role in inflammatory chemical milieu. Primary hyperalgesia, which is the major symptom of inflammation and tissue injury, probably caused by acetic acid, was the result of sensitization of nociceptors by a variety of inflammatory mediators. One of these mediators is 5-HT, and it has been shown that it can sensitize peripheral nerve fibers to other inflammatory mediators such as bradykinin (35).

In our study, while ondansetron inhibited the antinociceptive effect of paroxetine, ketanserin could not. This finding suggests a contribution of 5-HT₃-receptors, rather than 5-HT₂ types, in the antinociceptive effect of paroxetine in our study. Peripheral 5-HT₂ and presynaptic 5-HT_{1A} receptors have been clearly shown to be involved in 5-HT-induced hyperalgesia (6). There are controversial results about the roles of 5-HT₃-receptors in nociception. Zeitz et al. (7), Giordano et al. (12, 36), and Sufka et al. (37) showed a hyperalgesic effect for 5-HT₃ agonists, but some authors reported that they had an analgesic effect (10, 19, 38–40).

When 5-HT₂-receptors were blocked by ketanserin, which does not have any algesic or analgesic effect alone, the antinociceptive effect of paroxetine was potentiated. However, when 5-HT₃-receptors were blocked by ondansetron, which does not have any algesic or analgesic effect alone, the antinociceptive effect of paroxetine was inhibited. These findings together suggest an involvement of 5-HT₂-receptors in hyperalgesia and 5-HT₃-receptors in analgesia in chemically stimulated pain.

In conclusion, our results indicate that the antinoci-

ceptive effect of paroxetine mainly depends on central opioidergic and/or serotonergic mechanisms. The contribution of peripheral serotonergic mechanisms or receptors, especially 5-HT₃ subtypes, cannot be excluded in the antinociceptive effect of paroxetine.

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