J Pharmacol Sci **94**, 161 – 165 (2004)

Journal of Pharmacological Sciences
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Full Paper

Possible Involvement of Opioidergic and Serotonergic Mechanisms in Antinociceptive Effect of Paroxetine in Acute Pain

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Received October 15, 2003; Accepted December 22, 2003

Abstract. Antidepressant drugs, especially tricyclics have been widely used in the treatment of chronic pain, but not in acute pain. Because of numerous undesirable side effects, the selective serotonin reuptake inhibitors (SSRIs), with their favorable side effect profile, are preferred nowadays. An activation of the endogenous opioid mechanisms or potentiation of the analgesic effect mediated by serotonergic and/or noradrenergic pathways are thought to be involved in the antinociceptive action of SSRIs. In this study, the potential antinociceptive effect of paroxetine and its interaction with opioidergic system and serotonin receptors were evaluated. The antinociceptive effect of paroxetine was tested using a hot plate test in mice. Paroxetine, a SSRI antidepressant drug, induced an antinociceptive effect following i.p. administration. This antinociception was significantly inhibited by naloxone, an opioid receptor antagonist, suggesting the involvement of opioidergic mechanisms. While ondansetron (a 5-HT_3-receptor antagonist) inhibited the effect of paroxetine, ketanserin (a 5-HT_2-receptor antagonist) could not. In conclusion, paroxetine-induced antinociception, similar to morphine, suggests an involvement of direct or indirect action (via an increase in release of endogenous opioid peptide(s)) at opioid receptor sites and an involvement of serotonergic mechanisms mainly at the receptor level.

Keywords: paroxetine, nociception, hot plate test

Introduction

Antidepressant drugs are widely used in the treatment of chronic pain states as an adjuvant or alone (1 – 3). The analgesic effect of antidepressants is not parallel to their antidepressant action. The antidepressant action occurs after several weeks of treatment (4) while analgesic action is prominent after an acute single dose administration, which is at a lower dosage than that required for its antidepressant effect (4, 5).

Tricyclic antidepressants, even though their antinociceptive mechanism is not clear, have been used for decades in the treatment of chronic pain patients without depression. Because of numerous undesirable side effects of traditional tricyclics, the selective serotonin reuptake inhibitors (SSRIs), with a favorable side effect profile, are preferred. A direct activation of the endogenous opioid system or potentiation of a mixed analgesic effect mediated by serotonergic and/or noradrenergic pathways or combinations of these mechanisms are thought to be involved in the antinociceptive action mechanisms of SSRIs. Paroxetine is the most potent inhibitor of serotonin re-uptake among SSRIs (6, 7). In clinical practice, paroxetine is used in the treatment of depressive disorders and as an adjuvant drug in chronic pain (2, 6).

The present study was designed to investigate if paroxetine's clinically positive analgesic effect on chronic pain (1, 2) was still relevant in acute nociception and the possible roles of opioidergic and serotonergic systems in the antinociceptive effect of the drug. The central antinociceptive activity was evaluated by using a hot plate test as an animal (mouse) model in order to evaluate the pain relieving property of paroxetine.

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Materials and Methods

Animals

All of the animals were housed in cages with free access to food and water. The cages were placed in a quiet and temperature-humidity controlled room (22 ± 2°C and 60 ± 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 School of Medicine, Karadeniz Technical University (2002/48).

Procedure

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. Group 1 was the control group. Saline at the same volume of other drugs was injected into the Group 1 animals.

In preliminary studies, the submaximal antinociceptive effective dosage of paroxetine that did not induce sedation (rotarod test) was found to be 5 mg/kg. Depending on this finding, paroxetine in a dose of 5 mg /kg was used in the whole study. In Group 2, paroxetine (5 mg/kg) alone was intraperitoneally (i.p.) injected. Group 3 mice were injected i.p. morphine at a dose of 0.5 mg/kg (8). Animals in Group 4 were i.p. injected with both paroxetine (5 mg/kg) and naloxone (5 mg /kg). In Group 5, both morphine (0.5 mg/kg) and naloxone (5 mg/kg) (8) were i.p. injected.

Animals in both Groups 6 and 7 received i.p. injections of paroxetine (5 mg/kg). Thirty minutes after receiving a paroxetine injection, 5-HT-receptor antagonists ondansetron (0.1 mg/kg) in Group 6 and ketanserin (0.3 mg/kg) in Group 7 were i.p. injected into the animals.

Hot-plate test

The test was based on that described by Singh et al. and Eddy and Leinbach (8, 9). A transparent glass cylinder (16-cm-high, 16-cm diameter) was used to keep the mouse on the heated surface of the plate. The temperature of the hot plate was set to 55 ± 0.5°C (8) by using a thermoregulated water-circulating pump. The time of latency was defined as the time period between the zero point when the animal was placed on the hot plate surface and the time when the animal licked its back paw or jumped off to avoid thermal pain. Baseline latency (pretreatment value) was determined before experimental treatment (preinjection) for each mouse. Post treatment latencies were determined after 60 min. for saline, paroxetine (5 mg/kg), and morphine (0.5 mg /kg) injections. For naloxone (5 mg/kg), ketanserin (0.3 mg/kg) and ondansetron (0.1 mg/kg), which were injected as second drugs, post treatment latencies were determined 30 min after the second and 60 min after the first injection. The accepted "zero time" of this study means 60 min after the first drug injection or if necessary 30 min after the second drug injection. To minimize tissue damage, a cut-off time (removing from the plate) of 30 s was adopted. The latencies of both forepaw licking or jumping were measured for each animal at 0, 30, 60, 90, and 120 min in order to calculate the percent maximum possible effect (% MPE) values for these time points (Fig. 1).

Drugs

Paroxetine hydrochloride (Novartis, Istanbul, Turkey), morphine sulphate (Galen, Istanbul, Turkey), naloxone hydrochloride (Abbott, Istanbul, Turkey), ketanserin tartrate (Janssen Pharmaceutica, Basel, Switzerland), and ondansetron hydrochloride (Glaxo-Welcome, Istanbul,

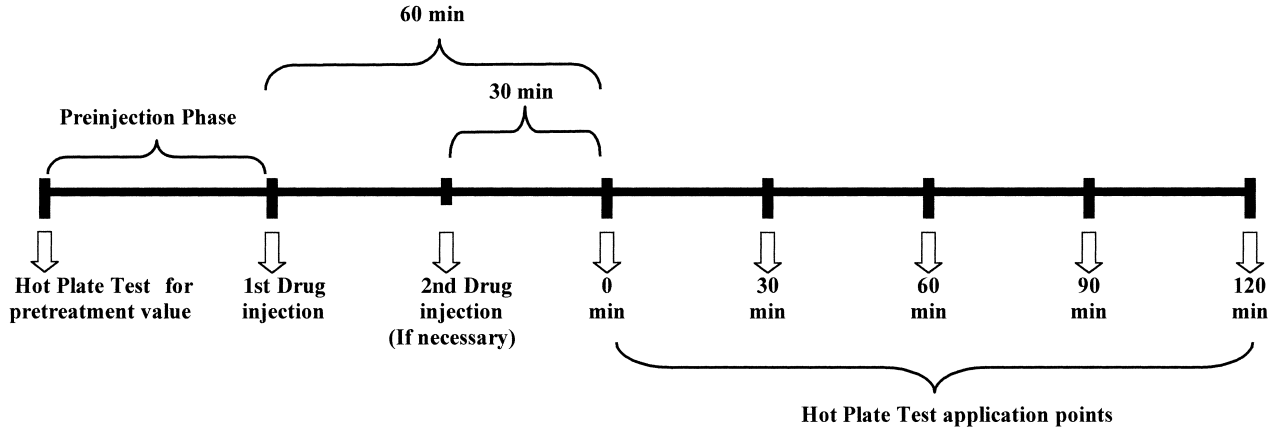


Fig. 1. Time schedule of the study protocol. 0 min shows the "zero time" mentioned as the accepted "zero time" of the study that means 60 min after the first drug injection or if necessary 30 min after the second drug injection.

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

Nociceptive thresholds for the hot plate test were converted to % MPE according to the following formula (10):

$$\% \text{ MPE} = \frac{(\text{Post treatment value}) - (\text{Pretreatment value})}{(\text{Cut off value}) - (\text{Pretreatment value})} \times 100$$

The data were expressed as the mean ± S.E.M. Statistical analyses were carried out by the one way ANOVA test. The level of significance was set at $P<0.05$.

Results

The effect of paroxetine against thermal nociception in mice

Systemic administration of paroxetine (5 mg/kg) produced a significant antinociceptive effect against thermal nociception in the hot plate test at 0, 30, and 60 min (Fig. 2). The antinociceptive effect of paroxetine was similar to that of morphine (0.5 mg/kg) in the hot plate test (Fig. 2).

The effect of naloxone, ketanserin, and ondansetron on the antinociceptive effect of paroxetine in mice

The antinociceptive effect produced by morphine (0.5 mg/kg) was antagonized by naloxone (5 mg/kg), an opioid antagonist. Naloxone inhibited not only the antinociceptive effect of morphine but also the antinociceptive effect of paroxetine (5 mg/kg) in a comparable manner (Fig. 3). Ondansetron (0.1 mg/kg), a selective 5-HT_3-receptor antagonist, also antagonized the antinociceptive effect of paroxetine. However, ketanserin (0.3 mg/kg), a selective 5-HT_2-receptor antagonist, could not inhibit the antinociceptive effect of paroxetine (Fig. 4).

Discussion

The analgesic effect of SSRIs has been shown both in animal models (8, 11 – 13) and human cases suffering from different types of chronic (1 – 3, 5, 14) but not acute pain. Each SSRI drug may have a different mechanism of analgesic action. Among the possible mechanisms, opioidergic (11, 12, 15) and noradrenergic (12, 16) mechanisms are listed in addition to serotonergic ones. In our study, paroxetine induced an antinociceptive effect starting approximately 60 min after injection and lasting for 60 min following i.p. administration in the hot plate test. The antinociceptive effect of paroxetine was similar to that of morphine in magnitude. Naloxone, an opioid receptor antagonist, decreased both of the antinociceptive effects of morphine and paroxetine, in a comparable manner. This inhibition clearly shows the interaction between opioidergic mechanisms/receptors and the antinociceptive action of paroxetine.

The antidepressant mechanisms of SSRI drugs are attributed to an increase in the amount and action of serotonin in the synaptic gap due to its re-uptake inhibitory effect of serotonin on the presynaptic site (6, 7). SSRI drugs indirectly act on presynaptic or postsynaptic 5-HT receptors activated by serotonin. In our study, while ondansetron inhibited the antinociceptive effect of paroxetine, ketanserin could not. This finding suggests a contribution of 5-HT_3 receptors rather than 5-HT_2 types, in the antinociceptive action of paroxetine.

Control of analgesia is performed by the descending inhibitory pathways in the central nervous system (CNS). The key part of this descending system is the periaqueductal grey area (PAG). PAG receives inputs from different brain regions and is assumed to be a gate in control of nociception, especially in the dorsal horn. PAG mainly stimulates the nucleus raphe magnus (NRM) and some fibers in the spinal cord, which form synaptic connections on dorsal horn interneurons. 5-HT is the major transmitter both at these synapses and the pathway from the NRM to the substantia gelatinosa of the dorsal horn. Activation of this pathway inhibits transmission specifically in nociceptive pathways (17). The 5-HT_3 receptors located in the dorsal horn of the rat spinal cord have been shown to mediate an antinociceptive effect (18).

The descending inhibitory pathway is probably an important spinal site of action for opioid analgesics. It was shown that naloxone, an opioid antagonist, prevented electrically induced analgesia, which would suggest that opioid peptides may function as transmitters in this system (19).

It is thought that the 5-HT descending system plays an important role in morphine analgesia (8, 19). It is suggested that the analgesic action of morphine was found to be independent of central 5-HT levels. On the other hand, the analgesic action of systemic opioids can be blocked by depletion of 5-HT by inhibiting its synthesis (8).

Both 5-HT_2 and 5-HT_3 receptors are present in the CNS. 5-HT_3 receptors are presynaptically located and mediated fast synaptic transmission in the CNS (20, 21). Most of the authors reported that 5-HT_3-receptor activation had an antinociceptive action (22 – 24). Only Ali et al. (25) showed a facilitating effect of 5-HT_3-

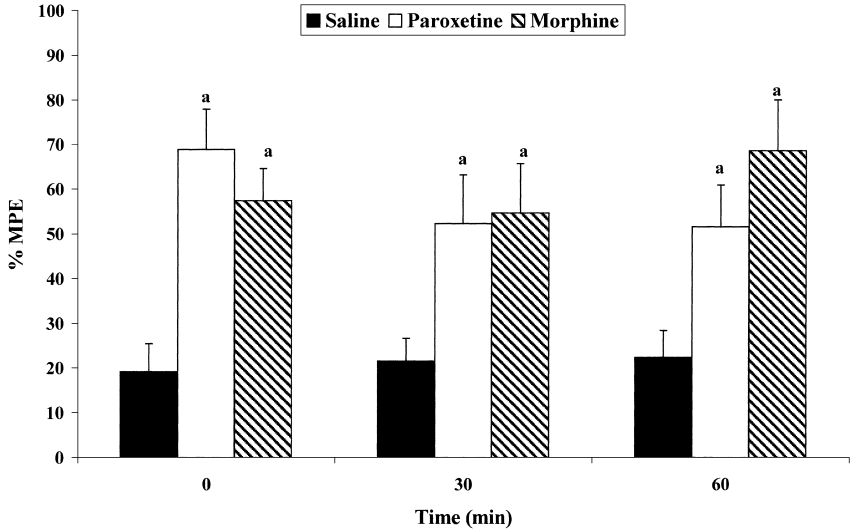


Fig. 2. Antinociceptive effects of paroxetine (5 mg/kg) and morphine (0.5 mg/kg) on thermal pain evaluated as percent maximum possible effect (% MPE) in the hot plate test. Vertical lines show means ± S.E.M. (n = 8 – 10). [a]*P*<0.05 as compared to control.

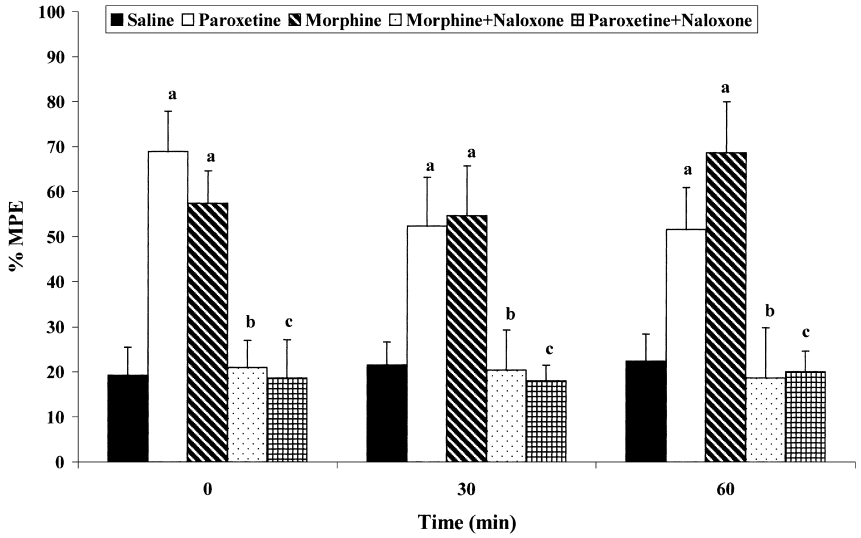


Fig. 3. The inhibitory effect of naloxone (5 mg/kg) on the antinociception induced by paroxetine (5 mg/kg) and morphine (0.5 mg/kg) on thermal pain evaluated as percent maximum possible effect (% MPE) in the hot plate test. Vertical lines show means ± S.E.M. (n = 8 – 10). [a]*P*<0.05, as compared to the control; [b]*P*<0.05, as compared to morphine per se; [c]*P*<0.05, as compared to paroxetine per se.

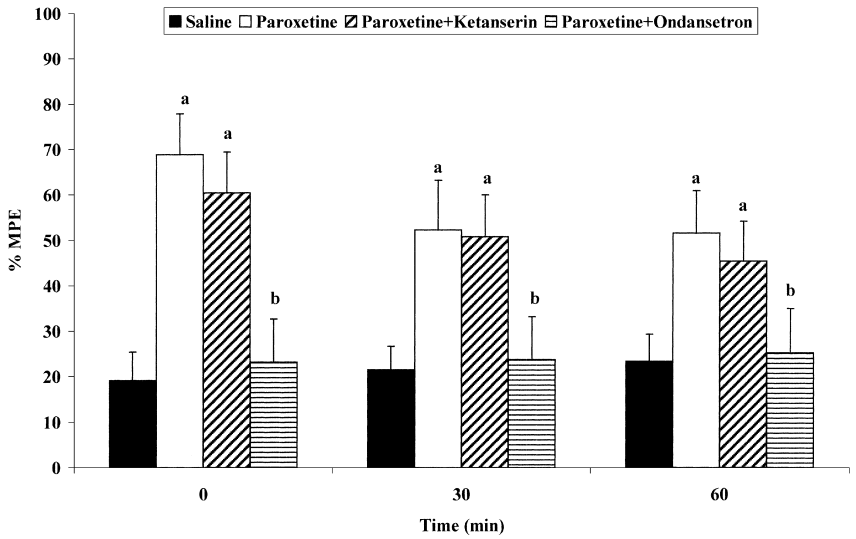


Fig. 4. The contrary effects of ketanserin (0.3 mg/kg) and ondansetron (0.1 mg/kg) on the antinociception induced by paroxetine (5 mg/kg) on thermal pain evaluated as percent maximum possible effect (% MPE) in the hot plate test. Vertical lines show means ± S.E.M. (n = 8 – 10). [a]*P*<0.05, as compared to the control; [b]*P*<0.05, as compared to paroxetine per se.

receptor activation on nociception. The 5-HT_3-receptor antagonists did not show any nociceptive or antinociceptive action when they were used alone (26), but these drugs can inhibit the antinociceptive action of some compounds thought to activate the 5-HT receptors (22, 23, 26).

In conclusion, paroxetine has a clear antinociception, which is inhibited by ondansetron and naloxone. This combined finding suggests an involvement of serotonergic mechanisms, serotonergic receptors (5-HT_3 subtype), and the opioidergic system (via an increase in release of endogenous opioid peptide(s)) or opioid receptors in antinociception induced by a SSRI drug, paroxetine.

Acknowledgments

This study was supported by Karadeniz Technical University Research Fund (Grant No. 22.114.002.4). The authors wish to thank Mr. F. Aydın for his special contribution. The authors are also thankful to Ms. Janice O. Vantrease for reviewing the English grammar of this manuscript.

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