Dose-Dependent Antinociceptive Effects of Tramadol Over Time and Contribution of Noradrenergic, Serotonergic, and Opioidergic Receptors

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Abstract: Our goal was to demonstrate the analgesic efficacy of two different doses of tramadol over time and the contribution of opioidergic, noradrenergic, and serotonergic mechanisms in the antinociceptive effects of tramadol using the thermal plantar withdrawal latency test in rats. α_2-adrenoceptor, 5-HT$_3$, and opioid receptor antagonists were used to assess the mechanisms of antinociception. Forty-eight rats received intraperitoneal injection of tramadol alone or together with antagonist according to the allocated group (n=6). Group 1: tramadol (10 mg kg^{-1}); Group 2: tramadol (10 mg kg^{-1}) + naloxone (1.2 mg kg^{-1}); Group 3: tramadol (10 mg kg^{-1}) + yohimbine (1 mg kg^{-1}); Group 4: tramadol (10 mg kg^{-1}) + ondansetron (1 mg kg^{-1}); Group 5: tramadol (20 mg kg^{-1}); Group 6: tramadol (20 mg kg^{-1}) + naloxone (1.2 mg kg^{-1}); Group 7: tramadol (20 mg kg^{-1}) + yohimbine (1 mg kg^{-1}); Group 8: tramadol (20 mg kg^{-1}) + ondansetron (1 mg kg^{-1}). Thermal withdrawal latency was recorded with the plantar test apparatus before and at 15, 30, 60, 90, and 120 minutes after injection. $p < 0.05$ was considered significant. Peak antinociceptive effects of low and high dose tramadol were seen at 30 and 60 minutes, respectively. The antinociceptive effects of tramadol were attenuated at different levels through opioidergic, α_2-adrenergic, and serotonergic-5-HT$_3$ receptors. The time to reach the peak analgesic effect is longer with the higher tramadol dose. Antinociceptive effectiveness of tramadol and the contribution of different receptors are affected by the dose of tramadol.

INTRODUCTION

Tramadol is a commonly used opioid drug to treat a variety of pain syndromes. It provides pain therapy at multiple levels with its opioidergic and monoaminergic action mechanisms[1,2]. O-desmethyl-tramadol's primary metabolite O-desmethyl tramadol provides analgesia through interaction with opioid receptors and serotonin blockade (5-HT). Tramadol also has antinociceptive effects at the spinal level through norepinephrine and serotonin uptake inhibition[3].

Information from animal studies demonstrates a consistent correlation to human analgesia[4]. The pharmacological properties (e. g., efficacy, potency, mechanisms of action) of tramadol have been demonstrated previously in rodent models of acute nociception and chronic pain[2,5-7]. In previous rodent studies, there are differences concerning the analgesic efficacy of tramadol doses and the contribution of different receptors in tramadol's antinociceptive effects[8-10].

Using the thermal withdrawal latency test (Hargreaves' method), the study evaluates two tramadol dosages in Wistar-Albino rats[11]. Our goal was to demonstrate the analgesic efficacy and the antinociceptive time effect of two different doses of tramadol and the contribution of opioidergic, noradrenergic, and serotonergic mechanisms.

METHODS

Animal subjects

Institutional Animal Experiments Ethics Board approved the study (2019-40). The rats were treated according to the "Guide for the care and use of laboratory animals"[12]. Forty-eight male Wistar-Albino rats (350-450 g) were used in the experiments. The rats were housed in separate cages with water and food available at libitum. The animals were randomized into eight groups, which consisted of six rats in each group.

Drugs and their administration

According to the allocated group, rats received an intraperitoneal (i.p.) injection of 10 or 20 mg kg^{-1} of tramadol alone or with an antagonist drug. Group 1 received 10 mg kg^{-1} of tramadol; Group 2 received 10 mg kg^{-1} of tramadol + 1.2 mg kg^{-1} of naloxone; Group 3 received 10 mg kg^{-1} of tramadol + 1 mg kg^{-1} of yohimbine; Group 4 received 10 mg kg^{-1} of tramadol + 1 mg kg^{-1} of ondansetron; Group

5 received 20 mg kg^{-1} of tramadol; Group 6 received 20 mg kg^{-1} of tramadol + 1.2 mg kg^{-1} of naloxone; Group 7 received 20 mg kg^{-1} of tramadol + 1 mg kg^{-1} of yohimbine; Group 8 received 20 mg kg^{-1} of tramadol + 1 mg kg^{-1} of ondansetron. The doses of the drugs were based on previous studies[13-15]. The receptor antagonists were used to test the probable mechanisms of tramadol's analgesic efficacy. The selected antagonist drugs were effective against the alpha-adrenergic receptors (yohimbine), 5-HT receptors (ondansetron), and opioid receptors (naloxone). The selected antagonist was administered at the same time with tramadol injection.

Behavioural testing

Experimental procedures: The thermal withdrawal latency was determined in all groups of rats before and at 15, 30, 60, 90, and 120 minutes after the drugs' injection. Thermal hyperalgesia was assessed with the plantar test apparatus (MAY PWAM 0903 ®, Ankara, TR) as described earlier[16]. Animals were placed individually into a glass-floored clear plastic cage and accommodated for 15 minutes. Paw withdrawal latencies were measured by heating the right hind paw's plantar surface with an infrared radiant heat source beam. The device automatically recorded the time taken from the onset of the stimulus to the withdrawal of the paw. As described earlier, the heat intensity was adjusted to produce a withdrawal latency of 10 seconds in naïve rats, and tissue damage was avoided by setting a cut-off at 15 seconds[17].

Drugs

Tramadol (Tradolex ®, Menta Pharma, Istanbul, TR), naloxone (naloxone hydrochloride dihydrate) and yohimbine (yohimbine hydrochloride) (Sigma-Aldrich, MO, USA), and ondansetron (Ondaren®, Vem, Ankara, TR) were dissolved to the calculated concentration in 0.9% NaCl solution and injected in 2 ml of volume.

Statistical analysis

Statistical data analysis was conducted using version 22.0 of the SPSS for Windows (SPSS, Inc, Chicago, IL, USA). Latencies are presented as the mean ± SEM. Per cent changes in the mean values of successive measurement times tested were compared with baseline values measured before injection. The Friedman test was used if there was significant differentiation between time-dependent measurements of each group. In the Friedman test, Wilcoxon sequential signs test was used to determine which time periods the differentiation was significant in significant differentiation cases. $p < 0.05$ was considered significant.

FINDINGS

Compared to baseline (before injection), i.p. tramadol administration resulted in significantly longer thermal withdrawal latency at 15th, 30th, 60th, and 90th min in rats that received 10 mg/kg (Group 1), and at 30th, 60th, and 90th min in rats which received 20 mg/kg of tramadol (Group 5) ($p < 0.05$) (Figure 1, Figure 2)

Low dose tramadol (10 mg kg^{-1}):
Thermal withdrawal latency was significantly longer only at the 30th min in rats that received tramadol (Group 1) compared to rats receiving tramadol + naloxone (Group 2) ($p < 0.05$). Latency was significantly longer at the 15th, 30th, 60th, 90th, and 120th min in rats that received tramadol (Group 1) than in rats receiving tramadol + yohimbine (Group 3) ($p < 0.05$). Latency was significantly longer in rats that received only tramadol (Group 1) compared to rats receiving tramadol + ondansetron (Group 4) at the 15th, 30th, 60th, and 90th min ($p < 0.05$) (Figure 1).

High dose tramadol (20 mg kg^{-1}):
Thermal withdrawal latency was significantly longer at the 30th and 60th min in rats that received tramadol (Group 5) compared to rats receiving tramadol + naloxone (Group 6) and tramadol + ondansetron (Group 8) ($p < 0.05$). Latency was also significantly longer at 30th and 60th min in rats that received tramadol (Group 1) compared to rats receiving tramadol + yohimbine (Group 7) ($p < 0.05$) (Figure 2).

DISCUSSION

We studied the antinociceptive effect of tramadol in an acute pain model produced by thermal hyperalgesia. Our results show that tramadol's antinociceptive effect is dose-dependent. The peak antinociceptive effect of higher dose tramadol is observed later than the lower dose. Furthermore, the antinociceptive effect of different doses of tramadol is attenuated at different levels by nonselective opioid receptor antagonist naloxone, selective α_2-adrenoceptor
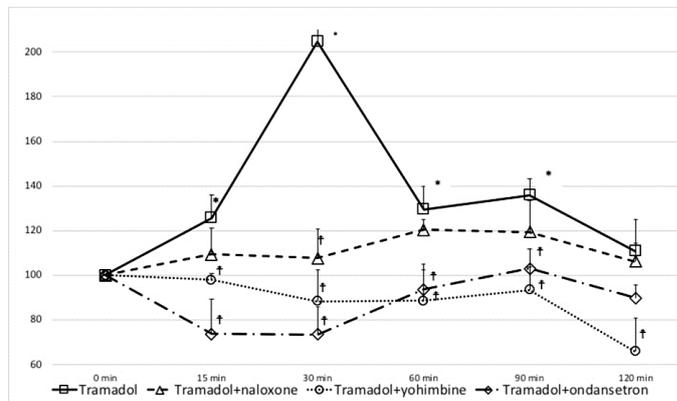

Figure 1. Effects of 10 mg kg-1 supercript tramadol (n=6) on thermal withdrawal latency and the effects of naloxone (n=6), yohimbine (n=6), and ondansetron (n=6) on tramadol elicited latencies.
Results are given as % changes compared with baseline values measured before injection. Data are presented as mean ± SEM, * $p < 0.05$ compared to baseline, † $p < 0.05$ compared to tramadol
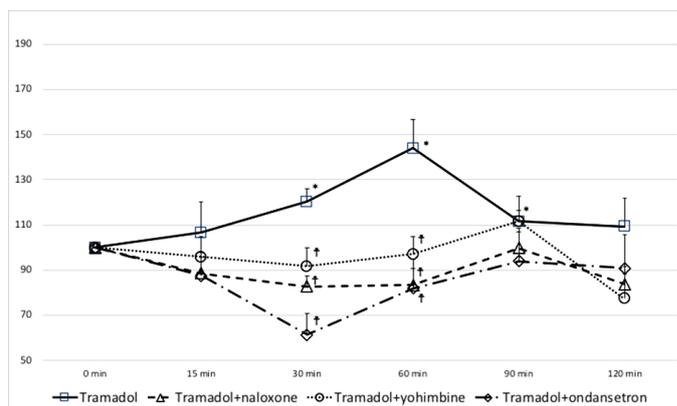

Figure 2. Effects of 20 mg kg-1 supercript tramadol (n=6) on thermal withdrawal latency and the effects of naloxone (n=6), yohimbine (n=6), and ondansetron (n=6) on tramadol elicited latencies.
Results are given as % changes compared with baseline values measured before injection. Data are presented as mean ± SEM, * $p < 0.05$ compared to baseline, † $p < 0.05$ compared to tramadol.

antagonist yohimbine, and serotonin-5HT$_3$ receptor antagonist ondansetron at different time points.

Researchers have developed various tests to evaluate different types of pain in animal models[1]. These tests evaluate the drug's ability to increase nociceptive thresholds. The studies utilize behavioural assays such as the tail-flick and hot plate tests[8]. The present study assessed tramadol's analgesic efficacy by using the thermal withdrawal latency test. The thermal withdrawal latency test involves recording the latency for withdrawing the paw from the thermal stimulus, and besides spinal pathways, it principally measures the supraspinal responses to a peripheral stimulus. In the present study, i.p., administration of tramadol significantly prolonged the rat's paw withdrawal latency.

Tramadol shows a low affinity for opioid receptors. (+) -O-desmethyl tramadol, the major metabolite of tramadol, is a potent μ-opioid receptor agonist[18]. In our study, co-administered naloxone, a nonselective opioid receptor antagonist, partially blocked the effects

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of 10 mg kg^{-1} and 20 mg kg^{-1} of tramadol. This partial blockade by naloxone was also reported by other researchers[5,19,20]. Tramadol's opioidergic effect is mainly mediated by spinal opioid receptors[13]. This partial blockade by naloxone points out that other mechanisms are also involved in tramadol analgesia besides the opioidergic mechanism. Previously tramadol's analgesic action has been suggested 40% via opioid mechanism[5,21] and 60% via activation of descending antinociceptive systems and suppression of amine reuptake[5,13,22].

According to previous knowledge, our results show that besides being an μ-opioid receptor agonist, tramadol antinociception is also partially inhibited by α_2-adrenergic antagonist yohimbine and 5-HT$_3$ antagonist ondansetron. Nociceptive transmission in the spinal cord is essentially suppressed by noradrenaline and serotonin. Activation of α_2-adrenergic receptors in the spinal cord inhibits nociceptive transmission by inhibiting the release of excitatory neurotransmitters from primary afferent terminals[23]. Antinociceptive action of 5-HT in the spinal cord involves several receptor subtypes, including the 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{2A}$, 5-HT$_{2C}$, and 5-HT$_3$ receptors[24]. Kimura et al.[13] have suggested that the increase of 5-HT$_3$ in the spinal cord plays an essential role in tramadol's antinociceptive effects. Our results support Kimura et al.[13] and Arcioni et al.[25] findings. In the present study, selective 5-HT$_3$ antagonist ondansetron significantly attenuated the antinociceptive effects of tramadol. Ondansetron is a commonly used antiemetic drug that prevents and treats nausea and vomiting. Clinically relevant interactions between the two drugs have been reported[24,25].

The effects of yohimbine on tramadol's analgesic properties may vary with species. While yohimbine blocks the antinociceptive effect of tramadol in rats and humans[26], mice studies' results are inconsistent. Pre-treatment of mice with yohimbine either increases[27], blocks[20], or does not affect tramadol's antinociception[5]. These differences in the effect of yohimbine on tramadol antinociception observed in mice may be due to different experimental pain models, drug doses, routes of administration, and strains of mice. Antinociception elicited by tramadol at the spinal level may be mainly due to its indirect activation of spinal α_2-adrenoceptors. Supraspinal activation of the monoaminergic inhibitory neuronal system has also been considered as an essential source of tramadol's antinociception[26].

With Wistar-Albino rats and the thermal plantar test method, we observed the peak antinociceptive effect of 10 mg kg^{-1} and 20 mg kg^{-1} of tramadol at the 30th and 60th min, respectively. The delay in the peak analgesic effect with the higher dose needs commenting. This finding is similar to Jang et al.[28], which observed peak analgesia with i.p. tramadol at 30th min with 10 mg kg^{-1} and at 60th min with 20 mg kg^{-1} in rats as assessed by the tail-flick method. Moreover, Dhasmana et al.[10] studied the analgesic properties of 25, 50, and 75 mg kg^{-1} of subcutaneous tramadol using the hot-plate method. Like our results, the higher dose of tramadol's peak effect was observed later than the lower dose. They determined the peak effect at the 20th min with 50 mg kg^{-1} and the 40th min with 75 mg kg^{-1} of tramadol. These results consist of a discrepancy with other rat studies[7,10,11]. In Guneli et al.[29] study, the peak antinociceptive effect of 10 mg kg^{-1} and 20 mg kg^{-1} of tramadol was achieved at the 45th and 30th min, respectively. The mentioned study used Wistar rats and the neuropathic pain model of acute pain. Bianchi et al.[7] used Sprague–Dawley CD rats to assess whether tramadol effects are pre-emptive or fully anti-hyperalgesic. The researchers administered tramadol before or after the induction of hyperalgesia. When given before the hyperalgesia induction, the peak antinociceptive effect of 10 mg kg^{-1} of tramadol was recorded at the 30th min. The difference in the time of peak effect between different tramadol doses may be affected by the route of administration, dose, and experimental pain model [8-11].

The concentrations of substances which modulate antinociception may differ at different time points, and the individual receptors' contribution to pain modulation of tramadol may vary according to the tramadol dose. Tramadol produces analgesia via spinal and supraspinal pathways. Activation of the opioid receptors and the inhibition of neuronal uptake of noradrenaline and 5-HT are the main mechanisms contributing to the drug's antinociceptive action.[2] A microdialysis study in a postoperative pain model in rats by Kimura et al.[13] showed that noradrenaline and 5-HT concentrations of the spinal cord increased with time, peaking at 30 minutes after i.p. injection of tramadol. The activity of supraspinal (central) opioid receptors may also vary over time. We did not assess motor function because we aimed for an effective dose without the risk of impaired motor function based on previous studies[1,5]. Nevertheless, probable impaired motor function with the higher tramadol dose may be another compounding factor. Previously, Cannon et al.[1] found that receiving 25 mg kg^{-1} or 50 mg kg^{-1} of tramadol impaired motor function in rats. Loram et al.[30] observed significant impairment to motor function with increasing tramadol dose, affecting the behavioural test. Besides these factors, stress during the handling and habituation of animals may also have inhibitory effects on nociceptive sensitivity, affecting pain studies[1]. All the above factors can disrupt or change experimental measurements of nociception. The choice of rat strain can also profoundly affect the outcome of experimental pain research[8].

Our results showed that in low dose tramadol (10 mg kg^{-1}), naloxone only reduced the thermal withdrawal latency at the 30th minute, while yohimbine and ondansetron significantly reduced the thermal withdrawal latency during the peak effect attenuated the antinociceptive effect of tramadol in almost all time periods. Therefore, it can be speculated that opioidergic mechanisms are more prominent during the high-effect period at this tramadol dose, and the contribution of noradrenergic and serotonergic mechanisms appear to have similar efficacy at all times. On the contrary, with high dose tramadol (20 mg kg^{-1}), all three mechanisms of action, opioidergic, noradrenergic and serotonergic, similarly attenuated the peak effect (60th min), suggesting similar involvement of mechanisms of action as the dose of tramadol increases. Accordingly, it can be thought that the effects of tramadol on receptors change in a dose-dependent manner. Moreover, this is also supported by the finding that the least percentage changes in thermal withdrawal latency in low-dose tramadol was observed with naloxone, while the least changes were observed with yohimbine with the high-dose tramadol.

The study has limitations. Pain can be divided into three main classes; nociceptive, inflammatory and pathological. Nociceptive tests can be performed with thermal (hot plate and plantar test), mechanical (analgesimeter) and chemical (formalin test) stimuli. However, the present study's design only assesses nociceptive pain using the plantar test with two doses of tramadol and a single dose of each receptor antagonist. Also, the motor function of the rats was not evaluated with a motor coordination test.

Conclusion

In conclusion, our results show that tramadol's antinociceptive effectiveness and the contribution of opioidergic, α_2-adrenergic, and 5-HT$_3$ receptors are affected by the dose of tramadol. Furthermore, the time to reach the peak analgesic effect is longer when a higher tramadol dose is used. This finding warrants further studies.

Conflicts of Interest Statement
The authors have no conflicts of interest that may affect the study.

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