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AKADÉMIAI KIADÓ

Blockade of orexin receptor type-1 by SB-334867 and activation of orexin receptor type-2 attenuate morphine tolerance in rats

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ABSTRACT

Purpose: The interaction of orexinergic neurons with the opioidergic system and their effects on morphine analgesia and tolerance have not been fully elucidated. The purpose of the study was to evaluate the effects of the orexin-1 and orexin-2 receptor (OX1R and OX2R) agonist and antagonist on morphine analgesia and tolerance in rats. *Material and methods:* A total of 90 Wistar albino male rats weighing 180–220 g were used in the experiments. To induce morphine tolerance, rats were injected with a single dose of morphine (50 mg kg^{-1}, s.c.) for 3 days. Morphine tolerance was assessed on day 4 in randomly selected rats by analgesia tests. In order to evaluate morphine tolerance situation, orexin-A, SB-334867, orexin-B and TCS OX2 29 were administered together with morphine for 3 days. The analgesic effects of orexin-A (10 μg kg^{-1}), OXR1 antagonist SB-334867 (10 mg kg^{-1}), OXR2 agonist orexin-B (15 μg kg^{-1}), OXR2 antagonist TCS OX2 29 (0.5 mg kg^{-1}) and morphine (5 mg kg^{-1}) were measured at 15 or 30-min intervals by tail-flick and hot-plate antinociceptive tests. *Results:* The results suggested that the combination of orexin-1 receptor antagonist SB-334867 and orexin-B with morphine significantly increased the analgesic effect compared to morphine-tolerant rats. In addition, administration of orexin-A and -B alone showed significant analgesic effects compared to the saline group. However, co-administration of orexin-A and -B with morphine did not increase the analgesic efficacy of morphine. *Conclusions:* The results of this study demonstrated that co-administration of SB-334867 and orexin-B with morphine attenuated morphine tolerance. Further studies are needed to elucidate the details of the interaction between orexin receptors and the opioidergic system.

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KEYWORDS

orexin receptors, orexin-A, orexin-B, SB-334867, morphine tolerance

INTRODUCTION

Morphine is one of the most potent analgesics commonly used for the relief of severe and chronic pain, and repeated administration of morphine causes a decrease in the analgesic effect and the development of tolerance. Morphine tolerance is a complex physiological response involving opioid receptor desensitization, activation of the nitric oxide-cGMP signaling pathway, the alpha-2 noradrenergic system, calcium and potassium channels, and mitogen-activated protein kinase (MAPK) [1–5]. Recently, it has also been stated that orexins affect the development of tolerance to morphine [6, 7].

Orexins (hypocretins) are important neuropeptides with a wide variety of functions such as different neuroendocrine functions, appetite regulation and pain modulation [8–10]. Orexin-A and orexin-B are two neuropeptides produced from prepro-orexin in the lateral hypothalamus (LH) by various enzymatic reactions. These two neuropeptides exert their effects through two G-protein coupled receptors (GPCR) called orexin-1 receptor (OX1R) and orexin-2 receptor (OX2R). Orexin-A activates both OX1R and OX2R receptors with similar affinity, and orexin-B specifically activates OX2R receptors [11]. There is evidence to suggest that orexins have antinociceptive effects in the brain and spinal cord in different types of pain, including thermal, mechanical, and chemical-induced nociceptions [12]. Orexin receptors have been identified in different brain areas known to be involved in pain sensation processing [13]. It has been reported that orexin-A has antinociceptive effects in the brain and spinal cord, whereas orexin-B has little or no antinociceptive effect [14]. The orexins, located in the LH region and produced by neurons play a role in pain regulation. Especially the OX1r and OX2r receptors to which they bind are concentrated in the ventral tegmental area (VTA). Although orexinergic receptors are found in various regions, the receptors found mostly in the VTA are effective in anti-nociception and pain modulation [15].

It has been shown that orexins have antinociceptive effects at the supraspinal level in different experimental pain models, and the ventral periaqueductal gray matter (vPAG) is one of the important regions in the realization of this modulation [12, 16]. It is stated that the orexinergic system extending from the hypothalamus to the rostral ventromedial medulla (RVM) and the orexinergic neurons in the LH have a potential role in the modulation of nociceptive transmission [17, 18]. Orexin neurons contain μ-opioid receptors, and administration of morphine elicits a variety of responses in these neurons [19]. OX1R is involved in morphine-induced antinociceptive effects, and morphine administration increases orexin-A levels in the plasma and the midbrain [20, 21]. Moreover, orexin-A receptors in the medial pre-optic area have been shown to play an important role in modulation of pain in morphine-treated rats. Various interactions between orexin-A and morphine have been demonstrated in this area of the hypothalamus [22, 23]. Generally, the orexin receptors and their orexinergic areas are located in regions where morphine tolerance and dependence are thought to occur. In addition, SB 334 867, an orexin-A antagonist, may inhibit the development of morphine tolerance [10].

The role of orexin neuropeptides in the development of opiate analgesia and tolerance has been extensively investigated in different studies. About 50% of orexin-containing neurons

highly express μ-opioid receptors [19]. It is also reported that orexinergic neurons are under tonic inhibition by endogenous opioids. This view is supported by the fact that administration of the μ-opioid receptor antagonist naloxone causes a dramatic increase in the firing frequency of these neurons [24]. On the other hand, behavioral studies have shown that OX1R activity has an important role in mediating tolerance to opiate antinociceptive activity. Administration of the OX1R antagonist SB-334867 has been shown to significantly inhibit the development of morphine tolerance in locus coeruleus (LC) neurons [25]. At the same time, in the process of physiological tolerance and dependence, the amount of cAMP decreases and orexin neurons are inhibited due to the continuous secretion of endogenous opioid peptides [26]. According to different scientific evidence, the analgesic effect of orexins is mainly attributed to orexin-A in various animal models. However, the antinociceptive effect of orexin-B is not fully elucidated. The analgesic activity of orexins is blocked by the orphan G proteins on the OX1r and OX2r receptors to which they bind. However, orexin-A and orexin-B have different analgesic effects due to their different affinities for receptors and their lipophilic properties [27]. Therefore, this study aims to investigate the effects of OX1R antagonist SB-334867 and OX2R agonist orexin-B on morphine analgesia and tolerance in male rats.

MATERIALS AND METHODS

Animals

A total number of 90 adult Wistar male rats weighing 180–220 g were purchased from Cumhuriyet University Experimental Animals Laboratory (Sivas, Turkey). Animals were housed in groups of four rats per cage under a 12-h light-dark cycle with the lights on between 6:00 and 18:00 in a temperature-controlled (23 ± 2 °C) room and a humidity of 52–56%. Food and water were provided ad libitum to the rats. The experimental protocols were approved by The Animal Ethics Committee of Cumhuriyet University (Ethic no: 2016/63). The housing and treatment of the rats followed the guidelines of the Animal Ethics Committee of Cumhuriyet University, which are based on the NIH Guide for the Care and Use of Laboratory Animals.

Drugs

To determine their analgesic activity, the OXR1 agonist orexin-A (10 μg kg^{-1}) and the selective OXR1 antagonist SB-334867 (10 mg kg^{-1}), the OXR2 agonist orexin-B (15 μg kg^{-1}) and the OXR2 antagonist TCS OX2 29 (0.5 mg kg^{-1}) were intraperitoneally (i.p.) injected into rats. Morphine HCl (Galen Medicine, Istanbul, Turkey) was injected at an effective dose of 5 mg kg^{-1} subcutaneously (s.c.). Drug doses determined in previous studies [28, 29] were used. Except for SB-334 867, the drugs were dissolved in 0.9% NaCl and the volume of solution administered to each rat was determined as 0.1 ml/100 g body weight. SB-334867 was dissolved in dimethyl sulfoxide (DMSO). The drugs used in the study were purchased from Sigma-Aldrich (Hamburg, Germany).

Experimental protocol

To induce morphine tolerance, rats were injected with a single dose of morphine (50 mg kg^{-1}, s.c.) for 3 days [30]. On the 4th day, morphine was administered at a test dose (5 mg kg^{-1}), and its analgesic effects were measured at 15 or 30-min intervals (15, 30, 60, 90, 120 min) by the

tail flick (TF) and the hot plate (HP) tests (Fig. 1). The effects of orexin-A, SB-334867 (selective OXR1 antagonist), orexin-B (OXR2 agonist) and TCS OX2 29 (selective OXR2 antagonist) on morphine analgesia and tolerance were simultaneously measured by antinociceptive tests. Saline group rats were administered saline (5 ml kg^{-1}, i.p.) instead of morphine during induction (Fig. 1).

Antinociceptive tests

Tail flick (May TF 0703 Tail flick Unit, Commat Company Ltd., Ankara, Turkey) and hot plate (May AHP 0603, Hot plate, Commat Company Ltd., Ankara, Turkey) tests were used to measure the antinociceptive effect of drugs. Different evidence has shown that analgesic responses to the tail flick test are often associated with central pain mechanisms [31]. In the TF test, the radiant heat source is focused on about 3 cm from the tail end of the rats, and then the TF latency (TFL) is recorded in seconds [32]. Infrared intensity was adjusted so that the baseline TFL was 2.8 ± 0.4 s. Animals with baseline TFL less than 2.4 s and greater than 3.2 s during testing were excluded from testing. The test cut-off time (cutoff latency) was accepted as 15 s to avoid tissue damage. Evaluation of peripheral and central analgesic effect was performed with HP thermal analgesia test [31]. In this test, each animal is placed in the test device and the table surface temperature is adjusted to 55 ± 0.5 °C. The heated table is mostly made of copper or aluminum. In this test, the time the animal starts to lick its feet for the first time or the time to jump off the hot plate is considered the test latency period. The test cut-off time was accepted as 30 s to avoid any damage to the feet of the rat.

Data analysis

In analgesia test (TF and HP tests) measurements, the tail and paw withdrawal latencies in seconds as the maximum possible effect (% MPE) of the animals was determined by the following equation:

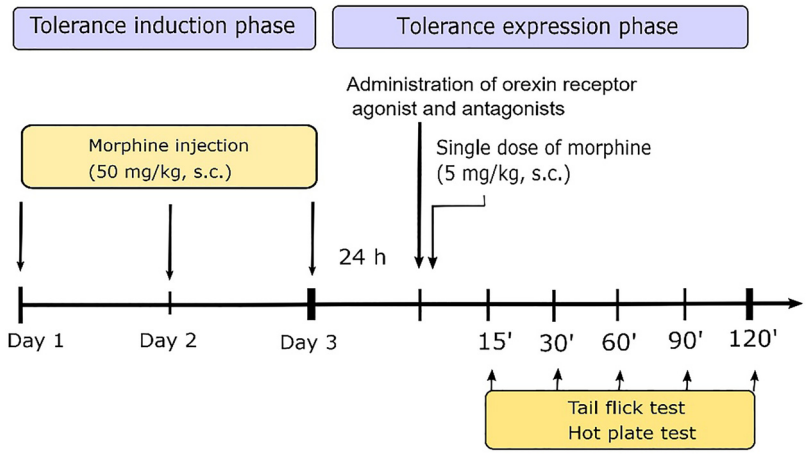

Fig. 1. Timeline diagram showing the experimental protocol of tolerance induction and analgesia tests

$$\% \text{ MPE} = [(\text{test latency} - \text{baseline}) \,/\, (\text{cutoff} - \text{baseline})] \times 100$$

Basal latencies and test latencies were determined in antinociceptive tests for each rat, and then % MPE values were determined with the formula. These values were means in each group for statistical analysis.

Statistical analysis

Antinociceptive test data were expressed as mean ± S.E.M. The normal distribution of the data was evaluated according to the Shapiro Wilk test. Statistical analysis of data from more than two groups was performed with two-way and repeated measures analysis of variance (ANOVA) and the Tukey post hoc test was used for multiple comparisons (SPSS computer program, version 22.0 for Chicago, IL, USA). $P < 0.05$ was considered statistically significant. The nonparametric method for Are Under Curve (AUC) analysis of the SPSS computer program was also used. Sensitivity and specificity in AUC analysis were evaluated between 0.5 and 1.0.

In order to observe the effects of orexin-A, orexin-B, antagonist TCS OX2 29 and antagonist SB 334 867 on morphine analgesia, %MPE values of morphine group, orexin-A group, orexin-A + morphine group, SB group, SB + morphine group, orexin-B group, orexin-B + morphine group, TCS group, TCS + morphine group and saline group were measured at 15, 30, 60, 90 and 120th minutes.

For the purpose of seeing the effects of orexin-A, orexin-B, TCS OX2 29, and SB 334 867 on morphine tolerance, group of orexin-A, orexin-B, orexin-A + morphine tolerance, orexin-B + morphine tolerance, SB + morphine tolerance, TCS + morphine tolerance, morphine group, morphine tolerance and saline group %MPE measurements were made at 15, 30, 60, 90 and 120th minutes.

RESULTS

Effects of orexin-A and SB-334 867 on morphine analgesia

When AUC analysis is performed with nonparametric method for all % MPE values of the orexin-A and SB-334867 groups, the results are the following: for orexin-A TF [AUC: 0.87; (0.72–1.00)] and HP [AUC: 0.90; (0.76–1.00)], for SB-334867 TF [AUC: 0.86; (0.69–1.00)] and HP [AUC: 0.87; (0.72–1.00)] (Fig. 2).

(A) shows the effect of orexin-A and SB-334 867 in the tail-flick test, and (B) shows the effect of orexin-A and SB-334 867 in the hot-plate test. Orexin-A (10 μg kg^{-1}; i.p.) and SB-334 867 (10 mg kg^{-1}; i.p.) in combination with morphine (5 mg kg^{-1}; s.c.) produce no significant increase in percent of maximal possible effect (% MPE) in either the tail-flick or the hot-plate assays as compared to the morphine-treated rats. Orexin-A alone has a significant analgesic effect compared to the saline group ($P < 0.05$). The maximum % MPE is observed at 60 min after administration of morphine. Each point represents the mean ± SEM of % MPE for 7–8 rats. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to the saline-treated group. Morp, morphine; SB, SB-334 867.

The mean % MPE values of the group treated with orexin-A (10 μg kg^{-1}), reaching the maximal level at the 60th minute, showed a statistically significant increase compared to the

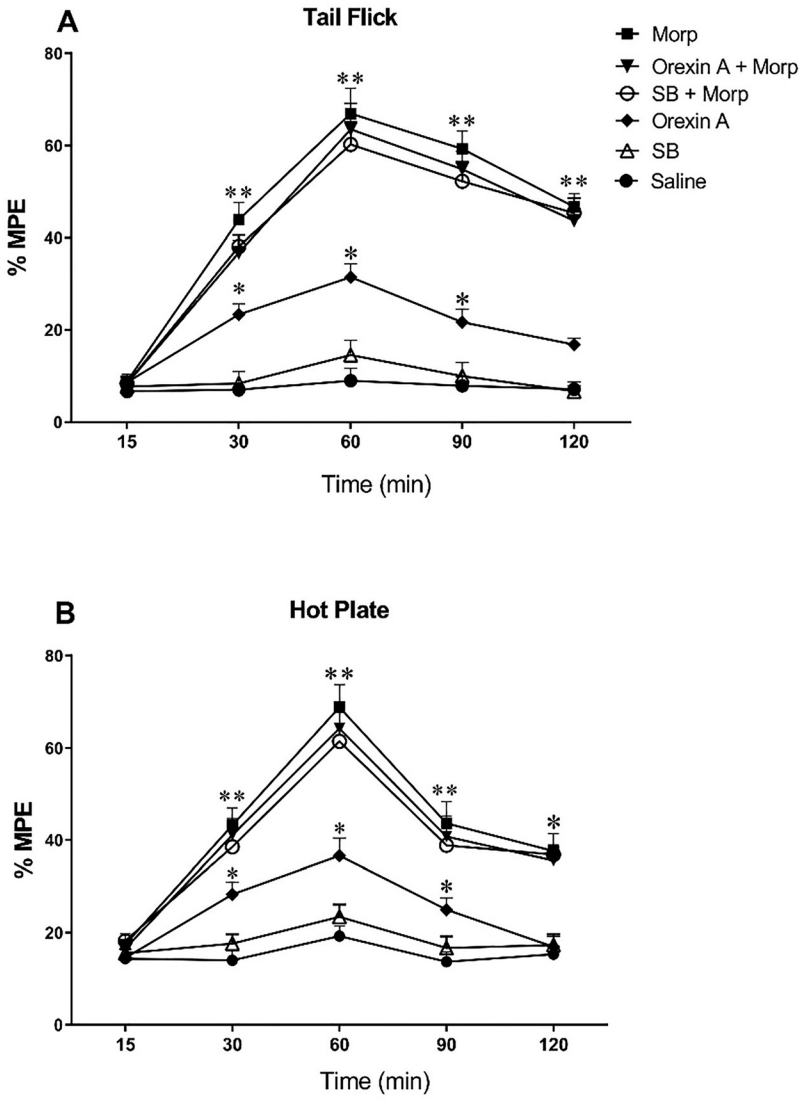

Fig. 2. Effects of orexin-A and SB-334 867 on morphine analgesia

saline group in both TF ($F_{5,24}$: 28.70, $P < 0.05$; Fig. 2A) and HP tests ($F_{5,24}$: 29.32, $P < 0.05$; Fig. 2B). Co-administration of orexin-A and morphine did not significantly increase the analgesic effect compared to the morphine group. There was a significant antinoceptive effect in the morphine-injected group compared to the saline group in both analgesia tests ($F_{5,24}$: 42.16, $P < 0.01$), however there was no significant effect in the SB-334867 (10 mg kg^{-1})-treated group of rats ($P > 0.05$) (Fig. 2).

Effects of orexin-B and TCS OX2 29 on morphine analgesia

Along the lines of the orexin-A and SB-334867 experiments, AUC analysis was performed with nonparametric method for all % MPE values of the orexin-B and TCS OX2 29 groups, with the following results: for orexin-B TF [AUC: 0.86; (0.68–1.00)] and HP [AUC: 0.81; (0.60–1.00)], for TCS OX2 29 TF [AUC: 0.81; (0.58–1.00)] and HP [AUC: 0.82; (0.58–1.00)].

Co-administration of morphine and the selective OXR2 agonist orexin-B (15 μg kg^{-1}) resulted in a significant reduction in analgesic effect compared to the morphine group ($F_{5,24}$: 24.21 for TF and $F_{5,24}$: 22.56 for HP, $P < 0.05$; Fig. 3A, B). However, administration of TCS OX2 29 (OXR2 antagonist; 0.5 mg kg^{-1}) with morphine did not significantly decrease % MPE compared to the morphine group in either the TF or the HP tests ($P > 0.05$). In addition, orexin-B produced a significant analgesic effect in behavioral tests compared to the saline group ($P < 0.05$) (Fig. 3).

(A) shows the effect of orexin-B (15 μg kg^{-1}; i.p.) and TCS OX2 29 (0.5 mg kg^{-1}; i.p.) in the tail-flick test, and (B) shows the effect of orexin-B and TCS OX2 29 in the hot-plate test. Orexin-B in combination with morphine produces a significant decrease in analgesic effect in both the tail-flick ($P < 0.05$; Fig. A) and the hot-plate assays ($P < 0.05$; Fig. B) as compared to the morphine-treated rats. The peak value of this group was also observed at 60 min after administration of morphine in analgesia tests. In addition, orexin-B produced a significant analgesic effect in behavioral tests compared to the saline group ($P < 0.05$). Each point represents the mean ± SEM of % MPE for 7–8 rats. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to the saline group and $\#P < 0.05$ compared to the morphine group of rats. Morp, Morphine; TCS, TCS OX2 29.

Effects of orexin-A and SB-334867 on morphine tolerance

When AUC analysis is performed with nonparametric method for all % MPE values of the orexin-A and SB-334867 groups, the results are the following: for orexin-A TF [AUC: 0.71; (0.51–0.90)] and HP [AUC: 0.78; (0.61–0.94)], for SB-334867 TF [AUC: 0.76; (0.59–0.94)] and HP [AUC: 0.76; (0.60–0.93)].

The data obtained suggested that administration of OXR1 antagonist SB-334867 to morphine-tolerant rats showed a significant increase in analgesic effect in TF ($F_{4,20}$: 22.64, $P < 0.05$; Fig. 4A) and HP ($F_{4,20}$: 25.74, $P < 0.05$; Fig. 4B) tests compared to the morphine-tolerant group. However, administration of orexin-A to morphine-tolerant rats did not significantly increase antinociceptive activity in either the TF or the HT tests ($P > 0.05$) (Fig. 4).

(A) shows the effects of orexin-A and SB-334867 in the tail-flick and (B) the hot-plate test. Pretreatment of morphine-tolerant animals with SB-334867 (10 mg kg^{-1}; i.p.) significantly decreased tolerance to morphine in both the tail-flick ($P < 0.05$; Fig. A) and the hot-plate test ($P < 0.05$; Fig. B) compared to morphine-tolerant animals. In contrast, pretreatment of animals with orexin-A did not result in a significantly increase in % MPE in either the tail-flick or the hot-plate test. Each point represents the mean ± SEM of % MPE for 7–8 rats. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to the saline group and $\#P < 0.05$, compared to the morphine-tolerant rats. Morp tol, morphine-tolerant; SB, SB-334 867.

Effects of Orexin-B and TCS OX2 29 on morphine tolerance

Accordingly, like in the case of orexin-A and SB-334867, AUC analysis was performed with nonparametric method for all % MPE values of the orexin-B and TCS OX2 29 groups, with the

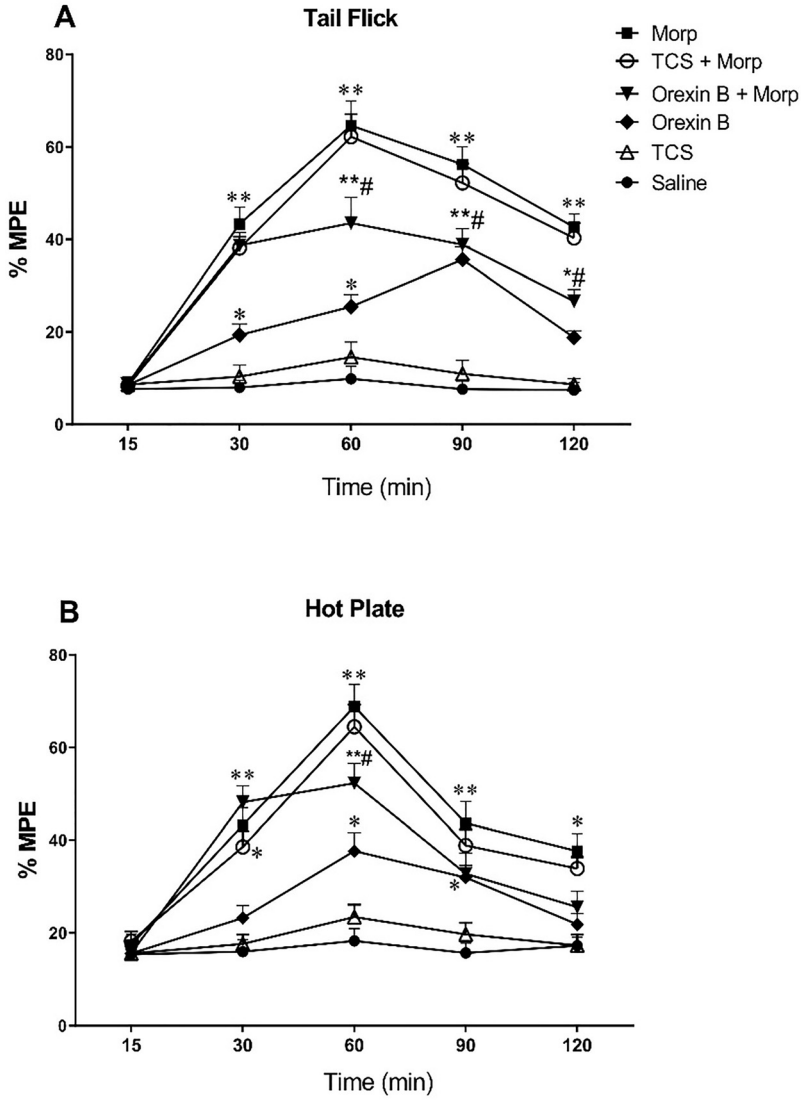

Fig. 3. Effects of orexin-B and TCS OX2 29 on morphine analgesia

following results: for orexin-B TF [AUC: 0.72; (0.51–0.92)] and HP [AUC: 0.72; (0.53–0.91)], for TCS OX2 29 TF [AUC: 0.70; (0.49–0.90)] and HP [AUC: 0.73; (0.54–0.93)].

Administration of orexin-B to morphine-tolerant rats demonstrated that the antinociceptive effect of morphine significantly increased in TF ($F_{4,20}$: 18.36, $P < 0.05$; Fig. 5A) and HP ($F_{4,20}$: 18.78, $P < 0.05$; Fig. 5B) analgesia tests. However, statistical analysis showed that TCS OX2 29 did not significantly modify the antinociceptive effect in morphine-tolerant rats ($P > 0.05$) (Fig. 5).

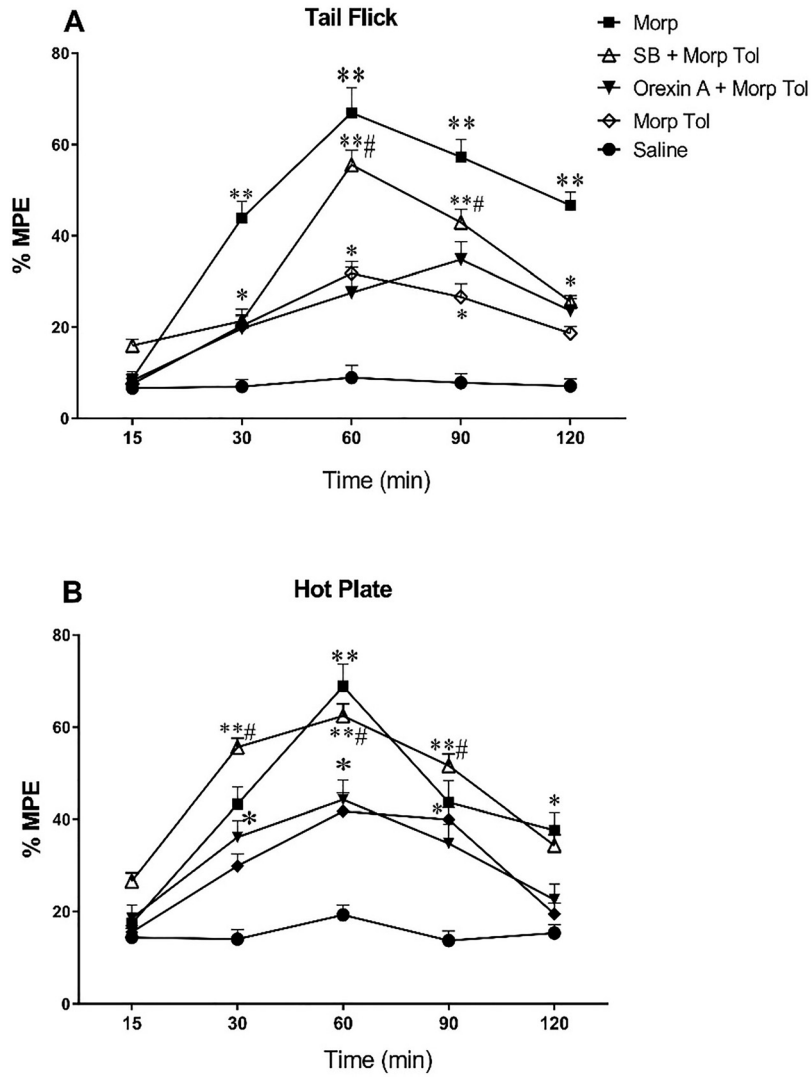

Fig. 4. Effects of orexin-A and SB-334867 on morphine tolerance

(A) shows the effects of orexin-B and TCS OX2 29 in the tail-flick and (B) the hot-plate test. Pretreatment of morphine-tolerant animals with orexin-B (15 µg kg^{-1}; i.p.) significantly decreased tolerance to morphine in both the tail-flick ($P < 0.05$; Fig. A) and the hot-plate test ($P < 0.05$; Fig. B) compared to morphine-tolerant animals. In contrast, pretreatment of animals with TCS OX2 29 did not significantly increase % MPE in either the tail-flick or the hot-plate test. Each point represents the mean ± SEM of % MPE for 7–8 rats. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to the saline group and $\#P < 0.05$, compared to the morphine-tolerant rats. Morp tol, morphine-tolerant; TCS, TCS OX2 29.

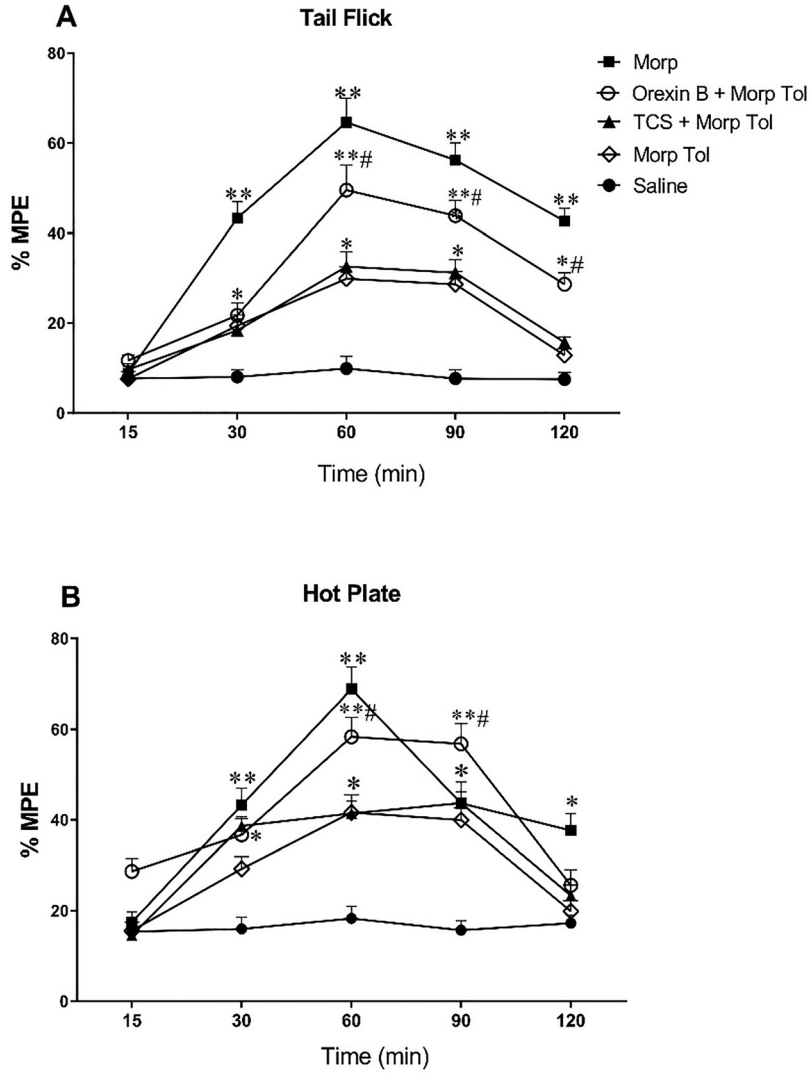

Fig. 5. Effects of orexin-B and TCS OX2 29 on morphine tolerance

Orexins – especially orexin-B – that bind to OX1r/OX2r receptors in the NAc reduce the efficacy of morphine. Especially, binding of morphine to any receptor in the NAc is largely inhibited and activation cannot be initiated. Inhibition of tolerance occurs due to the increase in morphine concentration, which cannot be activated in the NAc and will bind to mu receptors in different regions (Fig. 6).

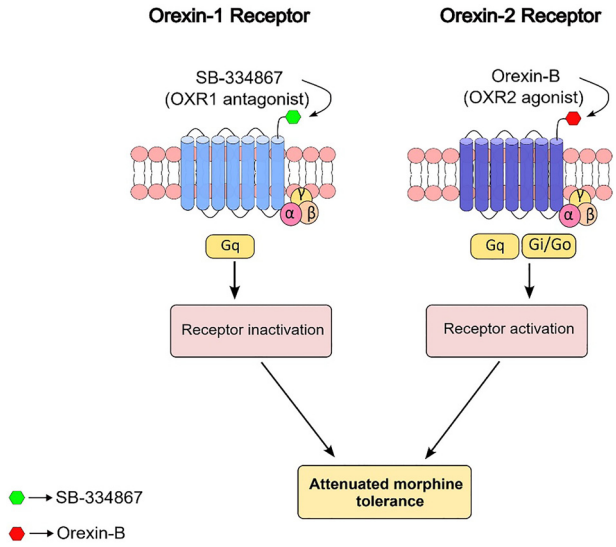

Fig. 6. Orexin receptors and morphine antinociceptive tolerance. OXR1, orexin-1 receptor; OXR2, orexin-2 receptor; Gq and Gi/Go, G protein-coupled receptors

Analgesic effects of morphine at different doses

The results of the analgesia tests we performed to determine the effective dose of morphine showed that the analgesic effect was maximal at the 60 min measurements and at the test dose of 5 mg kg^{-1} (TF: 63.98 ± 5.44; Fig. 7A and HP: 67.97 ± 4.56; Fig. 7B). When the mean % MPE values of the morphine group at a dose of 5 mg kg^{-1} were compared with the saline group (TF: 8.57 ± 1.08 and HP: 17.54 ± 1.36), the difference was found to be statistically significant ($P < 0.01$).

(A) shows the effects of morphine in the tail-flick and (B) the hot-plate test. The effective dose of morphine showed that the analgesic effect was maximal at the 60th minute measurements and at the test dose of 5 mg kg^{-1} (A and B). Each point represents the mean ± SEM of % MPE for 7–8 rats. $^{*}P < 0.01$ and $^{**}P < 0.001$ compared to the saline group of rats.

DISCUSSION

The aim of this study was to investigate the effects of the OX1 receptor ligands (orexin-A and SB-334867) and the OX2 receptor ligands (orexin-B and TCS OX2 29) on morphine analgesia and tolerance in rats. The results indicated that the OX1 receptor antagonist SB-334867 and the OX2 receptor agonist orexin-B attenuated morphine tolerance. However, OX1 receptor agonist orexin-A and OX2 receptor antagonist TCS OX2 29 did not have a significant effect on morphine tolerance. In addition, neither OX1 nor OX2 receptor ligands were able to increase the antinociceptive activity of morphine. On the contrary, co-administration of morphine with orexin-B showed a decrease in the analgesic activity of morphine.

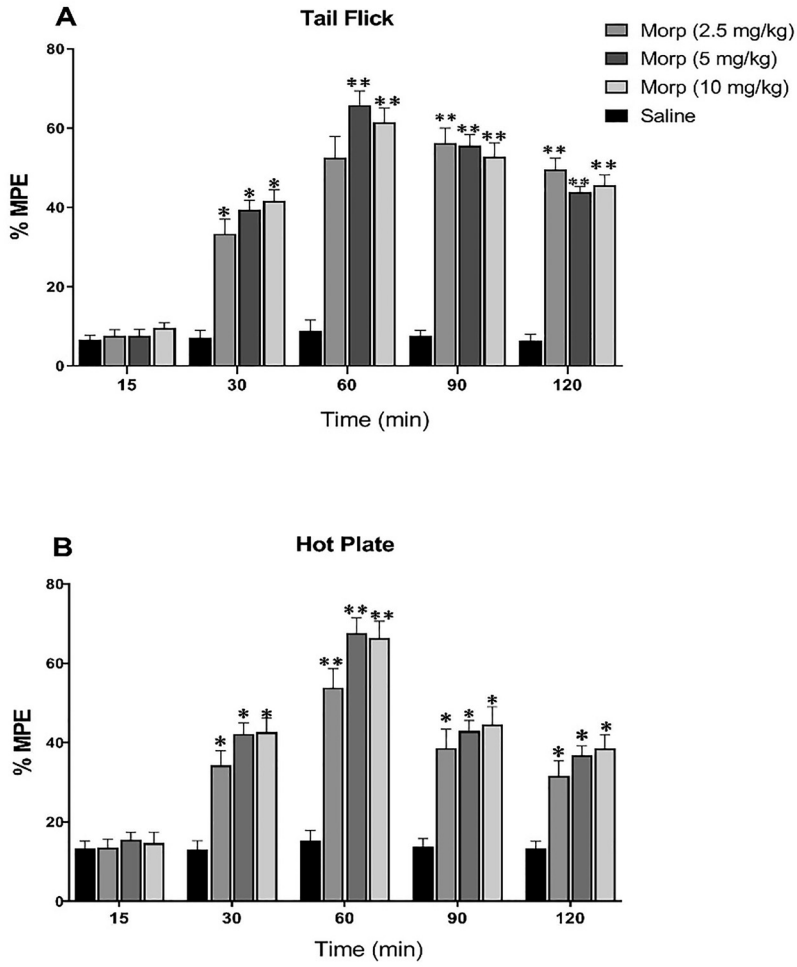

Fig. 7. Analgesic effects of morphine at different doses

Richardson et al. concluded that the decrease in cAMP inhibits orexin neurons due to the continuous secretion of endogenous opioid peptides in physiological tolerance and dependence states [26]. Therefore, exogenous orexin-B, which is not produced from orexinergic neurons, acts on the nucleus accumbens (NAc), reducing the tolerance to opiates depending on the receptors it affects in the reward center, the intensity of their concentration, and the differences in the style of administration. According to the findings we obtained in our study, morphine-induced effects are carried out by orexin-B, and therefore the physiological functions of morphine in the reward center are inhibited.

Opioid drugs such as morphine are frequently used to treat acute and severe pain [33]. However, the development of tolerance to the antinociceptive effect of morphine is the most important problem limiting its clinical use [34]. Although there is still no precise evidence of

the relationship between the orexinergic system and opioid antinociceptive tolerance, there is some evidence that orexin neurons may play a critical role in the development of morphine tolerance in animal models [35].

The role of the endogenous orexin system in a wide variety of brain functions has been extensively studied. Orexin-A and -B are synthesized and stored in the lateral hypothalamus (LH). LH has an important role in the modulation of pain together with the orexinergic system [36]. Evidence suggests that orexin-A released by stimulation of LH activates OX1R in the spinal dorsal horn [37]. An experimental study showed that administration of orexin-A, but not orexin-B, reduced formalin-induced nociceptive behaviors by producing an analgesic effect in the hot plate test [38]. In this study, injection of SB-334867 (an OX1R antagonist) did not produce any analgesic activity in the hot plate test, but inhibited the analgesic effect of orexin-A. Our study results suggested that SB-334867 and TCS OX2 29 (an OX2R antagonist) did not have any analgesic effect, whereas both orexin-A and orexin-B had antinociceptive activity.

A growing body of evidence suggests a functional interaction between orexinergic neurons and the opioid system in pain modulation. LH orexinergic neurons also express μ-opioid receptors and these neurons respond to morphine [19]. Recent studies demonstrate that OX1R antagonists can attenuate morphine-induced analgesia [39]. For example, co-administration of SB-334867 with morphine to rats reduced the efficacy of morphine in formalin-induced nociceptive behavior [40]. These results suggest that at least part of the analgesic effect of morphine is through activation of OX1R. Also, orexin-A activates reactions that play a role in the inhibition of adenylate cyclase, similarly to morphine. It is thought that the analgesic effect occurs in this way. In contrast, our study data showed that co-administration of SB-334867 with morphine did not reduce analgesic activity in rats. Also, co-administration of orexin-A and -B with morphine did not increase the analgesic efficacy of morphine.

It has been stated that chronic co-administration of morphine with an OXR1 agonist may contribute to morphine tolerance by accelerating adaptive changes [41]. Consistent with this, central administration of the OX1R antagonist SB-334867 inhibited the antinociceptive tolerance of morphine [42]. In this study, intracerebroventricular injection of SB-334867 alone had no significant effect on nociception. In our study, morphine tolerance was decreased in rats treated with SB-334867, but no antinociceptive effect was observed in rats treated with SB-334867 alone. Evidence indicates that OX1 receptors have an important role in mediating the modulatory effects of orexin on the development of opioid tolerance [43]. Erami et al. state that the reduction or partial elimination of tolerance to morphine with different chains of reactions that have not yet been fully defined and activated chemicals are closely related to the inhibition processes of SB 334 867 [10]. The more intense involvement of endogenous opioids or neuropeptides released into the reward center as a result of the binding of SB 334 867 to the OX1r receptor on the VTA offers a reasonable approach to how tolerance-reducing mechanisms work.

The locus coeruleus (LC) containing noradrenergic nerves is considered an important site in the modulation of opiate effects in the central nervous system. It appears that activation of OX1R may lead to direct and indirect effects on LC neurons by influencing a number of modulatory mechanisms that are still not fully understood. One study showed that OX1R blockade by SB-334867 prevented the development of morphine tolerance in LC neurons [25]. In our study, administration of orexin-A and TCS OX2 29 did not bring about any change in morphine tolerance in rats, whereas administration of SB 334867 and orexin-B decreased the tolerance. Similarly, inhibition of OX1R in neurons in the LC nucleus in rats reduced the

development of morphine addiction [44]. In the tolerance mechanism formed by the desensitization of mu opiate receptors, orexin-A also contributes to the desensitization of opiate receptors and is thought to increase tolerance. However, the type of pathways by which these neuropeptides reach the receptors are also important in their desensitization function. At the same time, orexin-A alone may contribute to this depersonalization phenomenon as a result of different reactions, especially some secondary messengers [6]. Ghaemi-Jandabi et al. obtained results supporting this view after intracerebroventricular (icv) administration of orexin-1 and antagonist SB-334 867 in their studies. In our study, no remarkable results were obtained on the morphine tolerance of orexin-A, as our route of administration was intraperitonal (ip), not icv, and as a result of the activations we think took place in the pathways here.

In addition, it is stated that strong stimuli from the lateral paragigantocellularis (LPGi) affect the LC neuronal response in the emergence of opioid dependence and tolerance. Administration of SB-334867 to LPG in rats significantly reduced naloxone-induced morphine withdrawal symptoms [45].

Morphine desensitization is considered the first stage in the development of tolerance to opioids. OX1R activation results in increased intracellular calcium concentration, which causes morphine desensitization [46]. Increased intracellular calcium produces changes in G protein receptors that play a role in μ-opioid receptor desensitization [33]. G protein receptors activate many protein kinases, such as protein kinase A (PKA), protein kinase C (PKC), mitogen-activated protein kinase (MAPKs), and calmodulin kinase II (CaMKII) [47–49]. PKC-induced morphine desensitization plays a critical role in the development of tolerance in neurons after prolonged opioid drug exposure [1]. The development of tolerance to morphine has been shown to be abolished by PKC inhibitors [50, 51]. These study results are consistent with the view that orexin neuropeptides may induce morphine tolerance by affecting a mechanism dependent on the G-protein coupled OX1 receptor and PKC. In addition, N-methyl-D-aspartate receptor (NMDAR)-mediated signals induce morphine tolerance through MOR desensitization [52, 53]. However, NMDAR antagonists cause a decrease in morphine tolerance [54]. Furthermore, activation of the OX1 receptor has been reported to increase NMDAR-mediated opioid tolerance [53, 55]. In previous studies observing the effects of orexin-A and orexin-B on morphine analgesia and tolerance, the routes of orexin administration may be different from that employed in our current study. It should be emphasized that, particularly because orexin-b is lipophilic and orexin-A has a large number of receptors and numerous pathways in the central nervous system, the route of administration is of special importance.

CONCLUSION

Our study results suggested that the administration of OX1R antagonist SB-334867 and OX2R agonist orexin-B with morphine attenuated morphine tolerance in antinociceptive tests. In addition, orexin-A and -B showed a significant analgesic effect when administered alone. However, orexin-A and -B co-administered with morphine did not increase the analgesic efficacy of morphine. Moreover, these results demonstrate that there is a significant interaction between the orexinergic neurons and the opioidergic system in morphine analgesia and tolerance. Further studies are needed to elucidate the details of this interaction.

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