Journal of Radiation Research and Applied Sciences 16 (2023) 100682

Contents lists available at ScienceDirect

Journal of Radiation Research and Applied Sciences

journal homepage: www.journals.elsevier.com/journal-of-radiation-research-and-applied-sciences

Ondansetron and AS19 attenuate morphine tolerance by modulating serotonin 5-HT_3 and 5-HT_7 receptor expressions in rat dorsal root ganglia

Seyma Ozsoy [a], Ercan Ozdemir [b,*], Handan Gunes [b], Fikret Gevrek [c], Kader Gulmez [d]

[a] Departments of Physiology, Faculty of Medicine, Tokat Gaziosmanpasa University, Tokat, Turkey
[b] Departments of Physiology, Faculty of Medicine, Sivas Cumhuriyet University, Sivas, Turkey
[c] Departments of Histology and Embryology, Faculty of Medicine, Tokat Gaziosmanpasa University, Tokat, Turkey
[d] Departments of Neuroscience, Faculty of Medicine, Sivas Cumhuriyet University, Sivas, Turkey

ARTICLE INFO

Keywords:
5-HT receptors
Ondansetron
AS19
Dorsal root ganglia
Morphine tolerance

ABSTRACT

Purpose: Opioid drugs are frequently preferred drugs for severe pain, but their long-term use causes tolerance to their analgesic effects. However, the factors that contribute to the development of tolerance to opioids are diverse and not fully understood. In this study, our aim was to investigate the effects of 5-HT_3 receptor antagonist ondansetron (ODN) and 5-HT_7 receptor agonist AS19 on morphine analgesia and tolerance in rats.

Material and methods: Eighty-four randomly selected Wistar Albino (230–260 g) male rats were included in the study. To induce morphine tolerance in rats, morphine (10 mg/kg) was administered subcutaneously twice daily for 7 days, and tolerance was assessed by thermal analgesia tests on day 8 for 120 min. After thermal analgesia tests, dorsal root ganglia (DRGs) at spinal L_4-S_5 level were isolated. 5-HT_3 and 5-HT_7 receptor expressions in DRGs were measured by immunohistochemical method.

Results: The results show that co-administration of ODN or AS19 with morphine indicated a significant increase in analgesic effect compared to morphine alone In addition, the difference between the mean of ODN or AS19 combined with morphine and the morphine-tolerant group was statistically significant. H-score analysis significantly increased 5-HT_3 receptor expressions in the morphine-tolerant rats, and administration of ODN prevented this. Similarly, 5-HT_7 receptor expressions were significantly decreased in the morphine tolerant group, and the administration of AS19 increased this expression.

Conclusion: These study results suggest that both ODN and AS19 alter the 5-HT_3 and 5-HT_7 receptor expressions in the DRG and possibly attenuate morphine tolerance in this way.

1. Introduction

Opioid agents such as morphine are clinically used for their potent analgesic activity in severe and chronic pain, but prolonged use of opioids causes loss of analgesic effectiveness over time. Patients affected by pain for a long time often require higher doses of opioids to maintain a pain-free state, resulting in various side effects, dependence and development of tolerance (Bardin & Colpaert, 2004; Nayebi & Charkhpour, 2006). There are many mechanisms proposed for the development of tolerance to the antinociceptive activity of morphine. These mechanisms include opioid receptor desensitization, induction of nitric oxide-cGMP pathway, alpha-2 noradrenergic, orexinergic, and descending serotonergic systems (Allouche et al., 2014; Lilius et al., 2012; Ozdemir et al., 2011, 2022). However, these mechanisms have not fully clarified the development of tolerance to opioids.

The opioid tolerance is classically considered to be mediated by the central nervous systems, but recent reports suggest that nociceptive dorsal root ganglion (DRG) neurons also play a critical role in this process (Muchhala et al., 2021). Removal of μ-opioid receptors from DRG neurons blocks the acute spinal and supraspinal opioid analgesia (Sun et al., 2019, 2020). Ablation of DRG neurons expressing the transient receptor potential vanilloid-1 (TRPV1) channel attenuates opioid tolerance (Chen et al., 2007). In addition, inhibition of morphine-induced apoptosis in rat DRG neurons led to a decrease in morphine tolerance (Avci et al., 2022; Ozdemir et al., 2023). These evidences suggest that μ-opioid receptors in primary afferent DRG neurons are an important target in inhibiting antinociceptive tolerance.

Considerable evidence indicate that serotonin (5-

* Corresponding author.
E-mail addresses: seyma.ozsoy@hotmail.com (S. Ozsoy), ercan_ozdemir@hotmail.com (E. Ozdemir).

https://doi.org/10.1016/j.jrras.2023.100682
Received 26 August 2023; Received in revised form 12 September 2023; Accepted 13 September 2023
Available online 22 September 2023
1687-8507/© 2023 Published by Elsevier B.V. on behalf of The Egyptian Society of Radiation Sciences and Applications. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

hydroxytryptamine, 5-HT) is critical modulator of nociceptive signaling in the spinal cord (Bardin et al., 2000; Cortes-Altamirano et al., 2018; Jeong et al., 2004). The relationship between opioid analgesia and descending 5-HT pathway has been known for a long time, and inhibition of serotonergic pathway has been reported to play an important role in decreasing opioid antinociception (Dogrul & Seyrek, 2006). Supraspinal opioidergic centers tonically control serotonergic neurons, and acute administration of morphine increases 5-HT release, while chronic administration decreases its release (Caillé et al., 2003; Neal & Sparber, 1986). Evidence regarding the effects of 5-HT morphine on analgesia and tolerance is conflicting. For example, co-administration of fenfluramine (5-HT agonist) with morphine attenuates morphine tolerance and increases morphine analgesia (Arends et al., 1998). Conversely, co-administration of morphine with 1-tryptophan (5-HT precursor) accelerates morphine tolerance (Contreras et al., 1973).

Activation of 5-HT receptor subtypes has differential effects on opioid analgesia and tolerance. Particularly, the effects of 5-HT_3 and 5-HT_7 receptor agonists on morphine analgesia are controversial (Dogrul et al., 2009). Experimental studies have shown that the 5-HT_3 receptor has pronociceptive functions and systemic administration of ondansetron (5-HT_3 receptor antagonist) inhibits this effect. Similarly, ondansetron significantly reduces opioid tolerance and blocks several morphine-induced hyperalgesic genes in mouse DRG (Liang et al., 2011). In contrast, ondansetron had no effect on acute opioid tolerance in patients receiving intrathecal opioids prior to cesarean section (Greer et al., 2017). It has also been reported that 5-HT_{3A} expressions are increased in the DRG ganglia of opioid-tolerant rats (Liang et al., 2011). It has been shown that 5-HT_7, the recently identified serotonin receptor, plays an important role in the analgesic effect of many drugs such as tramadol (Lee et al., 2015). In addition, spinal blockade of 5-HT_7 receptors inhibits the antinociceptive effect of opioids, while activation of 5-HT_7 receptors increases opioid analgesic activity (Brenchat et al., 2011).

Although there is different evidence for effect of 5-HT_3 and 5-HT_7 receptor agonists on morphine antinociception, the effects of these receptor agonists on opioid tolerance are not fully understood. Therefore, our aim in this study is to investigate the effects of ondansetron and AS19 on 5-HT receptor expressions in the rat dorsal root ganglia and morphine analgesia and tolerance.

2. Materials and methods

2.1. Animals

In the study, 84 adult male Wistar Albino rats aged 6–8 months (230–260 g) and housed in cages in conforming to the standards were used. The animals were obtained from the local Experimental Animal Center (Cumhuriyet University, Sivas, Turkey). The housing and treatment of the rats followed the guidelines of the “Guide for the Care and Use of Laboratory Rats” (Institute of Laboratory Animal Resources, Commission on Life Sciences 2011). Before the study, the experimental protocols were approved by the Animal Ethics Committee of Cumhuriyet University (Ethic no: 2021/01). The rats were kept in our animal care facility for an additional 7–10 days prior to use to allow them to acclimate. Rats were housed at 4–6 per cage under pathogen-free conditions. All rats were housed under a 12:12 h light/dark (lights on at 8:00 a.m.) schedule at room temperature (23 ± 2 °C) and a humidity of 50–55%. Standard laboratory chow and tap water were available ad libitum. The experiments were carried out blindly between 09.00 and 15.00 h.

2.2. Drugs

To determine their analgesic activity, the selective 5-HT_{3A} receptor agonist 1-phenylbiguanide (PBG) (Sigma-Aldrich, Germany) 7.5 mg/kg intraperitoneally (i.p.) and the 5-HT_{3A} antagonist ondansetron (ODN) (Med Chem Express, NJ, USA) 2 mg/kg subcutaneously (s.c.) injected into rats. The other group rats were administered the selective 5-HT_7 receptor agonist AS19 (Tocris Bioscience, UK) 2.5 mg/kg (s.c.) and the 5-HT_7 antagonist SB-269970 (Cayman Chemicals, MI, USA) 3 mg/kg (i.p.). Morphine HCl (Galen Medicine, Istanbul, Turkey) was injected at an effective dose of 5 mg/kg (s.c.). Previous similar studies were used to determine the doses of drugs (Brenchat et al., 2011; Liang et al., 2011; Roczniak et al., 2015). All drugs were dissolved in 0.9% NaCl and solution volumes were measured as 0.1 ml/100 g body weight for each rat.

2.3. Experimental protocol

The rats were divided into 14 groups, each consisting of 6 rats. First, the control group rats were injected intraperitoneally with saline solution. Then, tail-flick (TF) and hot-plate (HP) pain tests were performed by administering drugs to drug groups at predetermined doses, and latency times were recorded in seconds. In addition, morphine was injected into rats for 7 days to induce morphine tolerance. Measurements were made at 30-min intervals (0, 30, 60, 90, 120 min) to evaluate the antinociceptive effects of 5-HT receptor drugs ODN (selective 5-HT_3 antagonist), PBG (5-HT_3 agonist), AS19 (selective 5-HT_7 agonist) and SB-269970 (5-HT_7 antagonist) on morphine analgesia and tolerance. Immediately after the analgesia tests, the DRG ganglion tissues of the rats were isolated and prepared for immunohistochemical study. H-score analysis was used to evaluate the obtained microscopic data (Fig. 1).

2.4. Induction of morphine tolerance

To induce morphine tolerance, rats received twice-daily injections of morphine 10 mg/kg, subcutaneous (s.c.) for 7 consecutive days, and the control (saline) group was injected with the same dose of saline (Huang et al., 2019). To assess the degree of tolerance, the antinociceptive effect of a test dose of morphine (5 mg/kg) was measured by the analgesia tests (TF and HP tests) 24 h (day 8) after the last morphine injection.

2.5. Antinociceptive tests

Tail flick (TF) (May TF 0703 Tail flick Unit, Commat Company Ltd., Ankara, Turkey) and hot plate (HP) (May AHP 0603, Analgesic Hot Plate, Commat Company Ltd., Ankara, Turkey) tests, which are thermal analgesia test devices, were used to measure the antinociceptive effect. In the TF test, which mostly shows spinal reflex arc activity, 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 (Kanaan et al., 1996). Infrared intensity was adjusted so that the baseline TFL was 2.8 ± 0.4 s. Animals with a baseline TFL below 2.4 s and above 3.2 s and animals that did not pull their tails for more than 15 s during the test were excluded from the tests. The test cut-off time (cutoff latency) was accepted as 15 s to avoid tissue damage. It has been stated that analgesic responses to the tail flick test are generally associated with central pain mechanisms (Ramabadran et al., 1989).

The measurement of peripheral and central analgesic effect was performed with another thermal analgesia test device, the HP test (Kanaan et al., 1996). 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. The HP test measurements were performed at the same intervals (30 min) for 120 min in all animals, 1 min after the TF test measurements.

2.6. Data analysis

In analgesia test (TF and HP tests) measurements, the determination of the tail and paw withdrawal latencies in seconds as the maximum

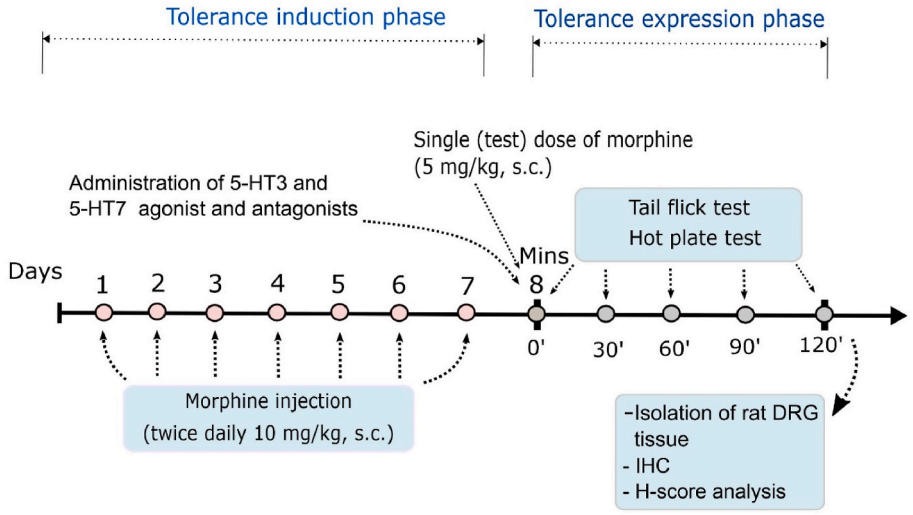


Fig. 1. Timeline diagram showing the experimental protocol of tolerance induction and immunohistochemical analysis. DRG, dorsal root ganglion; IHC, immunohistochemistry; s.c., subcutaneously.

possible effect (% MPE) of the animals was provided by the following equation:

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

First of all, basal latencies and test latencies were determined for each rat in antinociceptive tests, and then % MPE values were determined with the formula. For statistical analysis, the mean of these values in each group was obtained.

2.7. Isolation of DRG ganglion tissue

After thermal analgesia measurements, rats were sacrificed by cervical dislocation and lumbosacral spinal cord (L_4-S_5 level) and DRGs were isolated. The neuronal tissues were placed in petri dishes and washed 3 times with phosphate buffered saline (PBS) buffer (pH 7.4). Then, all the DRGs were completely isolated with forceps by removing other spinal tissue sections under stereo dissection microscope (Leica EZ4D, Heidelberg, Germany) and placed in a Petri dish.

The DRG tissues were fixed in 10% neutral buffered formalin and washed for total histological examination. Then, it was passed through a series of increasing alcohol (70%, 80%, 90% and 100%) and triple xylene series and embedded in paraffin. Sagittal and horizantal serial sections of 5 μm were taken from tissue samples with a microtome (Leica SM 2000R, Heidelberg, Germany) and they were placed on slides and incubated overnight at 56 °C. The sections were then subjected to Mayer's hematoxylin and eosin staining for histological examination.

2.8. Immunohistochemistry

5-HT_{3A} and 5-HT_7 receptor expressions were determined using routine immunohistochemical techniques after dehydration and deparaffinization in sections of control (saline) and drug groups. The endogenous peroxidase activity of neuronal sections was blocked with 3% H_2O_2 and epitopes were exposed by immersing them in 10 mM sodium citrate buffer (pH 6) in a microwave oven. Protein blockade was performed to prevent non-specific binding. Then, rabbit polyclonal 5-HT_{3A} (5 μg/ml; Abcam Biotech, Cambridge, UK) and rabbit polyclonal 5-HT_7 (1:300; Bioss Antibodies Inc., Massachusetts, USA) primary antibodies were applied to the sections. Afterwards, 5-HT_{3A} and 5-HT_7 expressions were determined by applying secondary antibody, streptavidin peroxidase complex (Scytek Laboratories Inc., Utah, USA) and chromogen, respectively. For negative controls, sections were incubated with PBS at a similar concentration as the primary antibody. Stained sections were photographed with a microscope (Leica DM2500, Nussloch, Germany). No staining was observed in negative controls.

2.9. H-score analysis

Five areas were randomly selected for analysis in each specimen under the light microscope (magnification, ×400), and 5-HT_3 and 5-HT_7 immunoreactive cells in each group were determined by the quantitative H-score method. Cells were classified into 4 categories according to the intensity of staining (Jr McCarty et al., 1985): 0 (no staining), +1 (weak but detectable staining), +2 (moderate staining), +3 (intense staining). The percentage of cells at each staining intensity level was calculated and the H-score was determined using the following formula (Cheon et al., 2001): H-score = 0 × (0% of cells staining) + 1 × (% of cells) staining 1) + 2 × (cell staining %2) + 3 × (cell staining %3). The H-score ranged from 0 to 300. H-score analysis was performed independently by two experienced researchers.

2.10. Statistical analysis

Antinociceptive test and semiquantitative H-score data were expressed as mean ± S.E.M. The normal distribution of the data was evaluated according to the Shapiro Wilk's test. Statistical analysis of data from more than two groups was performed with one-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 in all groups.

3. Results

3.1. The effects of ODN and PBG on morphine analgesia

Maximum analgesic effect (% MPE) was obtained in 60 min measurements in all group rats (Fig. 2). Administration of ODN (2 mg/kg, i.p.) with morphine to rats significantly increased the analgesic activity in both TF ($F_{5,24}$: 58.70, $p < 0.05$; Fig. 2A) and HP ($F_{5,24}$:10.49, $p< 0.05$; Fig. 2B) tests compared to the morphine group. On the other hand, co-administration of morphine and selektive 5-HT_3 agonist PBG (7.5 mg/kg, i.p.) caused a significant decrease in the analgesic effect compared to the morphine group ($F_{5,24}$: 11.58 for TF and $F_{5,24}$: 17.07 for HP, $p < 0.05$). Furthermore, these data demonstrated that ODN alone has a significant analgesia in both TF and HP tests compared to the saline

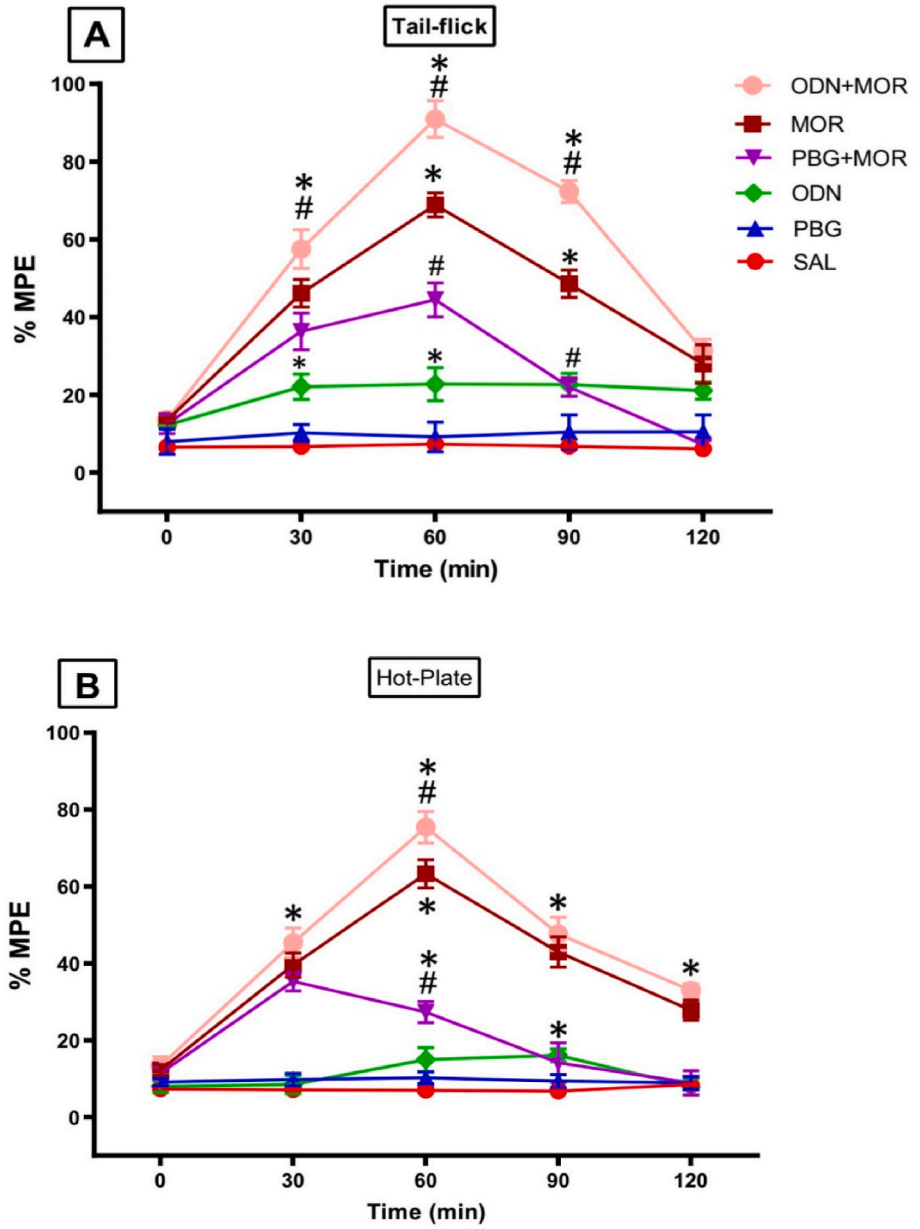


Fig. 2. Effects of ODN (2 mg/kg) and PBG (7.5 mg/kg) on morphine analgesia. The analgesic effect (%MPE) of ODN + MOR group was significantly higher in both tail-flick (A) and hot-plate test (B) compared to the MOR group ($p < 0.05$). However, the analgesic effect of PBG + MOR group was significantly decreased compared to the morphine group rats ($p < 0.05$). Each point represents the mean ± SEM of % MPE for 6 rats. *$p < 0.01$ vs. saline group and #$p < 0.05$ vs. morphine group (two way ANOVA followed by Tukey HSD post hoc test). Morphine test dose: 5 mg/kg % MPE, Percent maximum possible effect; MOR, morphine; ODN, ondansetron; PBG, 1-phenylbiguanide; SAL, saline.

group ($p < 0.05$). However, no significant increase was observed in the PBG group.

3.2. The effects of AS19 and SB-269970 on morphine analgesia

Co-administration of morphine and the selective 5-HT_7 agonist AS19 (2.5 mg/kg, s.c.) resulted in a significant increase in analgesic effect compared to the morphine group ($F_{5,24}$: 67.95 for TF and $F_{5,24}$: 42.55 for HP, $p < 0.05$; Fig. 3A–B). However, administration of SB-269970 (5-HT_7 antagonist) with morphine did not significantly decrease % MPE compared to the morphine group in both TF and HP tests ($p > 0.05$). Neither the AS19 nor the SB-269970 groups alone produced an anti-nociceptive effect compared to saline.

3.3. The effects of ODN, PBG, AS19 and SB-269970 on morphine tolerance

ODN administration to morphine-tolerant rats significantly increased the antinociceptive effect in both TF ($F_{4,20}$: 12.25, $p < 0.05$; Fig. 4A) and HP ($F_{4,20}$: 13.20, $p<0.05$; Fig. 4B) tests compared to the morphine-tolerant group. The maximal antinociceptive effect was measured at 60 min measurements and at the same time, a significant increase was observed in % MPE values at 30 min and 90 min. However, the difference between the mean % MPE of the PBG group and the mean of the morphine tolerant group was not statistically significant ($p > 0.05$).

Combination of morphine with the selective 5-HT_7 agonist AS19 showed a statistically significant increase in antinociceptive activity compared to the morphine tolerant group ($F_{4,20}$: 11.55 for TF and $F_{4,20}$:

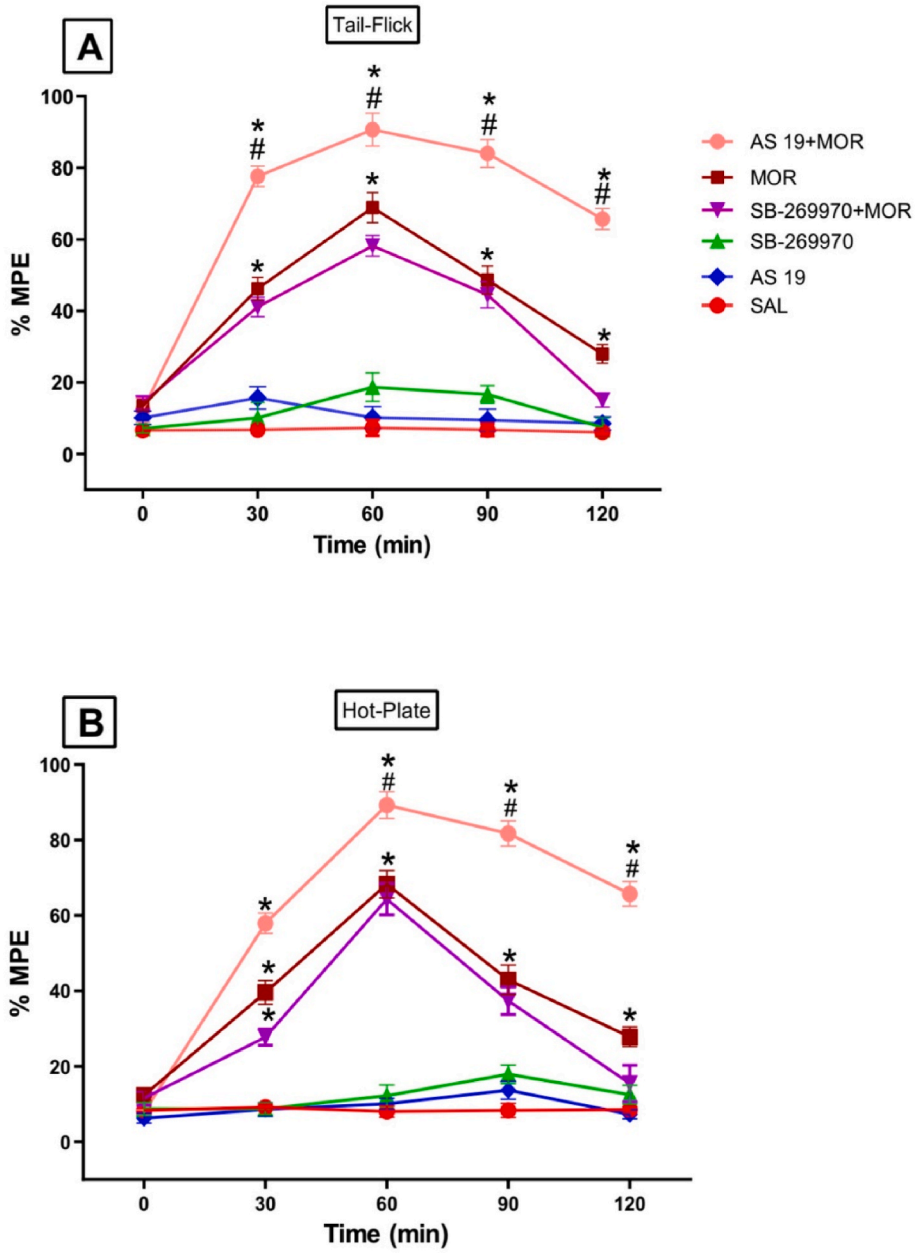


Fig. 3. Effects of A S19 (2.5 mg/kg) and SB-269970 (3 mg/kg) on morphine analgesia. Co-administration of AS 19 with MOR to rats indicated a significant increase in analgesic effect compared to that injected with morphine alone both tail-flick (A) and hot-plate (B) tests ($p < 0.05$). The analgesic effect of SB-269970 + MOR group was significantly decreased compared to the morphine group rats ($p < 0.05$). Each point represents the mean ± SEM of % MPE for 6 rats. *$p < 0.01$ vs. saline treated group, and #$p < 0.05$ vs. morphine group. MOR, morphine; SAL, saline.

14.02 for HP, $p < 0.05$; Fig. 4C–D). In addition, in the group in which AS19 and morphine were combined, maximal analgesic effect was observed only in the TF test.

3.4. Effects of drugs on 5-HT$_{3A}$ receptor expressions in dorsal root ganglia (DRGs)

When the stained preparations were examined under the microscope (magnification, ×400) after immunohistochemical analysis, it was observed that 5-HT$_{3A}$ immunoreactivity in DRG increased more in the morphine tolerant group than in the saline and morphine groups (Fig. 5A).

There was no significant increase in 5-HT$_{3A}$ receptor protein expressions in the saline and morphine groups. While 5-HT$_{3A}$ immunoreactivity was decreased in ODN groups, it was increased in PBG groups.

H-score analysis showed that 5-HT$_{3A}$ immunoreactivity was significantly reduced by administration of ODN to morphine-tolerant rats ($F_{8,45}$: 76.72, $p < 0.01$; Fig. 5B). However, 5-HT$_{3A}$ immunoreactivity was found to be significantly higher in the group given PBG to morphine-tolerant rats than the mean of the morphine-tolerant group ($F_{8,45}$: 36.52, $p < 0.05$). In addition, 5-HT$_{3A}$ receptor protein expressions of the morphine tolerant group showed a significant increase compared to the saline ($p < 0.001$).

3.5. Effects of drugs on 5-HT$_7$ receptor expressions in DRGs

It was determined that 5-HT$_7$ expression in stained DRG tissue sections was significantly decreased in the morphine tolerant group compared to the saline group (Fig. 6A). However, significant increases in 5-HT$_7$ receptor protein expressions were detected in the microscopic

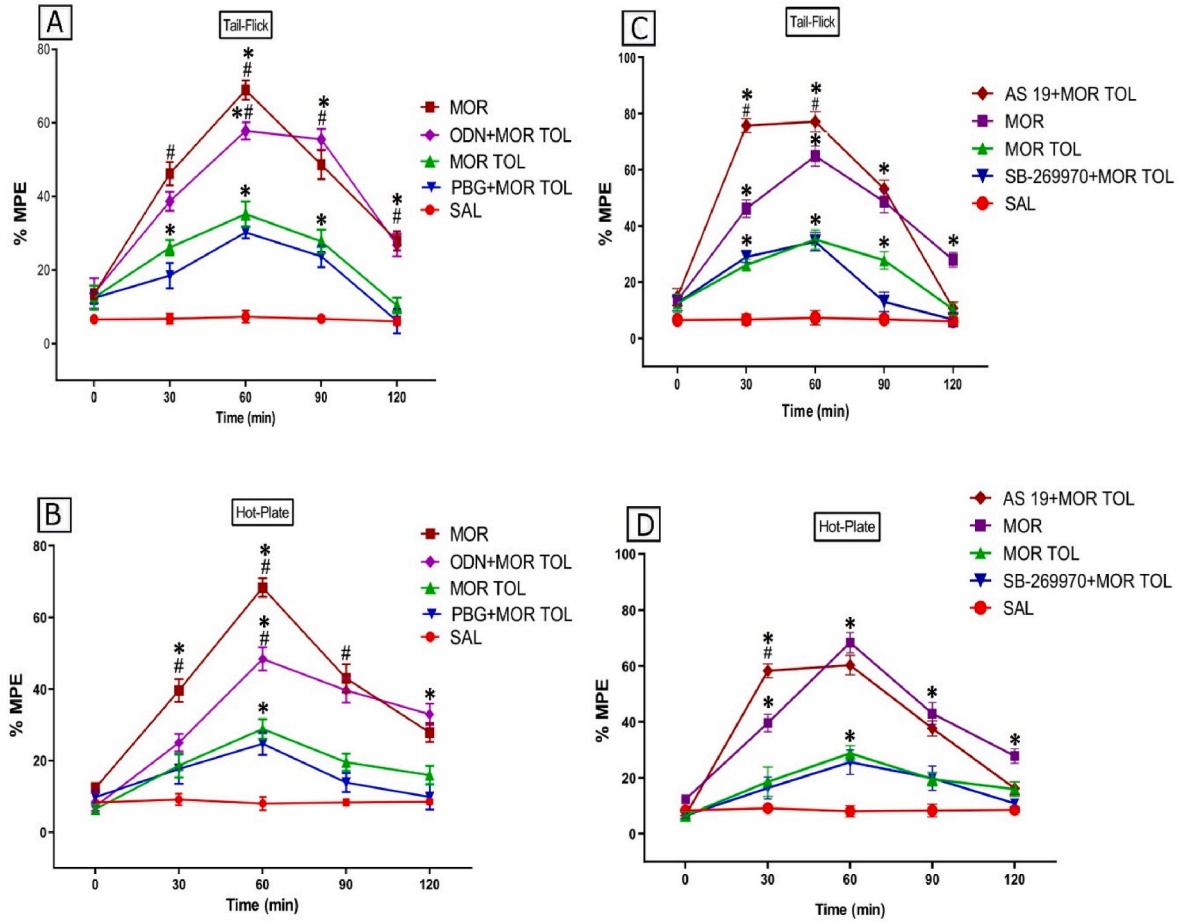


Fig. 4. Effects of ODN, PBG, AS19 and SB-269970 on morphine tolerance. Administration of ODN to morphine-tolerant rats significantly increased the analgesic efficiency in both tail-flick (A) and hot-plate (B) tests compared to the morphine tolerant group ($p < 0.05$). On the other hand, PBG did not provide a significant reduction in morphine tolerance. Similarly, administration of AS19 to morphine-tolerant rats reduced morphine tolerance in both the tail-flick (C) and hot-plate (D) tests, but not when injected with SB-269970. Each point represents the mean ± SEM of % MPE for 6 rats. *$p < 0.01$ vs. saline treated group, and #$p < 0.05$ vs. morphine group. MOR, morphine; ODN, ondansetron; PBG, 1-phenylbiguanide; SAL, saline.

images of the stained sections of the AS19-treated groups.

H-score analysis demonstrated that 5-HT_7 receptor expression was significantly decreased in the morphine tolerant group compared to the saline group ($F_{8,45}$: 9.15, $p < 0.01$; Fig. 6B). On the other hand, administration of 5-HT_7 receptor agonist AS19 to morphine tolerant group rats resulted in a significant increase in 5-HT_7 receptor expression compared to the morphine tolerant group ($F_{8,45}$: 7.14, $p < 0.05$). Administration of SB-269970 to morphine tolerant group rats caused a decrease in 5-HT_7 receptor expression.

4. Discussion

The results of this study demonstrated that the selective 5-HT_3 receptor antagonist ODN increased the analgesic activity of morphine and attenuated morphine tolerance in thermal analgesia tests. Similarly, the 5-HT_7 receptor agonist AS19 decreased morphine tolerance by increasing the analgesic activity of morphine. The effects of 5-HT_3 and 5-HT_7 receptor agonists and antagonists would be expected to be opposite to each other (Dogrul et al., 2009). However, antinociceptive test results showed this opposite relationship in rats without morphine tolerance induced. These results suggest that not only 5-HT_3 and 5-HT_7 receptors but also other mechanisms may be effective in morphine tolerance. Moreover, it can be determined that the 5-HT_3 receptor agonist and antagonist are opposite each other in their effects on the morphine antinociceptive activity. Immunohistochemical analysis of DRG sections indicated that 5-HT_3 receptor expression was enhanced in morphine-tolerant rats. Administration of ODN to morphine-tolerant rats caused a decrease in 5-HT_3 expression. However, a decrease in H-score of 5-HT_7 receptor was detected in DRG sections of morphine-tolerant rats. Injection of AS19 to morphine-tolerant rats increased the expression of 5-HT_7 receptors. These results may suggest that changes in 5-HT receptor expression are independent of the antinociceptive effects of morphine.

It has been suggested for many years that there is a relationship between opioid analgesia and 5-HT, and it has been reported that descending serotonergic pathways play an important role in the antinociceptive effect of opioid (Millan, 2002; Ozdemir et al., 2012). It is stated that some of the analgesic effects of opioids are achieved by the activation of the serotonergic system. In addition, acute morphine administration increases 5-HT synthesis, release and metabolism (Arends et al., 1998). However, the effects of serotonin 5-HT_3 and 5-HT_7 subtypes on morphine analgesia are not fully understood and are controversial.

The 5-HT_{3A} receptor subunits are expressed in brain, spinal cord and DRG tissue (Kia et al., 1995; Morales et al., 2001). Expression of 5-HT_{3A} in DRGs supports the role of 5-HT_{3A} receptors in the modulation of activity in primary sensory afferent fibers (Tecott et al., 1993). The 5-HT_3 receptor is a ligand-gated ion channel and, when activated by agonist, elicits rapid depolarizing responses in neurons (Cortes-Altamirano et al., 2018). Administration of morphine increases 5-HT in the spinal dorsal horn, which attenuates the analgesic effects of morphine on neuropathic pain via spinal 5-HT_3 receptors. It has been shown that ondansetron

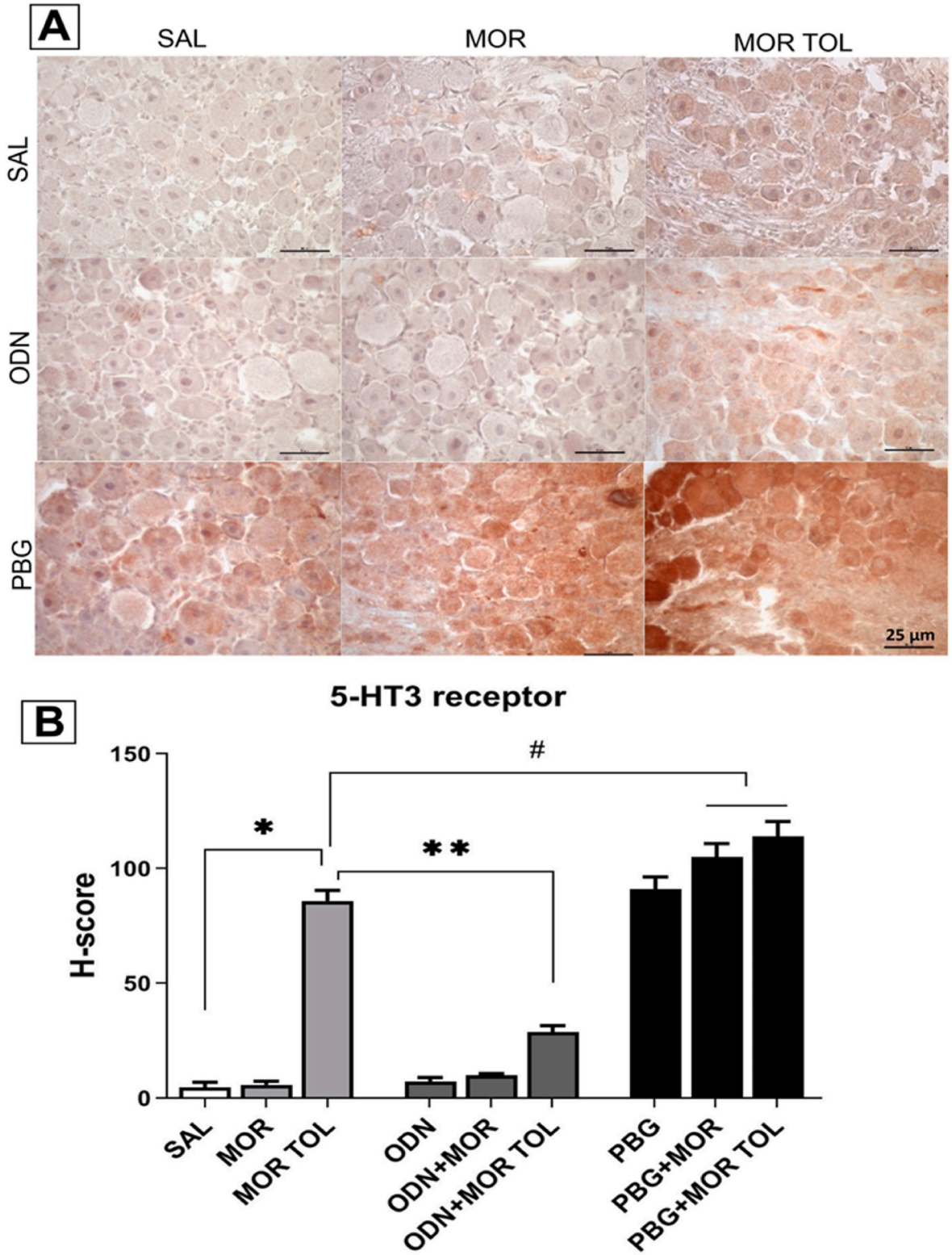
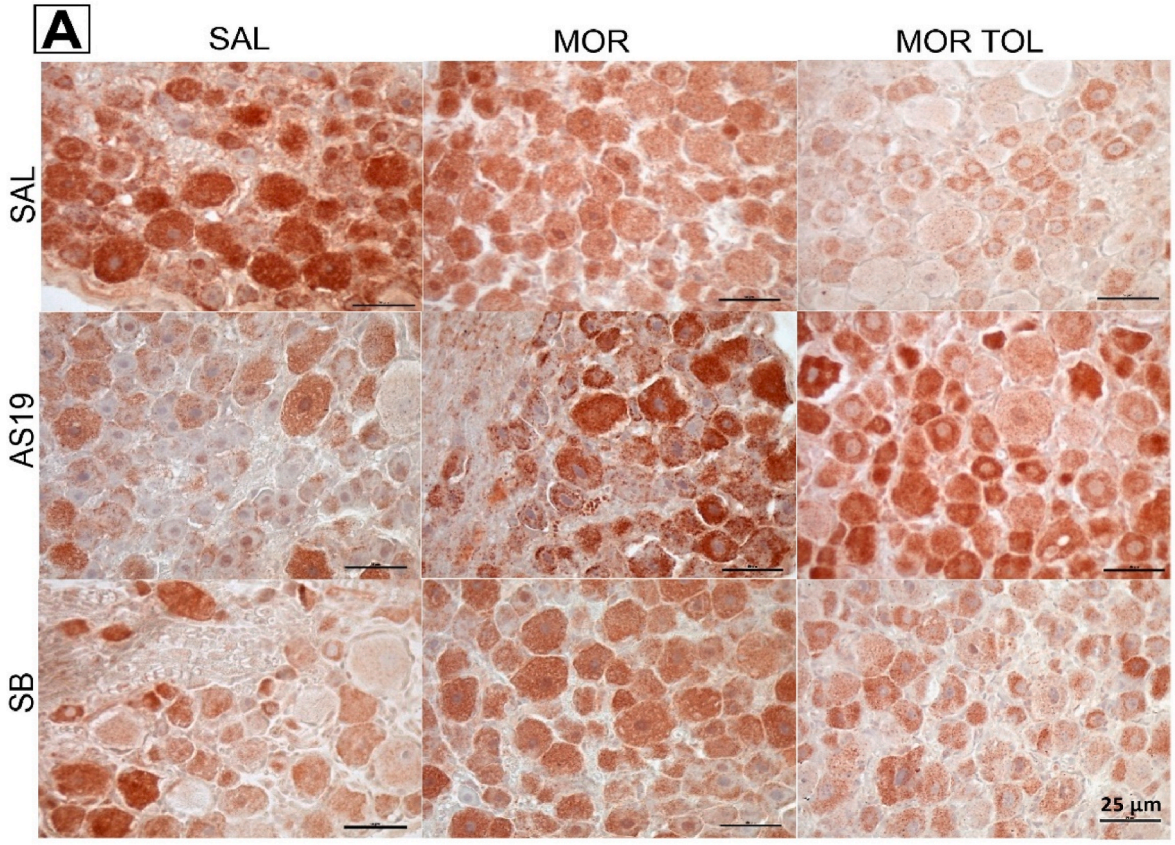


Fig. 5. Effects of drugs on 5-HT3A receptor expressions in dorsal root ganglia. (A) image shows 5-HT3 receptor expressions immunohistochemically in DRG sections (magnification, ×400). (B) image shows the H-score means of 5-HT3 receptor expressions (n = 6). *p < 0.001 vs. saline treated group, **p < 0.01 vs. morphine-tolerant group, #p < 0.05 vs. morphine-tolerant group. MOR, morphine; ODN, ondansetron; PBG, 1-phenylbiguanide; SAL, saline.

blocks morphine-induced increases in mice of multiple genes involved in hyperalgesia and tolerance in the DRG and spinal cord (Liang et al., 2011). Dysfunction of the descending serotonergic system contributes to reducing the efficacy of morphine on neuropathic pain via spinal 5-HT_3 receptors and GABA neurons (Hiroki et al., 2021; Kimura et al., 2014). Animal studies have shown that spinal 5-HT_3 receptor blockade reduces hypersensitivity caused by nerve damage, and a clinical trial has shown that ondansetron relieves chronic pain (McCleane et al., 2003; Suzuki et al., 2004). Consistent with this study, our study results showed that administration of ondansetron to rats caused an increase in the analgesic



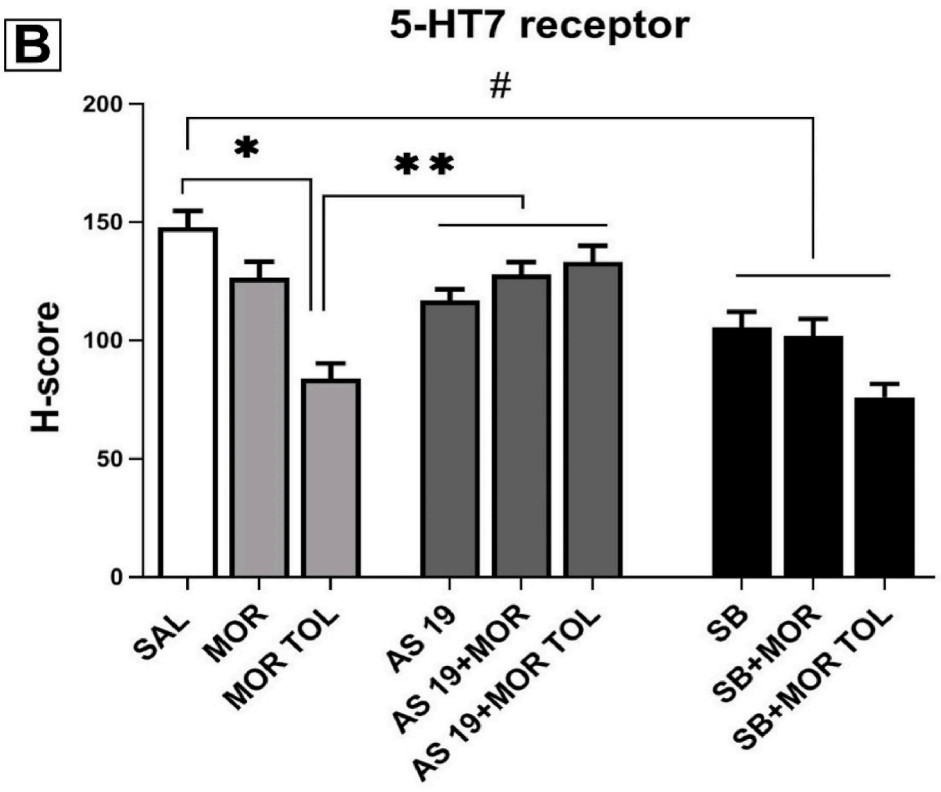


Fig. 6. Effects of drugs on 5-HT$_7$ receptor expressions in dorsal root ganglia. (A) image shows 5-HT$_7$ receptor expressions immunohistochemically in DRG sections (magnification, ×400). (B) image shows the H-score means of 5-HT$_7$ receptor expressions (n = 6). *p < 0.01 vs. saline treated group, **p < 0.05 vs. morphine-tolerant group, #p < 0.05 vs. morphine-tolerant group. MOR, morphine; MOR TOL, morphine tolerant; SB; SB-269970; SAL, saline.

effect of morphine. In addition, ondansetron caused a decrease in 5-HT receptor expressions in DRG neurons, possibly increasing the analgesic effect of morphine in this way. Likewise, PBG reduced these effects. However, in some experimental studies, it has been emphasized that 5-HT_3 receptor agonists cause a decrease in hypersensitivity (Okazaki et al., 2008).

The mechanism of tolerance expressed by the attenuated antinociceptive effect of morphine is not yet clearly understood, but it is thought to probably involve descending serotonergic pathways (Liang et al., 2011). Molecular studies indicate that the 5-HT_3 receptor plays an important role in the development of morphine antinociceptive tolerance. In this study, the spinally administered 5-HT_3 receptor antagonist ondansetron has been reported to block morphine-induced hyperalgesia and antinociceptive tolerance (Vera-Portocarrero et al., 2007). In our study, ODN, which we administered intraperitoneally, resulted in a decrease in morphine tolerance. However, in a clinical study in patients given intrathecal opioids prior to cesarean section, ODN had no effect on acute opioid tolerance (Greer et al., 2017).

Activation of spinal 5-HT_7 receptors mediates antinociception, while spinal 5-HT_3 receptors mediate pain transmission (Dogrul et al., 2009). Accordingly, the 5-HT_7 receptor agonist AS19 has been shown to have an antinociceptive role as well as a potential role in the treatment of diabetic neuropathic pain. In addition, the 5-HT_7 receptor has also been associated with the antinociceptive effects of commonly used analgesic drugs such as tramadol, acetaminophen, and morphine (Blattner et al., 2019; Yanarates et al., 2010). In addition to the antinociceptive effects of 5-HT_7 receptor activation, studies such as formalin test or spinal nerve ligation showing pronociceptive effects have also been reported (Godínez-Chaparro et al., 2012; Rocha-González et al., 2005). Current evidence indicates that spinal activation of 5-HT_7 receptors plays an important role in the expression of opiate-induced analgesia, possibly through activation of the descending inhibition pathway. In addition, 5-HT_7 receptor inactivation increases morphine withdrawal syndrome (Shahidi & Hashemi-Firouzi, 2014). In support of this, subcutaneous administration of the selective 5-HT_7 receptor agonist E−55888 was shown to potentiate the analgesic effect of morphine in mice (Brenchat et al., 2011). Consistent with this, our study results showed that the analgesic activity of morphine increased with the administration of the 5-HT_7 receptor agonist AS19 to rats. However, tail flick test data indicated that intrathecally administered 5-HT_7 antagonist SB-269970 inhibits the antinociceptive effects of systemic morphine (Brenchat et al., 2009). The 5-HT_3 receptor is an ion channel and mediates neuronal stimulation in the central and peripheral nervous systems and contains a pore that is permeable to Na^+, K^+ and Ca^{2+} ions (Liang et al., 2011). On the other hand, the 5-HT_7 receptor is a member of the Gs protein receptor family and its intracellular signaling molecule is cAMP. Blockade of 5-HT_7 receptors inhibits the antinociceptive effect of opioids (Lee et al., 2015). The opposite effects of these two receptors on morphine activity may be due to the difference in these action pathways. Immunohistochemical techniques have demonstrated 5-HT_7 expression in various nerve tissues including the thalamus, hypothalamus, hippocampus, and spinal cord dorsal horn (Hedlund & Sutcliffe, 2004). Our results showed that 5-HT_7 receptor expression was reduced in DRG neurons in morphine-tolerant rats. On the other hand, it was observed that the expression of 5-HT_7 increased with the administration of AS19. These results demonstrate that downregulation of 5-HT_7 receptors and upregulation of 5-HT_3 receptors is a possible mechanism for morphine tolerance (Fig. 7).

5. Conclusion

In conclusion, thermal analgesia tests showed that selective 5-HT_3 receptor antagonist ODN and 5-HT_7 agonist AS19 increased the analgesic effect of morphine and decreased morphine tolerance. In addition, 5-HT_3 receptor protein expression was increased in DRG neurons of morphine-tolerant rats, whereas 5-HT_7 receptor expression decreased. Furthermore, administration of ODN decreased 5-HT_3 receptor expression in morphine-tolerant rats, while AS19 increased 5-HT_7 receptor expression. The results indicate that increased 5-HT_3 receptor expression and decreased 5-HT_7 receptor expression in DRG sensory neurons play an important role in the development of morphine tolerance. Further studies are needed to clarify other mechanisms underlying the effect of ODN and AS19 on morphine analgesia and tolerance.

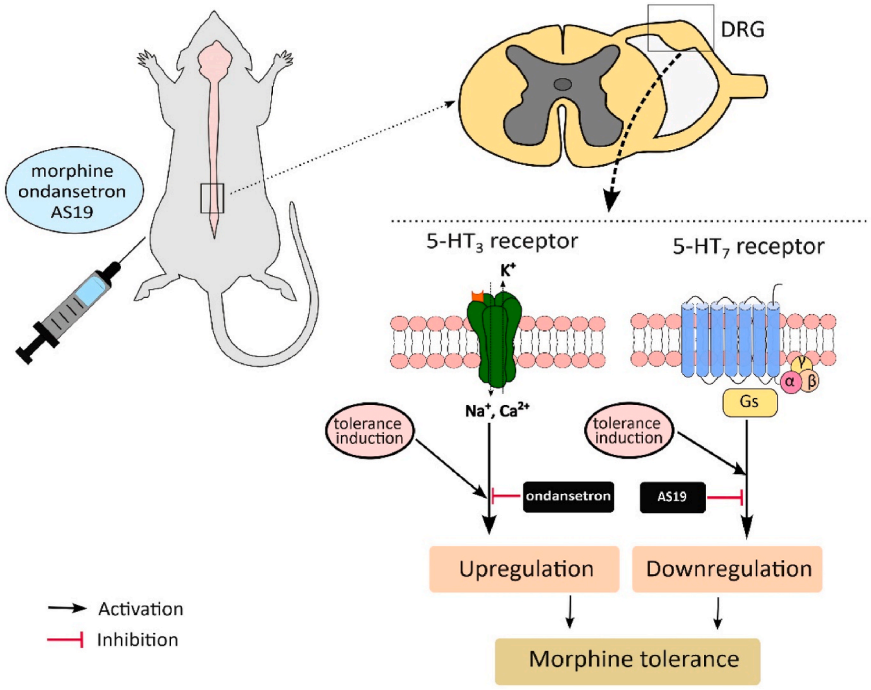


Fig. 7. Possible mechanism for ondansetron and AS19 to attenuate morphine anticiceptive tolerance. Ondansetron inhibits 5-HT3 receptor upregulation in the DRG and AS19 prevents 5-HT7 receptor downregulation. Both effects reduce morphine anticiceptive tolerance. DRG, dorsal root ganglion.

Funding

This study was supported by Cumhuriyet University Scientific Research Project (T-735, CUBAP, Sivas, Turkey).

Declaration of competing interest

All authors declare no conflict of interest.

Acknowledgments

The authors would like to thank Cumhuriyet University Scientific Research Project Presidency for supporting this study.

References

Allouche, S., Noble, F., & Marie, N. (2014). Opioid receptor desensitization: Mechanisms and its link to tolerance. *Frontiers in Pharmacology, 18*(5), 280.

Arends, R. H., Hayashi, T. G., Luger, T. J., & Shen, D. D. (1998). Cotreatment with racemic fenfluramine inhibits the development of tolerance to morphine analgesia in rats. *Journal of Pharmacology and Experimental Therapeutics, 286*, 585–592.

Avci, O., Ozdemir, E., Taskiran, A. S., Inan, Z. D. S., & Gursoy, S. (2022). Metformin prevents morphine-induced apoptosis in rats with diabetic neuropathy: A possible mechanism for attenuating morphine tolerance. *Naunyn-Schmiedeberg's Archives of Pharmacology, 395*(11), 1449–1462.

Bardin, L., & Colpaert, F. C. (2004). Role of spinal 5-HT (1A) receptors in morphine analgesia and tolerance in rats. *European Journal of Pain, 8*(3), 253–261.

Bardin, L., Lavarenne, J., & Eschalier, A. (2000). Serotonin receptor subtypes involved in the spinal antinociceptive effect of 5-HT in rats. *Pain, 86*(1–2), 11–18.

Blattner, K. M., Canney, D. J., Pippin, D. A., & Blass, B. E. (2019). Pharmacology and therapeutic potential of the 5-HT7 receptor. *ACS Chemical Neuroscience, 16*(10), 89–119.

Brenchat, A., Ejarque, M., Zamanillo, D., Vela, J. M., & Romero, L. (2011). Potentiation of morphine analgesia by adjuvant activation of 5-HT7 receptors. *Journal of Pharmacological Sciences, 116*(4), 388–391.

Brenchat, A., Romero, L., García, M., Pujol, M., Burgueño, J., Torrens, A., Hamon, M., et al. (2009). 5-HT7 receptor activation inhibits mechanical hypersensitivity secondary to capsaicin sensitization in mice. *Pain, 141*(3), 239–247.

Caillé, S., Stinus, L., Espejo, E. F., De Deurwaerdère, P., Spampinato, U., & Koob, G. F. (2003). Inhibition of 5-HT neurotransmission increases clonidine protective effects on naloxone-induced conditioned place aversion in morphine-dependent rats. *Neuropsychopharmacology, 28*(2), 276–283.

Chen, S. R., Prunean, A., Pan, H. M., Welker, K. L., & Pan, H. L. (2007). Resistance to morphine analgesic tolerance in rats with deleted transient receptor potential vanilloid type 1-expressing sensory neurons. *Neuroscience, 16*(145), 676–685.

Cheon, K. W., Lee, H. S., Parhar, I. S., & Kang, I. S. (2001). Expression of the second isoform of gonadotrophin-releasing hormone (GnRH-II) in human endometrium throughout the menstrual cycle. *Molecular Human Reproduction, 7*(5), 447–452.

Contreras, E., Tamayo, L., Quijada, L., & Silva, E. (1973). Decrease of tolerance development to morphine by 5-hydroxytryptophan and some related drugs. *European Journal of Pharmacology, 22*(3), 339–343.

Cortes-Altamirano, J. L., Olmos-Hernandez, A., Jaime, H. B., Carrillo-Mora, P., Bandala, C., Reyes-Long, S., & Alfaro-Rodríguez, A. (2018). Review: 5-HT1, 5-HT2, 5-HT3 and 5-HT7 receptors and their role in the modulation of pain response in the central nervous system. *Current Neuropharmacology, 30*(16), 210–221.

Dogrul, A., Ossipov, M. H., & Porreca, F. (2009). Differential mediation of descending pain facilitation and inhibition by spinal 5HT-3 and 5HT-7 receptors. *Brain Research, 14*(1280), 52–59.

Dogrul, A., & Seyrek, M. (2006). Systemic morphine produce antinociception mediated by spinal 5-HT7, but not 5-HT1A and 5-HT2 receptors in the spinal cord. *British Journal of Pharmacology, 149*(5), 498–505.

Godínez-Chaparro, B., López-Santillán, F. J., Orduña, P., & Granados-Soto, V. (2012). Secondary mechanical allodynia and hyperalgesia depend on descending facilitation mediated by spinal 5-HT$_4$, 5-HT$_6$ and 5-HT$_7$ receptors. *Neuroscience, 11*(222), 379–391.

Greer, K. C., Terkawi, A. S., Tsang, S., Singla, P., Durieux, M. E., & Tiouririne, M. (2017). The effect of ondansetron on acute opioid tolerance in patients receiving intrathecal opioids prior to cesarean delivery. *Regional Anesthesia and Pain Medicine, 42*(5), 669–673.

Hedlund, P. B., & Sutcliffe, J. G. (2004). Functional, molecular and pharmacological advances in 5-HT7 receptor research. *Trends in Pharmacological Sciences, 25*(9), 481–486.

Hiroki, T., Suto, T., Ohta, J., Saito, S., & Obata, H. (2021). Spinal γ-aminobutyric acid interneuron plasticity is involved in the reduced analgesic effects of morphine on neuropathic pain. *The Journal of Pain, 20*. S1526-5900(21)00350-3.

Huang, J., Liang, X., Wang, J., Kong, Y., Zhang, Z., Ding, Z., & Zou, W. (2019). miR-873a-5p targets A20 to facilitate morphine tolerance in mice. *Frontiers in Neuroscience, 13*, 347.

Jeong, C. Y., Choi, J. I., & Yoon, M. H. (2004). Roles of serotonin receptor subtypes for the antinociception of 5-HT in the spinal cord of rats. *European Journal of Pharmacology, 19*(502), 205–211.

Jr McCarty, K. S., Miller, L. S., Cox, E. B., Konrath, J., & Sr McCarty, K. S. (1985). Estrogen receptor analyses. Correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. *Archives of Pathology and Laboratory Medicine, 109*, 716–721.

Kanaan, S. A., Saadé, N. E., Haddad, J. J., Abdelnoor, A. M., Atweh, S. F., Jabbur, S. J., & Safieh-Garabedian, B. (1996). Endotoxin-induced local inflammation and hyperalgesia in rats and mice: A new model for inflammatory pain. *Pain, 66*(2–3), 373–379.

Kia, H. K., Miquel, M. C., McKernan, R. M., Laporte, A. M., Lombard, M. C., Bourgoin, S., Hamon, M., & Verge, D. (1995). Localization of 5-HT3 receptors in the rat spinal cord: Immunohistochemistry and in situ hybridization. *NeuroReport, 6*, 257–261.

Kimura, M., Obata, H., & Saito, S. (2014). Peripheral nerve injury reduces analgesic effects of systemic morphine via spinal 5-hydroxytryptamine 3 receptors. *Anesthesiology, 121*(2), 362–371.

Lee, H. G., Kim, W. M., Kim, J. M., Bae, H. B., & Choi, J. I. (2015). Intrathecal nefopam-induced antinociception through activation of descending serotonergic projections involving spinal 5-HT7 but not 5-HT3 receptors. *Neuroscience Letters, 5*(587), 120–125.

Liang, D. Y., Li, X., & Clark, J. D. (2011). 5-hydroxytryptamine type 3 receptor modulates opioid-induced hyperalgesia and tolerance in mice. *Anesthesiology, 114*(5), 1180–1189.

Lilius, T. O., Rauhala, P. V., Kambur, O., Rossi, S. M., Väänänen, A. J., & Kalso, E. A. (2012). Intrathecal atipamezole augments the antinociceptive effect of morphine in rats. *Anesthesia & Analgesia, 114*(6), 1353–1358.

McCleane, G. J., Suzuki, R., & Dickenson, A. H. (2003). Does a single intravenous injection of the 5HT3 receptor antagonist ondansetron have an analgesic effect in neuropathic pain? A double blinded, placebo-controlled cross-over study. *Anesthesia & Analgesia, 97*, 1474–1478.

Millan, M. J. (2002). Descending control of pain. *Progress in Neurobiology, 66*(6), 355–474.

Morales, M., McCollum, N., & Kirkness, E. F. (2001). 5-HT (3)-receptor subunits A and B are co-expressed in neurons of the dorsal root ganglion. *The Journal of Comparative Neurology, 438*(2), 163–172, 17.

Muchhala, K. H., Jacob, J. C., Dewey, W. L., & Akbarali, H. I. (2021). Role of β-arrestin-2 in short- and long-term opioid tolerance in the dorsal root ganglia. *European Journal of Pharmacology, 15*(899), Article 174007.

Nayebi, A. R., & Charkhpour, M. (2006). Role of 5-HT (1A) and 5-HT (2) receptors of dorsal and median raphe nucleus in tolerance to morphine analgesia in rats. *Pharmacology Biochemistry and Behavior, 83*(2), 203–207.

Neal, B. S., & Sparber, S. B. (1986). Mianserin attenuates naloxone-precipitated withdrawal signs in rats acutely or chronically dependent upon morphine. *Journal of Pharmacology and Experimental Therapeutics, 236*(1), 157–165.

Okazaki, R., Namba, H., Yoshida, H., Okai, H., Miura, T., & Kawamura, M. (2008). The antiallodynic effect of Neurotropin is mediated via activation of descending pain inhibitory systems in rats with spinal nerve ligation. *Anesthesia & Analgesia, 107*, 1064–1069.

Ozdemir, E., Avcı, O., Inan, Z. D. S., Taskiran, A. S., Gunes, H., & Gursoy, S. (2023). Aspirin attenuates morphine antinociceptive tolerance in rats with diabetic neuropathy by inhibiting apoptosis in the dorsal root ganglia. *Metabolic Brain Disease, 38*(6), 2145–2158.

Ozdemir, E., Bagcivan, I., Durmus, N., Altun, A., & Gursoy, S. (2011). The nitric oxide-cGMP signaling pathway plays a significant role in tolerance to the analgesic effect of morphine. *Canadian Journal of Physiology and Pharmacology, 89*(2), 89–95.

Ozdemir, E., Baser, T., & Taskiran, A. S. (2022). Blockade of orexin receptor type-1 by SB-334867 and activation of orexin receptor type-2 attenuate morphine tolerance in rats. *Physics International, 109*(4), 457–474.

Ozdemir, E., Gursoy, S., & Bagcivan, I. (2012). The effects of serotonin/norepinephrine reuptake inhibitors and serotonin receptor agonist on morphine analgesia and tolerance in rats. *The Journal of Physiological Sciences, 62*(4), 317–323.

Ramabadran, K., Bansinath, M., Turndorf, H., & Puig, M. M. (1989). The hyperalgesic effect of naloxone is attenuated in streptozotocin diabetic mice. *Psychopharmacology (Berl.), 97*, 169–17.

Rocha-González, H. I., Meneses, A., Carlton, S. M., & Granados-Soto, V. (2005). Pronociceptive role of peripheral and spinal 5-HT7 receptors in the formalin test. *Pain, 117*(1–2), 182–192.

Roczniak, W., Babuśka-Roczniak, M., Kwapuliński, J., Brodziak-Dopierała, B., Widuchowski, W., Cipora, E., & Oświęcimska, J. M. (2015). The effect of central noradrenergic system lesion on dopamine (DA) and serotonin (5-HT) synthesis rate following administration of 5-HT3 receptor ligands in chosen parts of the rat brain. *Pharmacological Reports, 67*(1), 146–151.

Shahidi, S., & Hashemi-Firouzi, N. (2014). The effects of a 5-HT7 receptor agonist and antagonist on morphine withdrawal syndrome in mice. *Neuroscience Letters, 22*(578), 27–32.

Sun, J., Chen, S. R., Chen, H., & Pan, H. L. (2019). μ-Opioid receptors in primary sensory neurons are essential for opioid analgesic effect on acute and inflammatory pain and opioid-induced hyperalgesia. *The Journal of Physiology, 597*, 1661–1675.

Sun, J., Chen, S. R., & Pan, H. L. (2020). μ-Opioid receptors in primary sensory neurons are involved in supraspinal opioid analgesia. *Brain Research, 1729*, Article 146623.

Suzuki, R., Rygh, L. J., & Dickenson, A. H. (2004). Bad news from the brain: Descending 5-HT pathways that control spinal pain processing. *Trends in Pharmacological Sciences, 25*, 613–617.

Tecott, L. H., Maricq, A. V., & Julius, D. (1993). Nervous system distribution of the serotonin 5-HT3 receptor mRNA. *Proceedings of the National Academy of Sciences of the United States of America, 90*(4), 1430–1434.

Vera-Portocarrero, L. P., Zhang, E. T., King, T., Ossipov, M. H., Vanderah, T. W., Lai, J., & Porreca, F. (2007). Spinal NK-1 receptor expressing neurons mediate opioid-induced hyperalgesia and antinociceptive tolerance via activation of descending pathways. *Pain, 129*(1–2), 35–45.

Yanarates, O., Dogrul, A., Yildirim, V., Sahin, A., Sizlan, A., Seyrek, M., Akgül, O., et al. (2010). Spinal 5-HT$_7$ receptors play an important role in the antinociceptive and antihyperalgesic effects of tramadol and its metabolite, O-Desmethyltramadol, via activation of descending serotonergic pathways. *Anesthesiology, 112*(3), 696–710.