Attenuation of morphine antinociceptive tolerance by cannabinoid CB1 and CB2 receptor antagonists

Ahmet Altun1 • Kemal Yıldırım1 • Ercan Ozdemir2 • Ihsan Bagcivan1 • Sinan Gursoy3 • Nedim Durmus4

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Abstract Cannabinoid CB1 and CB2 receptor antagonists may be useful for their potential to increase or prolong opioid analgesia while attenuating the development of opioid tolerance. The aim of this study was to investigate the effects of AM251 (a selective CB1 antagonist) and JTE907 (a selective CB2 antagonist) on morphine analgesia and tolerance in rats. Adult male Wistar albino rats weighing 205–225 g were used in these experiments. To constitute morphine tolerance, we used a 3 day cumulative dosing regimen. After the last dose of morphine was injected on day 4, morphine tolerance was evaluated by analgesia tests. The analgesic effects of morphine (5 mg/kg), ACEA (a CB1 receptor agonist, 5 mg/kg), JWH-015 (a CB2 receptor agonist, 5 mg/kg), AM251 (1 mg/kg) and JTE907 (5 mg/kg) were considered at 30-min intervals (0, 30, 60, 90, and 120 min) by tail-flick and hot-plate analgesia tests. Our findings indicate that ACEA and JWH015 significantly increased morphine analgesia and morphine antinociceptive tolerance in the analgesia tests. In contrast, the data suggested that AM251 and JTE907 significantly attenuated the expression of morphine tolerance. In conclusion, we observed that co-injection of AM251 and JTE907 with morphine attenuated expression of tolerance to morphine analgesic effects and decreased the morphine analgesia.

Keywords Cannabinoid receptors • AM251 • JTE907 • Morphine analgesia • Morphine tolerance

Introduction

Opioids such as morphine are the most effective treatment for most types of pain. However, the chronic use of opioids is restricted by the potential for addiction, adverse effects and the development of tolerance to opioids analgesia [1, 2]. The mechanisms underlying the development of morphine tolerance are complicated and not exactly understood. There are several possible explanations for the development of opioid tolerance, including receptor desensitization, up-regulation of the cAMP pathway, induction of nitric oxide–cGMP and alfa-2 noradrenergic systems, serotonergic systems, and protein kinase-dependent neuroadaptative changes in signal transduction cascades (G protein-coupled receptor kinases) [3–6].

One promising approach to enhance the antinociceptive effect of opioids is combination drug treatment in which low doses of different types of analgesics (such as opioids and cannabinoids) are administered. Although the analgesic effects of cannabinoids are relatively mild [7, 8], pretreatment with a non-analgesic dose of tetrahydrocannabinol (THC) has been shown to cause up to a 22-fold enhancement of the morphine analgesic effect [9]. The enhanced antinociception occurs following oral, intrathecal and systemic injections [10–12].
Cannabinoid (CB) receptors have been implicated in pain transduction and perception [13] as well as neuroinflammation [14]. These receptors are the most common G protein-coupled receptors in the brain and are expressed at various levels along the body in humans. There are two cannabinoid receptor subtypes, cannabinoid CB1, which is expressed in the brain as well as many peripheral tissues, and CB2, which is expressed mainly on immune cells and damaged tissues, and in the midbrain [15]. Opioids and cannabinoids produce analgesia through activity at spinal, supraspinal and peripheral sites. The convergence of the intracellular signaling pathway of the mu-opioid and CB1-receptors likely underlies both the mutual potentiation of the responses evoked by opioid and cannabinoid agonists and the reported cross-tolerance between these agonists [16]. While the mechanisms underlying such interactions remain inexplicit, a recent study indicates that formation of heteromeric receptor complexes between mu-opioid and CB1-receptors may contribute to functional interaction between the two classes of agonists [17]. In addition, prolonged exposure to morphine alters both CB1-receptor function and endocannabinoid levels [18]. Chronic morphine has also been reported to upregulate the CB1-receptor density and augment G-protein-coupled signaling [19, 20]. In addition, several reports have demonstrated antinociceptive efficacy of selective CB2 receptor agonists in models of acute and neuropathic pain [21, 22]. Results from studies have shown that selective CB2 receptor agonists may produce analgesia without exhibiting significant side effects, and support the potential development of selective CB2 receptor agonists as a viable alternative to cannabinoïd agonists for the treatment of pain.

In many studies, however, the effects of cannabinoid receptor agonists and antagonists include conflicting results on morphine antinociceptive tolerance. Fischer et al. [23] reported that cannabinoid CB1 receptor agonist (CP-55940) attenuate the development of morphine analgesic tolerance. In another study, it has been suggested that co-administration of acute or chronic morphine with a CB1 receptor antagonist (AM251) inhibited the development of both acute and chronic analgesic tolerance [1]. There are no studies on the effects of CB2 receptors on morphine tolerance. In addition, CB1/CB2 receptors have not been studied in combination with agonists and antagonists. In the light of these data, in the present study, we aimed to investigate the effects of CB1 and CB2 antagonists (AM251 and JTE907) and CB1 and CB2 agonists (ACEA and JWH-015) on morphine analgesia and tolerance in rats. Thus, we intended to compare the effects of cannabinoid antagonists with agonists on morphine tolerance.

### Materials and methods

#### Animals

The experiments were performed on adult male Wistar albino rats weighing 205–225 g. Animals were housed four per cage in a room maintained at 22 ± 1 °C with an alternating 12 h dark/12 h light cycles and free access to water and food. The experimental protocols were approved by the Cumhuriyet University Animal Ethics Committee (licence number: 87/Ethic). Animals were acclimatized to laboratory conditions before the test. All experiments were carried out blindly between 0900 and 1700 hours.

#### Drugs

Arasdonil-2’-kloroetilamid hidrat (ACEA), 1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3-carboxamide (AM251), 2-Methyl-1-propyl-1H-indol-3-yl)-1-naphthalenylmethanone (JWH-015) and (N-(1,3-Benzodioxol-5-ylmethyl)-1,2-dihydro-7-methoxy-2-oxo-8-(pentyloxy)-3-quinolinecarboxamide (JTE907) (Sigma-Aldrich, USA) and morphine sulphate (Cumhuriyet University Hospital, Sivas, Turkey) were dissolved in physiological saline. Solutions were freshly prepared on the days of experimentation. Subcutaneous (s.c.) morphine (5 mg/kg), intraperitoneal (i.p.) ACEA (a CB1 receptor agonist, 5 mg/kg), AM251 (a CB1 receptor antagonist, 1 mg/kg), JWH-015 (a CB2 receptor agonist, 5 mg/kg), and JTE907 (a CB2 receptor antagonist, 5 mg/kg) were administered before the analgesia tests.

#### Induction of morphine tolerance

To constitute morphine tolerance, was used a 3-day cumulative dosing regimen. The treatment schedule consisted of twice daily s.c. doses of morphine given at 30 mg/kg (am) and 45 mg/kg (pm) on days 1, 60 and 90 mg/kg on day 2, and 120 mg/kg twice on day 3. Animals were assessed for tolerance on day 4, as described by Way et al. [24]. Tolerance was assessed based on loss of the antinociceptive effects of a test dose (5 mg/kg) of morphine. On day 4, tail-flick and hot-plate tests were done for each rat to average them as a baseline latency, then a challenge dose of morphine (5 mg/kg, s.c.) was injected and, 30 min after morphine injection, further tail-flick and hot-plate tests were done and averaged to find the post-drug latency for each rat for evaluating the development of tolerance to morphine. In saline-treated rats, saline was administered twice daily for 3 days according to the same injection schedule.
Antinociceptive effects using the following equation: withdrawal latencies (tail-flick) were converted to percent effects (% MPE), lick/escape latencies (hot-plate) and tail-withdrawal test is generally attributed to central mechanisms [25, 26].

Second analgesia test is the hot-plate (HP). In this test, animals were individually placed on a HP (May AHP 0603 Analgesic Hot-plate; Commat) with the temperature adjusted to 55 ± 0.5 °C. The latency to the first sign of paw licking or jump response to avoid the heat was taken as an index of the pain threshold; the cut-off time was 30 s in order to avoid damage to the paw. The antinociceptive response on the hot-plate is considered to result from a combination of central and peripheral mechanisms [26].

Experimental protocols

The antinociceptive effects of morphine, ACEA, JWH-015, AM251 and JTE907 were considered at 30-min intervals (0, 30, 60, 90, and 120 min) by tail-flick and hot-plate tests in rats (n = 10). In the morphine-treated rats after induction of morphine tolerance, analgesic response to the challenge dose was determined again on day 4 at 30-min intervals after the same morphine (5 mg/kg challenge dose; s.c.) injection on the first day. To evaluate the effects of ACEA, JWH-015, AM251 and JTE907 on expression of morphine tolerance, morphine tolerant animals received ACEA (5 mg/kg; i.p.), JWH-015 (5 mg/kg; i.p.), AM251 (1 mg/kg; i.p.) and JTE907 (5 mg/kg; i.p.). In the saline-treated group, animals received saline (5 ml/kg) instead of morphine during the induction session.

Data analysis

In order to calculate percentage maximal antinociceptive effects (% MPE), lick/escape latencies (hot-plate) and tail-withdrawal latencies (tail-flick) were converted to percent antinociceptive effects using the following equation:

$$\% \text{ MPE} = \frac{(\text{test latency} - \text{baseline})}{(\text{cutoff} - \text{baseline})} \times 100.$$ 

Statistical analysis

The antinociceptive effects of the drugs were measured as tail-flick and hot-plate latencies in all groups for each rat and converted to % MPE. The data were analysed by two-way analysis of variance (ANOVA) and repeated measures ANOVA followed by a Tukey post hoc test for multiple comparisons between groups (SPSS 14.0 for Windows). All data are presented as mean ± SEM. The level of significance was set at p < 0.05.

Results

The antinociceptive effects of different doses of morphine

To determine the effective morphine dose, we measured the antinociceptive responses for the three different doses of morphine (2, 5, and 10 mg/kg, s.c.) at 30-min intervals by tail-flick and hot-plate test. The maximum % MPE was observed at 60 min after administration of a 5 mg/kg dose of morphine (62.3 ± 5.6 for the tail-flick and 66.2 ± 8.1 for the hot-plate test; Table 1). The % MPE produced by morphine (5 mg/kg) was significantly higher than in the other groups (2 mg/kg morphine and saline group) in both the tail-flick (p < 0.01) and hot-plate tests (p < 0.01) in rats.

Effect of ACEA on morphine analgesia

The data obtained indicated that pretreatment of animals with ACEA (a CB1 receptor agonist) significantly increased (increased mean of % MPE value) morphine antinociceptive effect in both tail-flick (p < 0.05; Fig. 1a) and hot-plate test (p < 0.05; Fig. 1b) compared to the morphine administration group. The peak value of this group was observed at 60 min after administration of drugs in analgesia tests (tail-flick 65.80 ± 6.10 and hot-plate 75.60 ± 5.10). In addition, these data demonstrated that ACEA (tail-flick 49.80 ± 4.60 and hot-plate 55.20 ± 5.60) alone has a significant analgesic effect compared to the saline group (p < 0.01).

Effect of JWH-015 on morphine analgesia

Administration of JWH-015 (a CB2 receptor agonist) with morphine produced a significant increase in % MPE in both the tail-flick (p < 0.05; Fig. 2a) and hot-plate (p < 0.05; Fig. 2b) assays as compared to morphine group rats (tail-flick 64.80 ± 5.70 and hot-plate 71.50 ± 5.40). In addition, JWH-015 alone has a significant analgesic effect compared to the saline group rats (p < 0.01).
Effect of AM251 on morphine analgesia

Statistical analysis suggested that the cannabinoid CB1 receptor antagonist AM251 significantly decreased the morphine analgesic effect in tail-flick ($p < 0.05$; Fig. 3a) and hot-plate tests ($p < 0.05$; Fig. 3b) compared to the morphine administration group. The peak value of this group was also observed at 60 min after administration of morphine in analgesia tests (tail-flick $54.60 \pm 5.30$ and hot-plate $56.60 \pm 6.10$). Furthermore, these data demonstrated that AM251 alone has no significant analgesic effect compared to the saline group rats.

Effect of JTE907 on morphine analgesia

Obtained data indicated that pretreatment of animals with JTE907 (a CB2 receptor antagonist) significantly decreased (decreased mean of % MPE value) morphine antinociceptive effect in both tail-flick ($p < 0.05$; Fig. 4a) and hot-plate test ($p < 0.05$; Fig. 4b) compared to morphine administration group. The peak value of this group was also observed at 60 min after administration of drugs in analgesia tests (tail-flick $49.60 \pm 5.60$ and hot-plate $55.40 \pm 6.10$). In addition, our data demonstrated that JTE907 alone has no significant analgesic effect compared to the saline group.

Effects of ACEA, JWH-015, AM251 and JTE907 on the tolerance to morphine analgesia

The % MPE of the morphine group was the statistically higher morphine-tolerant group ($p < 0.01$). AM251 and JTE907 in combination with morphine produced a significantly decreased expression analgesic tolerance to morphine in both the tail-flick (respectively, $p < 0.01$, $p < 0.05$; Figs. 5a, 6a) and hot-plate assays (respectively, $p < 0.01$, $p < 0.05$; Figs. 5b, 6b) as compared to the morphine-tolerant rats. However, the cannabinoid CB1 receptor agonist ACEA and CB2 receptor agonist JWH-015 in combination with morphine did not show a significantly decreased morphine analgesic tolerance in the tail-flick and hot-plate assays. The maximum % maximal antinociceptive effects (% MPE) was observed at 60 min after administration of morphine by analgesia tests in all groups rats.

Discussion

Morphine is the commonly used opioid for analgesic action, alone or in combination with an adjunct drug. Opioid and cannabinoid receptors share a similar analgesic profile. The overlapping activity of opioids and cannabinoids suggests possible interaction between these two groups of drugs. Analgesic effects of opioids and cannabinoids are well known to be related to central effects. Synergistic antinociceptive interaction between these two groups of drugs when given systemically is known to cocur [10, 27]. In this study, we determined that the cannabinoid receptors have a significant role in morphine analgesia and tolerance. The data obtained suggested that co-injection of morphine with ACEA (cannabinoid CB1 receptor agonist) and JWH-015 (CB2 agonist) increased the analgesic effects of morphine and enhanced the expression of tolerance to morphine analgesia. On the other hand, AM251 (cannabinoid CB1 antagonist) and JTE907 (CB2 antagonist) decreased the analgesic effects of morphine and attenuated the expression of tolerance to morphine.

Pain modulation is a dynamic process, which includes many interactions among complex ascending and descending neuronal systems [28, 29]. Opioidergic and cannabinoid signal pathways have very important roles in

Table 1 The antinociceptive effects of different doses of morphine

| Time (min) | 0     | 30    | 60    | 90    |
|-----------|-------|-------|-------|-------|
| Tail-flick|       |       |       |       |
| Saline    | 3.8 ± 1.1 | 4.4 ± 1.4 | 5.1 ± 0.4 | 4.5 ± 0.8 |
| Morphine (2 mg/kg) | 4.8 ± 1.3 | 19.3 ± 0.8 | 33.5 ± 2.1 | 14.4 ± 3.1 |
| Morphine (5 mg/kg) | 3.7 ± 1.4 | 25.3 ± 2.2 | 62.3 ± 5.6** | 38.3 ± 5.3* |
| Morphine (10 mg/kg) | 4.6 ± 0.9 | 24.8 ± 2.5 | 44.3 ± 5.3 | 32.6 ± 3.4 |
| Hot-plate  |       |       |       |       |
| Saline    | 7.3 ± 1.3 | 8.3 ± 0.9 | 7.9 ± 1.8 | 9.3 ± 1.4 |
| Morphine (2 mg/kg) | 9.5 ± 1.5 | 34.5 ± 3.1 | 38.3 ± 6.4 | 24.5 ± 2.1 |
| Morphine (5 mg/kg) | 10.2 ± 1.7 | 44.8 ± 4.5* | 66.2 ± 8.1** | 37.4 ± 2.4 |
| Morphine (10 mg/kg) | 10.1 ± 1.6 | 42.3 ± 4.3 | 54.4 ± 7.4 | 31.8 ± 3.5 |

Data are mean ± SEM. Analgesia is expressed in % MPE.

* $p < 0.05$, ** $p < 0.01$ as compared with the saline group ($n = 10$ in each group)
analgesia [17, 30]. Activation of opioid and cannabinoid receptors inhibits the transmission of pain sensation at spinal and supraspinal levels. While cannabinoid receptors inhibit nociception and have analgesic synergy with opioids, there is evidence that cannabinoid receptors may facilitate opioid analgesia [31].

Several studies have demonstrated the antinociceptive properties of cannabinoid receptor agonists in acute animal models of pain. In accordance with our findings, administration of cannabinoid receptor agonists increases tail-flick and hot-plate latencies [32, 33], and the analgesic effects are reversible with the cannabinoid CB1 receptor antagonist SR141716A [34]. Evidence indicates that spinal mechanisms are an important component of cannabinoid-induced analgesia. Intrathecal administration of Δ9-tetrahydrocannabinol (THC) produces antinoceception in the tail-flick test in spinally transected animals [35]. In addition, the intrathecal injection non-selective cannabinoid receptor agonist, WIN 55,212-2, produces a dose-dependent antinociceptive effect in the analgesia test [36]. On the other hand, there is considerable evidence that over-activity of calcitonin gene-related peptide (CGRP), a neuropeptide present in nociceptive primary afferents, contributes to the development of opioid analgesic tolerance. Chronic exposure to morphine markedly increases CGRP immunoreactivity in the dorsal horn, a response that coincides with a decline in the magnitude of antinociceptive effect [37, 38]. Consistent with our findings, Trang et al. [1] demonstrated that coupling repeated administration of intrathecal morphine with AM251 prevents both the
decline in the level of analgesia and the loss of agonist potency. At the biochemical level, this coupling prevents the morphine-induced increase in CGRP-immunoreactivity in the dorsal horn and in the cultured adult dorsal root ganglion (DRG) neurons, suggesting that its locus of action is at the level of sensory neurons. Interestingly, when co-administered with an analgesic dose of morphine to chronic tolerant animals, AM251 partially restored the actions of morphine and reversed the increase in spinal CGRP-immunoreactivity. Thus, cannabinoid CB1 receptor activity not only modulates responses associated with opioid withdrawal but also influences responses signaling the analgesic tolerance that is associated with increased expression of CGRP in sensory neurons [39]. The ability of AM-251 to prevent and reverse opioid tolerance appears consistent with a similar spinal distribution of CB1 and µ-opioid receptors and a convergence of their intracellular signaling processes [40, 41]. It is also congruent with studies showing that opioid and cannabinoid cross-tolerance, as well as cross-dependence, are sensitive to the actions of CB1-receptor antagonists, AM-251 and SR141716A [39, 42].

Fisher et al. [23] reported that co-administration of CB1 receptor agonists CP-55940 with morphine attenuated the development of morphine antinociceptive tolerance. In addition, this study demonstrated an interaction between the cannabinoid CB1 and NMDA receptor systems in the attenuation of morphine antinociceptive tolerance. Conversely, our findings suggested that cannabinoid CB1 receptor agonist ACEA in combination with morphine did
not decrease morphine analgesic tolerance in the analgesia tests.

Recent studies have indicated that CB2 receptors are involved in peripheral and central morphine antinociception [43, 44]. The cannabinoid CB2 receptor-selective agonists, HU-308 and AM1241, decreased inflammatory pain [45, 46]. In these studies, agonist efficacy was attenuated by co-administration of a selective cannabinoid CB2 receptor antagonist. Whiteside et al. [47] suggested that the analgesic effects of GW405833 (selective CB2 receptor agonist) are mediated via the cannabinoid CB2 receptor. However, it has been stated that the mechanism of action for GW405833 does not depend on the release of endogenous opioids. In contrast, our findings demonstrated that co-administration of JWH-015 (CB2 receptor agonist) with morphine significantly increased the analgesic effects of morphine. Accordingly, cannabinoid CB2 receptor antagonist JTE907 decreased the morphine analgesia in hot-plate and tail-flick tests. Similarly, JTE907 in combination with morphine produced a significantly decreased expression of analgesic tolerance to morphine. Our findings have demonstrated that CB2 antagonists also reduced the analgesic tolerance to morphine as CB1 antagonists.

In conclusion, although we have not identified the exact mechanisms by which cannabinoid receptors influence morphine-induced analgesia and tolerance, our findings further support the existence of a functional interaction between the cannabinoid and opioid systems. In addition, we also determined that AM251 (cannabinoid CB1 antagonist) and JTE907 (CB2 antagonist) decreased the expression of tolerance to morphine.

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**Fig. 5** Effects of ACEA and AM251 on the tolerance to morphine analgesia. a Effects of morphine, ACEA and AM251 in the tail-flick test, and b the hot-plate test. Pretreatment of morphine-tolerant animals with AM251 significantly increased % MPE (decreased tolerance to morphine) in both tail-flick (p < 0.01; a) and hot-plate tests (p < 0.01; b) compared to morphine-tolerant animals. However, pretreatment of animals with ACEA did not significantly increase % MPE in either tail-flick or hot-plate tests. Each point represents the mean ± SEM of % MPE for 10 rats. *p < 0.01, †p < 0.01, and ‡p > 0.05 compared to the morphine-tolerant group.

**Fig. 6** Effects of JWH-015 and JTE907 on the tolerance to morphine analgesia. a Effects of morphine JWH-015 and JTE907 in the tail-flick test, and b the hot-plate test. Pretreatment of morphine-tolerant animals with JTE907 significantly increased % MPE (decreased tolerance to morphine) in both tail-flick (p < 0.05; a) and hot-plate tests (p < 0.05; b) compared to morphine-tolerant animals. However, pretreatment of animals with JWH-015 did not significantly increase % MPE in either tail-flick or hot-plate tests. Each point represents the mean ± SEM of % MPE for 10 rats. *p < 0.01, †p < 0.05, and ‡p > 0.05 compared to the morphine-tolerant group.
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