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ORIGINAL PAPER

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

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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 JWH907 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].

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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 cannabinoid 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

Araşidonil-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 tests

To evaluate thermal nociception, we used a standardised tail-flick (TF) apparatus (May TF 0703 Tail-flick Unit; Commat, Turkey). The radiant heat source was focused on the distal portion of the tail at 3 cm after administration of the vehicle and study drugs. Following vehicle or compound administration, tail-flick latencies (TFL) were obtained. The infrared intensity was adjusted so that basal TFL occurred at 2.9 ± 0.5 s. Animals with a baseline TFL below 2.4 or above 3.4 s were excluded from further testing. The cutoff latency was set at 15 s to avoid tissue damage. Any animal not responding after 15 s was excluded from the study. The hyperalgesic response in the 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:

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

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 ($p < 0.01$).

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)

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 ± 5.30 and hot-plate 56.60 ± 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 ± 5.60 and hot-plate 55.40 ± 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

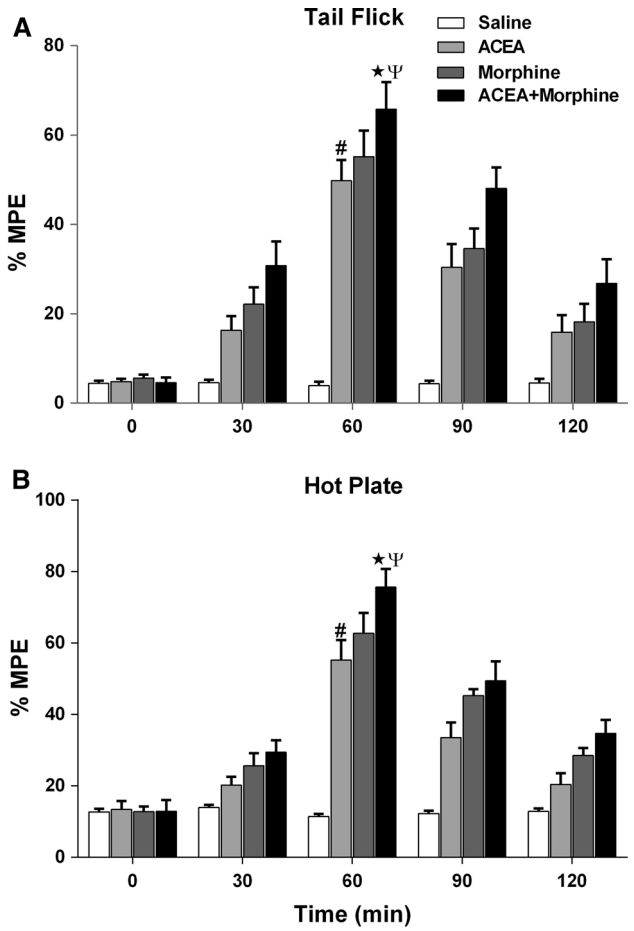

Fig. 1 Effect of ACEA on the morphine analgesia. **a** Effect of ACEA (5 mg/kg) in the tail-flick test, and **b** effect of ACEA in the hot-plate test. ACEA in combination with morphine produced a significant increase in percent of maximal possible effect (% MPE) in both the tail-flick ($p < 0.05$; **a**) and hot-plate assays ($p < 0.05$; **b**) as compared to the morphine-treated rats. ACEA alone has a significant analgesic effect compared to the saline group ($p < 0.01$). The maximum % MPE is observed at 60 min after administration of morphine. Each *point* represents the mean ± SEM of % MPE for 10 rats. $^{*}p < 0.05$, ACEA + morphine group compared to the morphine-treated group and $^{\Psi}p < 0.01$, ACEA + morphine group compared to the ACEA group and $^{\#}p < 0.01$, ACEA group compared to the saline-treated group

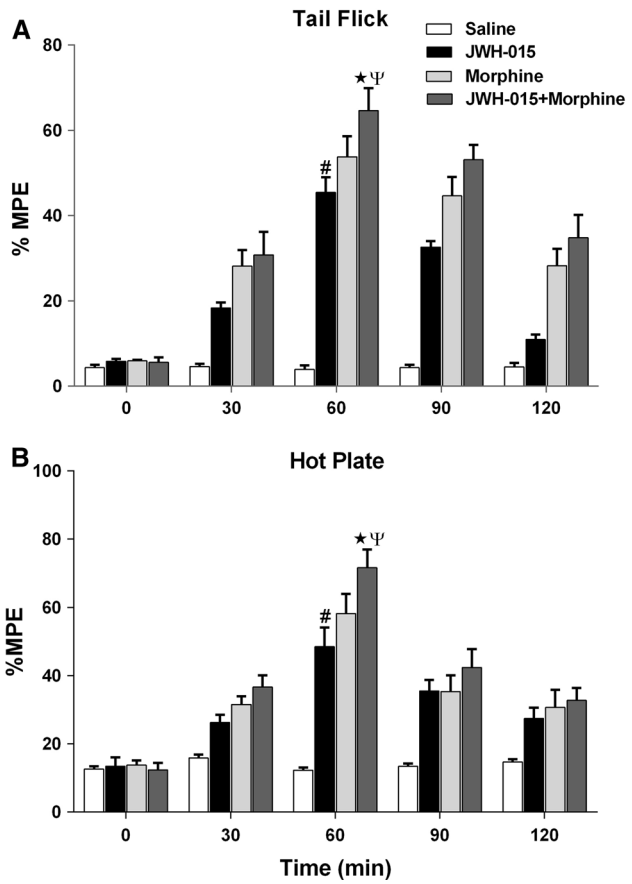

Fig. 2 Effect of JWH-015 on the morphine analgesia. **a** Effect of JWH-015 (5 mg/kg) in the tail-flick test, and **b** effect of JWH-015 in the hot-plate test. JWH-015 in combination with morphine produced a significant increase in % MPE in both the tail-flick ($p < 0.05$; **a**) and hot-plate assays ($p < 0.05$; **b**) as compared to the morphine-treated rats. Each *point* represents the mean ± SEM of percent of maximal possible effect (% MPE) for 10 rats. $^{*}p < 0.05$, JWH-015 + morphine group compared to the morphine-treated group and $^{\Psi}p < 0.01$, JWH-015 +morphine group compared to the JWH-015 group and $^{\#}p < 0.01$, JWH-015 group compared to the saline-treated 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-tetrahydrocanabinol (THC) produces antinociception 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

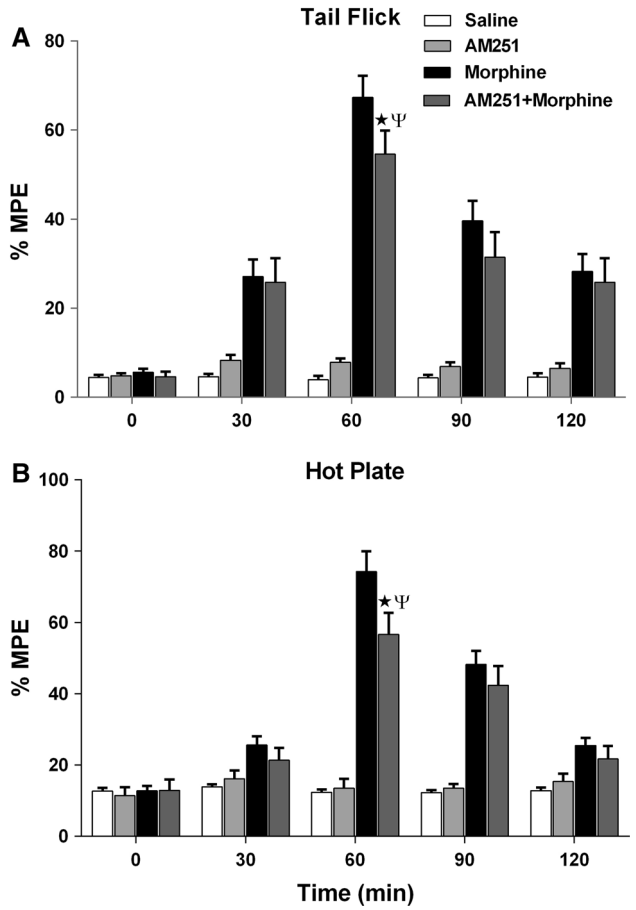

Fig. 3 Effect of AM251 on the morphine analgesia. **a** Effect of AM251 (1 mg/kg) in the tail-flick test, and **b** effect of AM251 in the hot-plate test. AM251 in combination with morphine produce a significant decrease in % MPE in both the tail-flick ($p < 0.05$; **a**) and hot-plate assays ($p < 0.05$; **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. Furthermore, these data demonstrated that AM251 alone has no significant analgesic effect compared to the saline group rats. Each *point* represents the mean ± SEM of % MPE for 10 rats. $^{*}p < 0.05$, AM251 + morphine group compared to the morphine group and $^{\Psi}p < 0.01$, AM251 + morphine group compared to the AM251 group

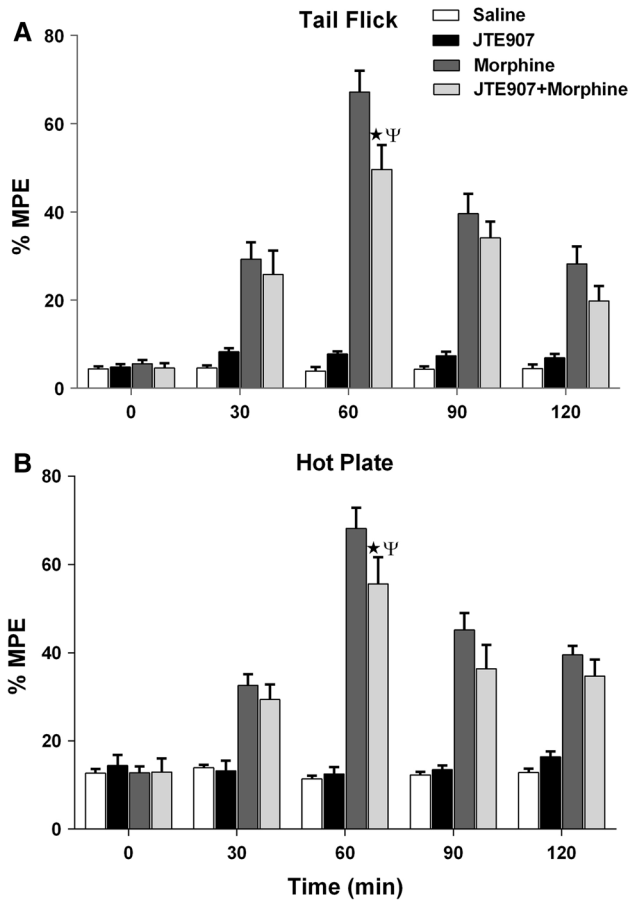

Fig. 4 Effect of JTE907 on the morphine analgesia. **a** Effect of JTE907 (5 mg/kg) in the tail-flick test, and **b** effect of JTE907 in the hot-plate test. JTE907 in combination with morphine produced a significant decrease in % MPE in both the tail-flick ($p < 0.05$; **a**) and hot-plate assays ($p < 0.05$; **b**) as compared to the morphine-treated rats. Each *point* represents the mean ± SEM of % MPE for 10 rats. $^{*}p < 0.05$, the JTE907 + morphine-treated group compared to the morphine group and $^{\Psi}p < 0.01$, JTE907 + morphine group compared to the JTE907 group

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 mu-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

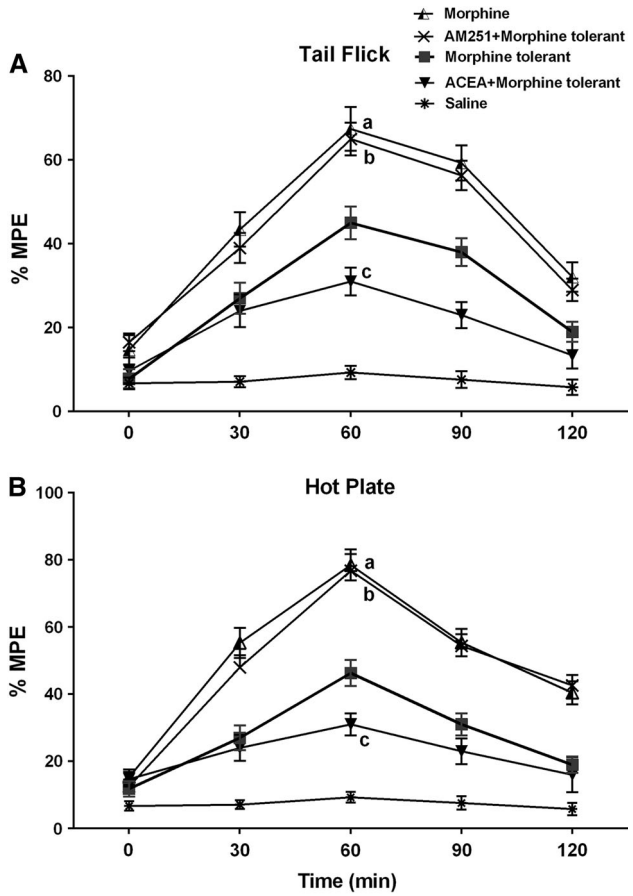

Fig. 5 Effects of ACEA and AM251 on the tolerance to morphine analgesia. **a** Effects of morphine, ACEA and AM251 in the tail-flick test, and **b** the hot-plate test. Pretreatment of morphine-tolerant animals with AM251 significantly increased % MPE (decreased tolerance to morphine) in both tail-flick ($p < 0.01$; **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. $^{a}p < 0.01$, $^{b}p < 0.01$, and $^{c}p > 0.05$ compared to the morphine-tolerant group

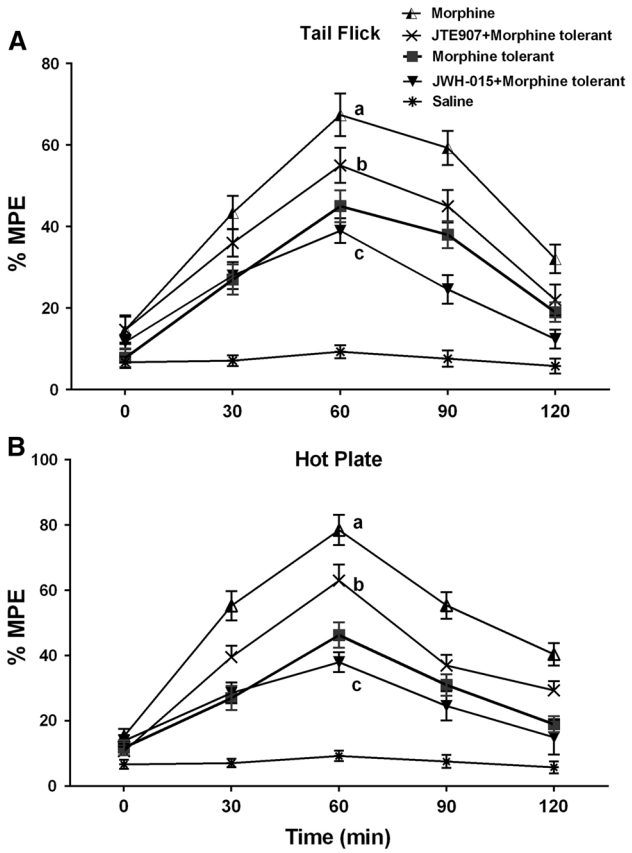

Fig. 6 Effects of JWH-015 and JTE907 on the tolerance to morphine analgesia. **a** Effects of morphine JWH-015 and JTE907 in the tail-flick test, and **b** the hot-plate test. Pretreatment of morphine-tolerant animals with JTE907 significantly increased % MPE (decreased tolerance to morphine) in both 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. $^{a}p < 0.01$, $^{b}p < 0.05$, and $^{c}p > 0.05$ compared to the morphine-tolerant group

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. There are no studies on the effects of cannabinoid CB2 receptor on morphine analgesic tolerance. 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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Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Conflict of ınterest The authors declare that they have no conflict of interest.

References

1. Trang T, Sutak M, Jhamandas K (2007) Involvement of cannabinoid (CB1)-receptors in the development and maintenance of opioid tolerance. Neuroscience 146:1275–1288
2. 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. J Physiol Sci 62:317–323
3. Liu JG, Anand KJ (2001) Protein kinases modulate the cellular adaptations associated with opioid tolerance and dependence. Brain Res Rev 38:1–19
4. Liu W, Wang CH, Cui Y, Mo LQ, Zhi JL, Sun SN, Wang YL, Yu HM, Zhao CM, Feng JQ, Chen PX (2006) Inhibition of neuronal nitric oxide synthase antagonizes morphine antinociceptive tolerance by decreasing activation of p38 MAPK in the spinal microglia. Neurosci Lett 410:174–177
5. Gursoy S, Ozdemir E, Bagcıvan I, Altun A, Durmus N (2011) Effects of alpha 2-adrenoceptor agonists dexmedetomidine and guanfacine on morphine analgesia and tolerance in rats. Upsala J Med Sci 116:238–246
6. 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. Can J Physiol Pharmacol 89:89–95
7. Frank B, Serpell MG, Hughes J, Matthews JN, Kapur D (2008) Comparison of analgesic effects and patient tolerability of nabilone and dihydrocodeine for chronic neuropathic pain: randomised, crossover, double blind study. BMJ 336:199–201
8. Hohmann AG, Herkenham M (1999) Localization of central cannabinoid CB1 receptor messenger RNA in neuronal subpopulations of rat dorsal root ganglia: a double-label in situ hybridization study. Neuroscience 90:923–931
9. Smith FL, Cichewicz D, Martin ZL, Welch SP (1998) The enhancement of morphine antinociception in mice by delta9-tetrahydrocannabinol. Pharmacol Biochem Behav 60:559–566
10. Cichewicz DL, Welch SP, Smith FL (2005) Enhancement of transdermal fentanyl and buprenorphine antinociception by transdermal delta9-tetrahydrocannabinol. Eur J Pharmacol 525:74–82
11. Finn DP, Beckett SR, Roe CH, Madjd A, Fone KC, Kendall DA, Marsden CA, Chapman V (2004) Effects of coadministration of cannabinoids and morphine on nociceptive behaviour, brain monoamines and HPA axis activity in a rat model of persistent pain. Eur J Neurosci 19:678–686
12. Welch SP, Stevens DL (1992) Antinociceptive activity of intrathecally administered cannabinoids alone, and in combination with morphine, in mice. J Pharmacol Exp Ther 262:10–18
13. Chapman V, Finn DP (2003) Analgesic effects of cannabinoids: sites and mechanisms of action. Rev Analg 7:25–39
14. Walter L, Stella N (2004) Cannabinoids and neuroinflammation. Br J Pharmacol 141:775–785
15. Mechoulam R, Parker LA (2013) The endocannabinoid system and the brain. Annu Rev Psychol 64:21–47
16. Pontieri FE, Monnazzi P, Scontrini A, Buttarelli FR, Patacchioli FR (2001) Behavioral sensitization to heroin by cannabinoid pretreatment in the rat. Eur J Pharmacol 421:R1–R3
17. Rios C, Gomes I, Devi LA (2006) Mu opioid and CB1 cannabinoid receptor interactions: reciprocal inhibition of receptor signaling and neuritogenesis. Br J Pharmacol 148:387–395
18. Vigano D, Grazia CM, Rubino T, Fezza F, Vaccani A, Di Marzo V, Parolaro D (2003) Chronic morphine modulates the contents of the endocannabinoid, 2-arachidonoyl glycerol, in rat brain. Neuropsychopharmacology 28:1160–1167
19. Lim G, Wang S, Mao J (2005) Central glucocorticoid receptors modulate the expression of spinal cannabinoid receptors induced by chronic morphine exposure. Brain Res 1059:20–27
20. Navarro M, Carrera MR, Fratta W, Valverde O, Cossu G, Fattore L, Chowen JA, Gomez R, del Arco I, Villanua MA, Maldonado R, Koob GF, Rodriguez de Fonseca F (2001) Functional interaction between opioid and cannabinoid receptors in drug self-administration. J Neurosci 21:5344–5350
21. Scott DA, Wright CE, Angus JA (2004) Evidence that CB-1 and CB-2 cannabinoid receptors mediate antinociception in neuropathic pain in the rat. Pain 109:124–131
22. Valenzano KJ, Tafesse L, Lee G, Harrison JE, Boulet JM, Gottshall SL, Mark L, Pearson MS, Miller W, Shan S, Rabadi L, Rotshteyn Y, Chaffer SM, Turchin PI, Elsemore DA, Toth M, Koetzner L, Whiteside GT (2005) Pharmacological and pharmacokinetic characterization of the cannabinoid receptor 2 agonist, GW405833, utilizing rodent models of acute and chronic pain, anxiety, ataxia and catalepsy. Neuropharmacology 48:658–672
23. Fischer BD, Ward SJ, Henry FE, Dykstra LA (2010) Attenuation of morphine antinociceptive tolerance by a CB1 receptor agonist and an NMDA receptor antagonist: interactive effects. Neuropharmacology 58:544–550
24. Way EL, Loh HH, Shen FH (1969) Simultaneous quantitative assessment of morphine tolerance and physical dependence. J Pharmacol Exp Ther 167:1–8
25. Kanaan SA, Saade NE, Haddad JJ, Abdelnoor AM, Atweh SF, Jabbur SJ, Safieh-Garabedian B (1996) Endotoxin-induced local inflammation and hyperalgesia in rats mince, a new model for inflammatory pain. Pain 66:373–379
26. Ramabadran K, Bansinath M, Turndorf H, Puig MM (1989) The hyperalgesic effect of naloxone is attenuated in streptozotocin-diabetic mice. Psychopharmacology 97:169–174
27. Cichewicz DL, McCarthy EA (2003) Antinociceptive synergy between delta9-tetrahydrocannabinol and opioids after oral administration. J Pharmacol Exp Ther 304:1010–1015
28. Christie MJ, Williams JT, North RA (1987) Cellular mechanisms of opioid tolerance: studies in single brain neurons. Mol Pharmacol 32:633–638
29. Bryant CD, Eitan S, Sinchak K, Fanselow MS, Evans CJ (2006) NMDA receptor antagonism disrupts the development of morphine analgesic tolerance in male, but not female C57BL/6J mice. Am J Physiol Regul Integr Comp Physiol 291:315–326
30. Rubino T, Tizzoni L, Vigano D, Massi P, Parolaro D (1997) Modulation of rat brain cannabinoid receptors after chronic morphine treatment. Neuroreport 8:3219–3223
31. Ibrahim MM, Porreca F, Lai J, Albrecht PJ, Rice F, Khodorova A, Davar G, Makriyannis A, Vanderah TW, Mata HP, Malan TP Jr (2005) CB2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. Proc Natl Acad Sci USA 102:3093–3098
32. Smith PB, Compton DR, Welch SP, Razdan RK, Mechoulam R, Martin BR (1994) The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice. J Pharmacol Exp Ther 270:219–227
33. Stein EA, Fuller SA, Edgemond WS, Campbell WB (1996) Physiological and behavioural effects of the endogenous

cannabinoid, arachidonylethanolamide (anandamide), in the rat. Br J Pharmacol 1:107–114
34. Welch SP, Huffman JW, Lowe J (1998) Differential blockade of the antinociceptive effects of centrally administered cannabinoids by SR141716A. J Pharmacol Exp Ther 286:1301–1308
35. Smith PB, Martin BR (1992) Spinal mechanisms of delta9-tetrahydrocannabinol- induced analgesia. Brain Res 578:8–12
36. Dogrul A, Gul H, Akar A, Yildiz O, Bilgin F, Guzeldemir E (2003) Topical cannabinoid antinociception: synergy with spinal sites. Pain 105:11–16
37. Menard DP, van Rossum D, Kar S, St Pierre S, Sutak M, Jhamandas K, Quirion R (1996) A calcitonin gene-related peptide receptor antagonist prevents the development of tolerance to spinal morphine analgesia. J Neurosci 16:2342–2351
38. Powell KJ, Quirion R, Jhamandas K (2003) Inhibition of neurokinin-1 substance P receptor and prostanoid activity prevents and reverses the development of morphine tolerance in vivo and the morphine-induced increase in CGRP expression in cultured dorsal root ganglion neurons. Eur J Neurosci 18:1572–1583
39. Trang T, Ma W, Chabot JG, Quirion R, Jhamandas K (2006) Spinal modulation of calcitonin gene-related peptide by endocannabinoids in the development of opioid physical dependence. Pain 126:256–271
40. Hohmann AG, Tsou K, Walker JM (1999) Intrathecal cannabinoid administration suppresses noxious stimulus-evoked Fos protein-like immunoreactivity in rat spinal cord: comparison with morphine. Zhongguo Yao Li Xue Bao 20:1132–1136
41. Salio C, Fischer J, Franzoni MF, Mackie K, Kaneko T, Conrath M (2001) CB1-cannabinoid and mu-opioid receptor co-localization on postsynaptic target in the rat dorsal horn. Neuroreport 12:3689–3692
42. Mas-Nieto M, Pommier B, Tzavara ET, Caneparo A, Da Nascimento S, Le Fur G, Roques BP, Noble F (2001) Reduction of opioid dependence by the CB(1) antagonist SR141716A in mice: evaluation of the interest in pharmacotherapy of opioid addiction. Br J Pharmacol 132:1809–1816
43. Paldy E, Bereczki E, Santha M, Wenger T, Borsodi A, Zimmer A, Benyhe S (2008) CB(2) cannabinoid receptor antagonist SR144528 decreases mu-opioid receptor expression and activation in mouse brainstem: role of CB(2) receptor in pain. Neurochem Int 53:309–316
44. Paldyova E, Bereczki E, Santha M, Wenger T, Borsodi A, Benyhe S (2008) Noladin ether, a putative endocannabinoid, inhibits mu opioid receptor activation via CB2 cannabinoid receptors. Neurochem Int 52:321–328
45. Hanus L, Breuer A, Tchilibon S, Shiloah S, Goldenberg D, Horowitz M, Pertwee RG, Ross RA, Mechoulam R, Fride E (1999) HU-308: aspecific agonist for CB2, a peripheral cannabinoid receptor. Proc Natl Acad Sci USA 96:14228–14233
46. Nackley AG, Makriyannis A, Hohmann AG (2003) Selective activation of cannabinoid CB(2) receptors suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. Neuroscience 119:747–757
47. Whiteside GT, Gottshall SL, Boulet JM, Chaffer SM, Harrison JE, Pearson MS, Turchin PI, Mark L, Garrison AE, Valenzano KJ (2005) A role for cannabinoid receptors, but not endogenous opioids, in the antinociceptive activity of the CB2 -selective agonist, GW405833. Eur J Pharmacol 528:65–72