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The effects of endocannabinoid receptor agonist anandamide and antagonist rimonabant on opioid analgesia and tolerance in rats

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Abstract. The role of the cannabinoid (CB) system in the tolerance to analgesic effect of opioid remains obscure. The aim of the present study was to evaluate the effects of the endocannabinoid nonselective receptor agonist anandamide (AEA) and CB1 receptor antagonist rimonabant (SR141716) on morphine analgesia and tolerance in rats. Male Wistar albino rats weighing 215–230 g were used in these experiments. To constitute morphine analgesic tolerance, a 3-day cumulative dosing regimen was used. The analgesic effects of AEA (10 mg/kg), SR141716 (10 mg/kg), and morphine (5 mg/kg) were considered at 30-min intervals by tail flick (TF) and hot plate (HP) analgesia tests. The analgesic effects of the drugs were measured as TF and HP latencies in all groups for each rat and converted to %MPE. The data were analysed by analysis of variance followed by Tukey test. The findings suggested that AEA in combination with morphine produced a significant increase in expression of analgesic tolerance to morphine. Conversely, cannabinoid receptor antagonist SR141716 attenuated morphine analgesic tolerance. In addition, administration of AEA with morphine increased morphine analgesia. In conclusion, we observed that the cannabinoid receptor agonist anandamide and CB1 receptor antagonist SR141716 plays a significant role in the opioid analgesia and tolerance.

Key words: Opioid — Cannabinoid receptors — Anandamide — SR141716 — Morphine tolerance

Abbreviations: AEA, anandamide; CB, cannabinoid; HP, hot plate; MOR, mu-opioid receptor; MPE, maximal antinociceptive effect; TF, tail flick; TFL, TF latencies; THC, tetrahydrocannabinol.

Introduction

Cannabinoid receptors constitute a part of the sensory receptor system in the brain and are involved in a variety of biological processes including mood, memory and nociception. Some physiological and pathological conditions cause an increase in brain endocannabinoids that arrange distinct physiological functions (Nagayama et al. 1999; Freund et al. 2003). One of the essential therapeutic targets of cannabinoids is analgesia. Cannabinoid analgesia is based on the repression of spinal and thalamic nociceptive neurons. In addition, it has also been determined that cannabinoids have analgesic effects on peripheral neurons (Zhang et al. 2003). In the last decade, in the role of the endocannabinoid system substantial advancement has been made in the pain modulation. It has been suggested that endocannabinoids act as analgesics in models of both acute nociception and chronic pain such as inflammation and painful neuropathy (Nackley et al. 2003; Sagar et al. 2005). In addition, the synthetic cannabinoid WIN 55,212-2 mesylate combination pregabalin exerted synergistic interaction in the mouse model of acute pain (Luszczki et al. 2011). The cannabinoid (CB) receptors are presented in areas of the nervous system important for pain processing. It is comprised of cannabinoid receptor type-1 (CB1) and type-2 (CB2), which are seven-transmembrane protein and

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G-protein coupled receptors (Matsuda et al. 1990). CB receptor also includes its endogenous ligand composed of lipid molecules, the endocannabinoids, of which anandamide (N-arachidonoylethanolamine, AEA) is commonly studied (Devane et al. 1992; Bisogno et al. 2008).

Alternating cannabinoid and opioid treatment could produce a longer lasting and more potent analgesia than either compound given alone (Wilson-Poe et al. 2013). Simultaneous administration of an opioid and cannabinoid produced synergy (Cichewicz 2004; Cox et al. 2007). The antinociceptive effects of cannabinoids are enhanced even when administered after morphine antinociception has dissipated. This is particularly true in morphine-tolerant animals (Williams et al. 2006). Opioids such as morphine are extensively used analgesics for chronic or persistent pain (Ozdemir et al. 2012). In addition, recent studies have obtained important information about the analgesic effects of cannabinoids in humans (Ware et al. 2010). Oral doses of tetrahydrocannabinol (THC) were no more effective than codeine for pain, and produced a significant amount of dysphoric side effects (Campbell et al. 2001). It was informed that cannabinoid could only produce antinociception at doses that were high enough to cause behavioral side effects. However, synthetic cannabinoid compounds have proven to demonstrate potent analgesic effects up to 10 times that of morphine in animal models of acute and neuropathic pain (Fuentes et al. 1999; Fox et al. 2001).

Opioids and cannabinoids both produce antinociception by G-protein-coupled mechanism that inhibits the release of pain-producing neurotransmitters in the brain and spinal cord (Seely et al. 2012). On the other hand, high doses of opioid drugs are accompanied by undesirable side effects. For this reason, a search for a better analgesic strategy led to the discovery that cannabinoid improves the potency of opioids. Furthermore, recent evidence has demonstrated that the analgesic effects of cannabinoids are mediated by delta and kappa opioid receptors, suggesting an intimate connection between cannabinoid and opioid signaling pathways in the modulation of pain perception (Cichewicz et al. 2004). Administration of different cannabinoids with morphine produces a greater-than-additive effect with respect to antinociception in mice as measured by the tail flick radiant heat test (Welch and Stevens 1992; Smith et al. 1994). THC enhances the analgesic effect of morphine in the spinal cord, whereas the synthetic cannabinoid CP 55,940, which is significantly more potent after intracerebroventricularly than spinally administration, does not increase (Welch et al. 1995). Administration of CP 55,940 with morphine also increases morphine antinociception by about 45% in animals (Massi et al. 2001).

Despite the production of cannabinoids synergy with opioid analgesics, the effects of cannabinoids on morphine analgesic tolerance are not completely understood. In the present study, we assessed the effects of endocannabinoid receptor agonist anandamide and selective CB1 receptor antagonist SR141716 on morphine analgesia and tolerance in rats.

Materials and Methods

Animals

Male adult Wistar albino rats weighing 215–230 g were used in the study. The rats were housed individually in a temperature controlled environment with a 12/12-h light/dark cycle (lights on at 6.00). Standard laboratory chow and tap water were available *ad libitum*. All experiments were carried out blindly between 09.00 and 16.00 h (n = 8 in each experimental group). Rats were handled in accordance with the Guide for the Care and Use of Laboratory Animals. The experimental protocols were approved by the Cumhuriyet University Animal Ethics Committee.

Drugs

Arachidonic acid N-(hydroxyethyl)amide (AEA, Anandamide), 5-(4-Chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide (SR141716) (Sig-ma-Aldrich, USA) and morphine HCl (Cumhuriyet University Hospital, Sivas) were dissolved in physiological saline. Solutions were freshly prepared on the days of experimentation. *Subcutaneous* (*s.c.*) morphine (5 mg/kg), *intraperitoneal* (*i.p.*) AEA (CB1 and CB2 receptor agonist, 10 mg/kg) and SR141716 (CB1 receptor antagonist/inverse agonist, 10 mg/kg) were administered before the nociception tests.

Induction of morphine tolerance

To constitute morphine tolerance, it 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 (a.m.) and 45 mg/kg (p.m.) on day 1; 60 and 90 mg/kg on day 2; and 120 mg/kg twice on day 3. Animals were assessed for tolerance on the 4th day, as described by Zarrindast et al. (2002). Tolerance was assessed based on loss of the antinociceptive effects of a test dose (5 mg/kg) of morphine. On day 4, tail flick (TF) and hot plate (HP) tests were performed on different rats for each test to average them as a baseline latency; then challenge dose of morphine (5 mg/kg) was injected; 30 min after morphine injection other TF and HP tests were done to average them to find 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 TF (May TF 0703 Tail-flick Unit, Commat) and HP (May AHP 0603 Analgesic Hot-plate, Commat) apparatus. In the TF test, 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, TF latencies (TFL) were obtained. The infrared intensity was adjusted so that basal TFL occurred at 3.0 ± 0.5 s. Animals with a baseline TFL below 2.5 or above 3.5 s were excluded from further testing. The cutoff latency was set at 20 s to avoid tissue damage. Any animal not responding after 20 s was excluded from the study. The nociceptive response in the TF test is generally attributed to central mechanisms (Ramabadran et al. 1989; Kanaan et al. 1996).

In the HP test, animals were individually placed on a plate with the temperature adjusted to 53 ± 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 nociceptive response on this test is considered to result from a combination of central and peripheral mechanisms (Ramabadran et al. 1989).

Experimental protocols

The antinociceptive effects of AEA, SR141716, and morphine were considered at 30-min intervals (0, 30, 60, 90, and 120 min) by TF and HP test in rats ($n = 8$). In the morphine-treated rats after induction of morphine tolerance, antinociceptive response to the challenge dose was determined again on day 4 at 30-min intervals after the same morphine (5 mg/kg; *s.c.*) injection on the first day. To evaluate the effects of AEA, and SR141716 on expression of morphine tolerance, morphine tolerant animals received AEA and SR141716 (10 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 % maximal antinociceptive effects (% MPE), lick/escape latencies (HP) and tail-withdrawal latencies (TF) were converted to percent antinociceptive effect using the following equation:

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

Statistical analysis

The means of MPEs in all groups were calculated after the antinociceptive effect was measured. The data were treated by analysis of variance followed by the Tukey test using the computer program SPSS (version 15.0 for Windows). The obtained data were expressed as means ± SEM. In all groups, the criterion for statistical significance was $p < 0.05$.

Results

Effect of AEA on morphine antinociception

The data indicated that pretreatment of animals with AEA (CB1 receptor agonist) significantly increased morphine antinociceptive effect in both TF ($p < 0.05$; Fig. 1A) and HP

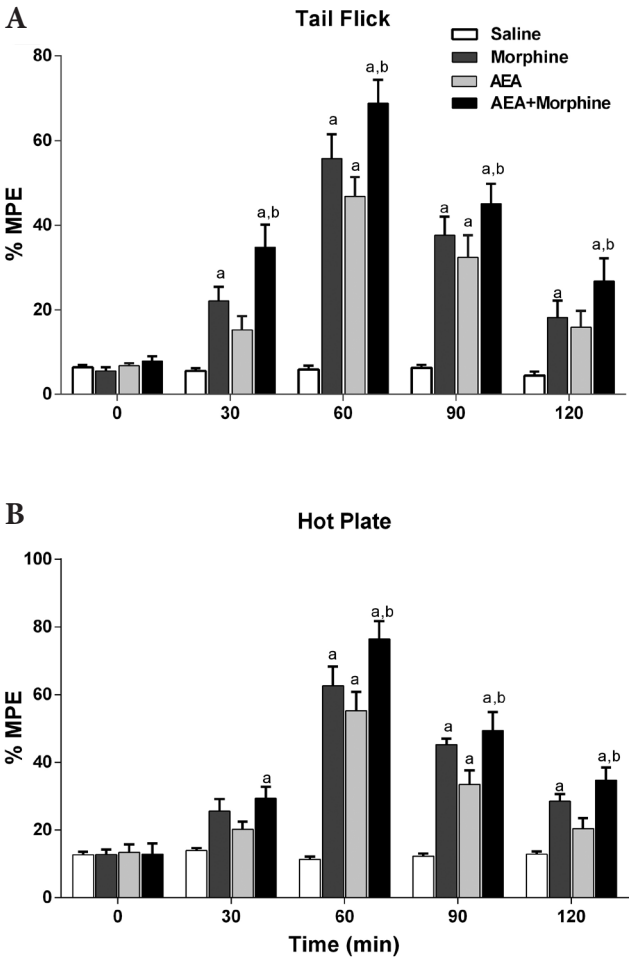

Figure 1. Effect of AEA on the morphine analgesia. Effect of anandamide (AEA) in the tail flick (TF) test (**A**) and in the hot plate (HP) test (**B**). AEA in combination with morphine produce a significant increase in percent of maximal possible effect (%MPE) in both the TF ($p < 0.05$) and HP assay ($p < 0.05$) as compared to the morphine treated rats. AEA 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 8 rats. [a] $p < 0.01$ compared to the saline-treated group and [b] $p < 0.05$ compared to morphine-treated group.

test ($p < 0.05$; Fig. 1B) compared to morphine administration group. The peak value of this group was observed at 60 min after administration of drugs in analgesia tests (TF: 68.80 ± 5.60 and HP: 76.45 ± 5.30). In addition, these data demonstrated that AEA (TF: 46.80 ± 4.60 and HP: 55.20 ± 5.60) alone has a significant analgesic effect compared to the saline group ($p < 0.01$).

Effect of SR141716 on morphine antinociception

Statistical analysis showed that cannabinoid CB1 receptor antagonist SR141716 significantly decreased morphine analgesic effect (TF: 69.35 ± 4.80 and HP: 76.20 ± 5.70) in TF ($p < 0.05$; Fig. 2A) and HP test ($p < 0.05$; Fig. 2B) compared to morphine administration group (TF: 52.60 ± 5.30 and HP: 55.60 ± 6.10). The peak value of this group was also observed at 60 min after administration of morphine in analgesia tests. Furthermore, these data demonstrated that SR141716 alone has no significant analgesic effect compared to the saline group rats.

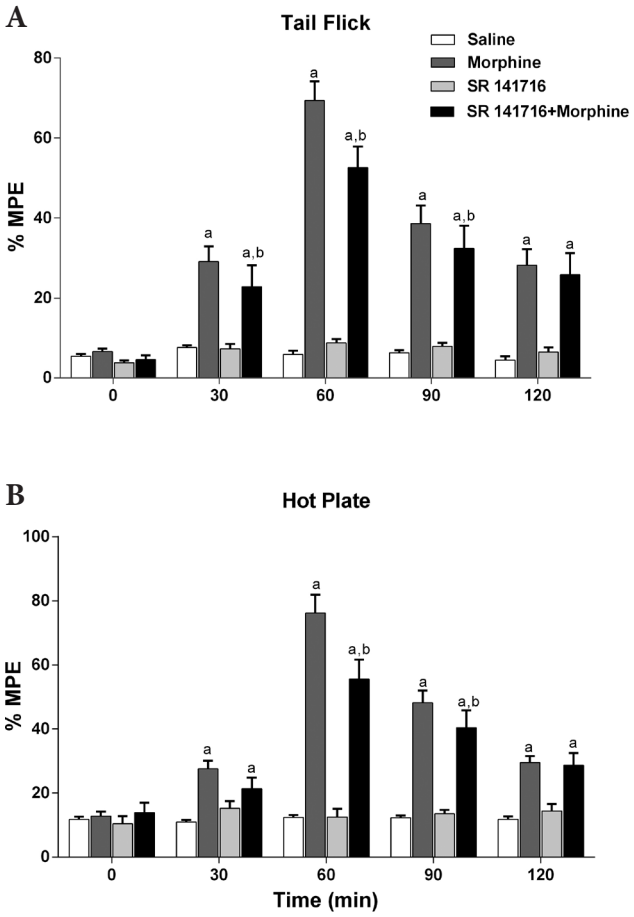

Figure 2. Effect of rimonabant (SR141716) on the morphine analgesia. Effect of SR141716 in the TF test (**A**) and in the HP test (**B**). SR141716 in combination with morphine produce a significant decrease analgesic effect in both the TF ($p < 0.05$) and HP assay ($p < 0.05$) as compared to the morphine treated rats. The peak value of this group was also observed at 60 min after administration of morphine in analgesia tests. Each point represents the mean ± SEM of %MPE for 8 rats. [a] $p < 0.01$ compared to the saline-treated group and [b] $p < 0.05$ compared to morphine-treated group.

Effects of AEA and SR141716 on the tolerance to morphine analgesia

AEA in combination with morphine produced a significant increase expression analgesic tolerance to morphine in both the TF ($p < 0.05$; Fig. 3A) and HP test assays ($p < 0.05$; Fig. 3B) as compared to the morphine tolerant rats. However, cannabinoid CB1 receptor antagonist/inverse agonist SR141716 in combination with morphine significant decreased morphine analgesic tolerance in the TF (Fig. 3A) and HP assays (Fig. 3B). The maximum %MPE was observed at 60 min after administration of morphine by analgesia tests in all groups.

The antinociceptive effects of different doses of morphine

To determine the effective morphine dose, we measured the antinociceptive responses for the 3 different doses of morphine (2.5, 5, and 7.5 mg/kg; *s.c.*) at 30 min intervals by TF and HP test. The maximum %MPE was observed at 60 min after administration of a 5 mg/kg dose of morphine (59.3 ± 6.5 for the TF and 68.2 ± 7.3 for the HP test; Table 1). The %MPE produced by morphine (5 mg/kg) was significantly higher than in the other groups (2.5 and 7.5 mg/kg morphine and saline group) in both the TF and HP tests in rats ($p < 0.05$).

Discussion

The purpose of the present study was to assess the effects of the cannabinoid receptor agonist AEA and selective CB1 receptor antagonist SR141716 on the attenuation of antinociceptive tolerance to morphine. The principal findings from these experiments were that the co-administration AEA with morphine increased morphine tolerance and the combination SR141716 with morphine attenuated morphine antinociceptive tolerance. Additionally, the nonselective receptor agonist AEA enhanced morphine analgesic effect.

It is widely known that opioids and cannabinoids share several pharmacological effects, including inhibition of locomotor activity, sedation and antinociception (Massi et al. 2001; Parolaro et al. 2010). Opioids such as morphine are commonly prescribed analgesics for acute and chronic pain, but the analgesic benefits of cannabinoids have not been well explored in humans (Cichewicz et al. 2004). Several studies

suggested that cannabinoids were no more effective than codeine for pain, and produced a significant amount of side effects (Noyes et al. 1975; Campbell et al. 2001). Thus, it was believed that cannabinoids could only produce analgesia at doses that were high enough to cause side effects.

Recent studies have demonstrated that cannabinoids can enhance the antinociceptive properties of opioids (Vigano et al. 2005; Desroches and Beaulieu 2010). The mechanisms underlying the loss of morphine analgesia are not clear but could include the release of endogenous cannabinoids in structures along the pain pathway or a disrupted endocannabinoid tone (Desroches et al. 2014). The analgesic effects of morphine have been found to be enhanced by crude cannabis extract (Ghosh and Bhattacharya 1979) and by orally administered THC (Mechoulam et al. 1984). It has been observed that administration of morphine and CB1 receptor (CB1R) agonists produces synergistic analgesic effects (Welch and Eades 1999). A common mechanism proposed to explain the synergism of analgesia observed when opioids and cannabinoids are co-administered is a direct interaction between mu-opioid receptors (MORs) and CB1Rs (Schoffelmeer et al. 2006). *In vitro* studies suggest that the constitutive activity of the CB1Rs negatively regulates MORs function (Canals and Milligan 2008). For example, the neutral CB1R antagonist O-2020 produces no effect on MOR activity, but the CB1R inverse agonist SR-141716 enhances MOR function. Pacheco et al. (2009) stated that selective CB1R inverse agonist AM-251 inhibits peripheral analgesia produced by morphine. Consistent with our findings, Trang et al. (2007) demonstrated that co-administration of selective CB1R inverse agonist with morphine also decreases the development of tolerance and dependence in chronically-treated mice. Evidence in recent years has suggested that cross-talk between these two signaling pathways shows promise for combination pain therapy as well as novel treatments for opioid addiction and tolerance (Ibrahim et al. 2005; Welch 2009). A combination of low-

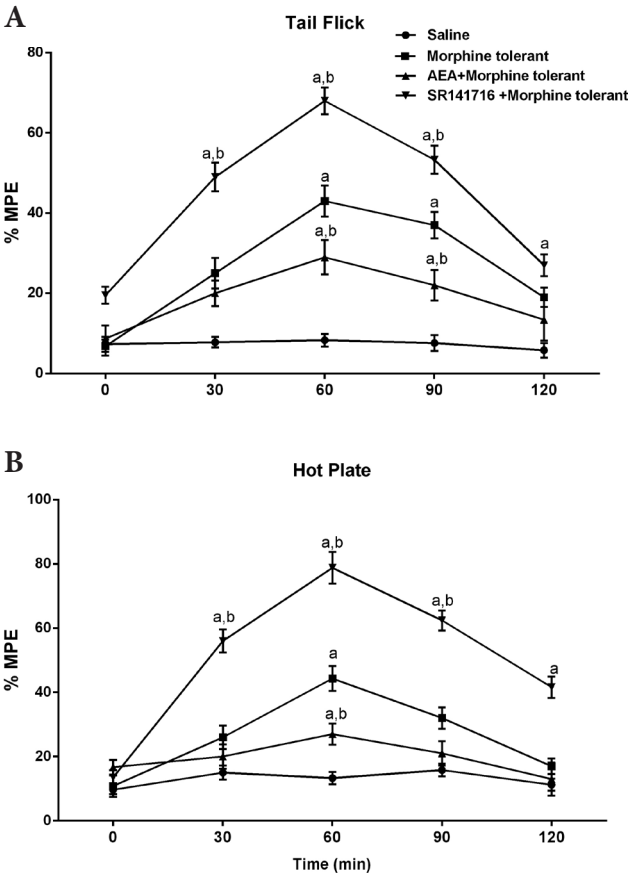

Figure 3. Effects of AEA and SR141716 on the tolerance to morphine analgesia. Effects of AEA and SR141716 in the TF test (**A**) and in the HP test (**B**). Pretreatment of morphine tolerant animals with SR141716 significantly decrease tolerance to morphine in both TF ($p < 0.05$) and HP test ($p < 0.05$) compared to morphine tolerant animals. In contrast, pretreatment of animals with AEA no significantly increase %MPE in both TF and HP test. Each point represents the mean ± SEM of %MPE for 8 rats. [a] $p < 0.01$, compared to the saline-treated group and [b] $p < 0.05$, compared to the morphine-treated group.

Table 1. The antinociceptive effects of different doses of morphine

Test	Solution	Time (min)			
		0	30	60	90
Tail-flick	Saline	4.5 ± 1.6	5.4 ± 1.3	4.3 ± 0.5	5.6 ± 0.3
	Morphine (2.5 mg/kg)	5.8 ± 1.2	18.3 ± 0.3	32.5 ± 2.3	16.4 ± 3.1
	Morphine (5 mg/kg)	4.7 ± 1.4	26.3 ± 2.2	59.3 ± 5.1**	35.3 ± 4.3*
	Morphine (7.5 mg/kg)	4.9 ± 0.9	22.8 ± 2.5	54.3 ± 5.3	32.6 ± 3.4
Hot-plate	Saline	8.3 ± 1.1	9.2 ± 0.8	8.2 ± 1.5	8.3 ± 1.2
	Morphine (2.5 mg/kg)	9.3 ± 1.2	33.5 ± 3.1	35.3 ± 6.4	23.5 ± 2.3
	Morphine (5 mg/kg)	9.2 ± 1.3	49.8 ± 4.5*	68.2 ± 8.3**	39.4 ± 2.4
	Morphine (7.5 mg/kg)	11.1 ± 1.6	41.3 ± 4.3	57.4 ± 7.3	36.8 ± 3.5

Data are mean ± SEM, * $p < 0.05$; ** $p < 0.01$ as compared with its saline group ($n = 8$ in each group). Analgesia was expressed in %MPE.

dose analgesics devoid of undesirable side effects would be ideal to replace high-dose analgesics that cause unnecessary sedation and respiratory depression.

Cannabinoids administered have been shown to release endogenous opioids which stimulate both delta and kappa opioid receptors (Smith et al. 1994; Pacheco et al. 2009). In addition, the discovery of a bi-directional cross-tolerance of cannabinoids and CP 55,940 to kappa agonists in the analgesia test confirms that cannabinoids interact with kappa opioids (Smith et al. 1994). It is believed that the synergistic effect with cannabinoid and morphine results from the initial release of dynorphin A by cannabinoid and the subsequent breakdown of dynorphin A to smaller dynorphin fragments and leucine-enkephalin metabolites (Mason et al. 1999). A time correlation between antinociception and increased dynorphin levels indicate that these opioids interact with the delta and kappa opioid receptors to mediate the antinociceptive effect of cannabinoid (Welch and Eades 1999).

Cannabinoid and opioid receptors are co-distributed in areas of the dorsal horn of the spinal cord, the periaqueductal gray (PAG) and raphe nuclei (Hohman et al. 1999; Salio et al. 2001; Wilson-Poe et al. 2012). Studies suggest that cannabinoids exhibit a similar binding distribution in the brain to that of morphine (Mailleux and Vanderhaeghen 1992). The blockade of THC-induced Fos immunoreactivity by naloxone in the periaqueductal gray indicates that these areas are important in cannabinoid-opioid interactions (Allen et al. 2003). Meng et al. (1998) reported that cannabinoids and opioids analgesia involve similar brainstem circuitry through modulation of rostal ventromedial medulla neuronal activity. Thus the spinal blockade of pain transmission becomes greater-than-additive as both opioid and cannabinoid receptor types are activated in the dorsal horn. Opioid-cannabinoid- interactions not only underlie synergy in acute analgesia, but persist after chronic drug administration. The CB1 receptor and mu opioid receptor have been found to be co-localized in areas important for the expression of morphine abstinence nucleus accumbens, PAG and amygdaloid nucleus (Seely et al. 2012). Thus, cannabinoids might alter the expression of morphine antinociceptive tolerance. After short-term treatment with low doses of cannabinoid and morphine in combination, there is a reduction in morphine tolerance without compromising the analgesic effect (Cichewicz and Welch 2003). Cichewicz et al. (2004) suggest that cannabinoids can alter the expression of morphine tolerance and may be useful long-term to provide pain relief in opioid-tolerant subjects. In contrast, our data demonstrated that co-injection of morphine with endogenous cannabinoid agonist AEA enhanced the expression of morphine tolerance. On the other hand, cannabinoid CB1 antagonist SR141716 attenuates the expression of tolerance to morphine.

In conclusion, this study presented here marks a potential use for active doses of cannabinoids to enhance the analgesic potency of opioid drugs. Since continued administration of morphine can lead to tolerance, an adjunct to morphine may be the key to prolong appropriate treatment. The administration of low doses of cannabinoid agonist anandamide in conjunction with morphine seems to be an alternative regimen that reduces the need to escalate opioid dose. Furthermore, the development of tolerance to morphine includes a different pathway and CB1 receptor plays a significant role in the morphine tolerance.

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Conflict of interest. The authors declare no competing financial interests.

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