Antinociceptive effects of HUF-101, a fluorinated cannabidiol derivative

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A B S T R A C T

Cannabidiol (CBD) is a phytocannabinoid with multiple pharmacological effects and several potential therapeutic properties. Its low oral bioavailability, however, can limit its clinical use. Preliminary results indicate that fluorination of the CBD molecule increases its pharmacological potency. Here, we investigated whether HUF-101 (3, 10, and 30 mg/kg), a fluorinated CBD analogue, would induce antinociceptive effects. HUF-101 effects were compared to those induced by CBD (10, 30, and 90 mg/kg) and the cannabimimetic CB1 receptor agonist WIN55,212-2 (1, 3, and 5 mg/kg). These drugs were tested in male Swiss mice subjected to the following models predictive of antinociceptive drugs hot plate, acetic acid-induced writhing, and carrageenan-induced inflammatory hyperalgesia. To evaluate the involvement of CB1 and CB2 receptors in HUF-101 and CBD effects, mice received the CB1 receptor antagonist AM251 (1 or 3 mg/kg) or the CB2 receptor antagonist AM65530 (1 or 3 mg/kg) 30 min before HUF-101, CBD, or WIN55,212-2. In the hot plate test, HUF-101 (30 mg/kg) and WIN55,212-2 (5 mg/kg) induced antinociceptive effects, which were attenuated by the pretreatment with AM251 and AM65530. In the abdominal writhing test, CBD (30 and 90 mg/kg), HUF-101 (30 mg/kg), and WIN55,212-2 (3 and 5 mg/kg) induced antinociceptive effects indicated by a reduction in the number of writhing. Whereas the pretreatment with AM65530 did not mitigate the effects induced by any drug in this test, the pretreatment with AM251 attenuated the effect caused by WIN55,212-2. In the carrageenan-induced hyperalgesia test, CBD (30 and 90 mg/kg), HUF-101 (3, 10 and 30 mg/kg) and WIN55,212-2 (1 mg/kg) decreased the intensity of mechanical hyperalgesia measured by the electronic von Frey method. The effects of all compounds were attenuated by the pretreatment with AM251 and AM65530. Additionally, we evaluated whether HUF-101 would induce the classic cannabinoid CB1 receptor-mediated tetrad (hypolocomotion, catalepsy, hypothermia, and antinociception). Unlike WIN55,212-2, CBD and HUF-101 did not induce the cannabinoid tetrad. These findings show that HUF-101 produced antinociceptive effects at lower doses than CBD, indicating that the addition of fluoride improved its pharmacological profile. Furthermore, some of the antinociceptive effects of CBD and HUF-101 effects seem to involve the activation of CB1 and CB2 receptors.

1. Introduction

The current pharmacological therapy to manage moderate to severe pain remains mostly based on the use of paracetamol, non-steroidal anti-inflammatory drugs and opioids (Stevens and Higgins, 2017). Also, antidepressant and antiepileptic drugs are employed in the treatment of the neuropathic pain. However, the relief using these drugs sometimes is not higher than 40–50% (Nascimento et al., 2016) and these treatments can cause several side effects (Luongo et al., 2017). Therefore, the search for new therapeutic strategies to manage acute and chronic pain is necessary (Stevens and Higgins, 2017).

Cannabis has been used to treat pain for centuries. Several studies show that its main psychoactive constituent, Δ9-tetrahydrocannabinol (THC), has antinociceptive activity in acute and chronic pain models in animals and humans (Bagiás et al., 2014; Britich et al., 2017; Cox and Welch, 2004; Greenwald and Sitzer, 2000; Lynch and Ware, 2015). Cannabidiol (CBD) is another abundant component of Cannabis sativa plant. Unlike THC, CBD does not cause the typical effects observed after cannabis use (Mechoulam et al., 2007). Actually, CBD attenuates some psychoactive effects induced by THC (Zuardi et al., 1982) and has a high potential for therapeutic use in several conditions, including pain (Izzo et al., 2009; Ligresti et al., 2016). In fact, Sativex, an oromucosal...
spray containing THC and CBD at an approximately 1:1 fixed ratio which is approved in Canada and several European countries for the treatment of spasticity in multiple sclerosis, has been found to be effective in the relief of pain (Johnson et al., 2013; Russo et al., 2016). There are, however, contradictory findings on the antinociceptive effects of this compound in acute pain (Booher et al., 2009; Costis et al., 2004b; Neelakantan et al., 2015; Sofia et al., 1975; Varvel et al., 2006), although it prevents the development of thermal and mechanical allodynia in different mouse model of neuropathic pain (Costis et al., 2007; King et al., 2017; Ward et al., 2014).

Despite the therapeutic properties of CBD and its safety profile, a potential limitation for its clinical use is CBD low and variable oral bioavailability in humans (Agurrell et al., 1981). In addition, studies with laboratory animals have indicated that CBD causes bell-shaped dose-response curves, indicating that this drug may possess a narrow therapeutic dose range (Guimardes et al., 1990; Zanella et al., 2010). The development of more potent CBD analogues could be a strategy to overcome these limitations (Ben-Shabat et al., 2006; Kozela et al., 2016).

A common approach used to enhance the activity of both endogenous and synthetic compounds is the addition of a fluorine atom to the molecule (Söhm et al., 2004; Park et al., 2001). Based on this strategy, fluorinated CBD derivatives have been developed (Breuer et al., 2016). One of these novel CBD derivatives, HUF-101, was found to be more potent than CBD in behavioral assays predictive of antinociceptive, antidepressant, anxiolytic and anticonvulsant effects in mice (Breuer et al., 2016). Moreover, even if the anticonvulsant-like effects induced by this drug were blocked by antagonists of CB1 and CB2 receptors (Breuer et al., 2016), the mechanisms of HUF-101 effects are largely unknown.

Here, we investigated the effects of the fluorinated analogue of CBD, HUF-101, in three models of acute pain (hot plate, acetic acid-induced writhing response, and carrageen-induced mechanical hyperalgesia). HUF-101 and CBD effects were compared to those produced by the synthetic mixed CB1,2 receptor agonist WIN55,212-2. We also evaluated if the antinociceptive effects induced by CBD, HUF-101, and WIN55,212-2 would involve the activation of CB1 and CB2 receptors. Finally, we investigated if these drugs induce the classic tetrad effects ("cannabinoid tetrad" - hypolocomotion, catalepsy, hypothermia, and antinociception), which is commonly observed after the administration of CB1 receptor agonists and is considered a good predictor of THC-like pharmacological activity (Martin et al., 1991). We found that HUF-101 induced antinociceptive effects in all models at lower doses than CBD, but failed in causing the cannabinoid tetrad. Also, some of antinociceptive effects were mediated by the activation of CB1 and CB2 receptors.

2. Material and methods

2.1. Animals

The experiments were performed using male Swiss mice weighing 30–45 g originated from the Central Animal Farm of the School of Medicine of Ribeirão Preto – University of São Paulo. Mice were housed in groups of 8 mice/cage under a 12 h light cycle (lights on at 7 am) with food and water ad libitum. Procedures were in accordance with the International Association for the Study of Pain (IASP) guidelines and were approved by the local Ethics Committee (CEUA, School of Medicine of Ribeirão Preto, Protocol number 058/2013), which follows Brazilian and International regulations. All efforts were made to minimize animal suffering.

2.2. Drugs

HUF-101 was synthesized by Prof. Raphael Mechoulam group, Hebrew University of Jerusalem, Israel. As described previously (Breuer et al., 2016), the synthesis involved the fluorination of the CBD molecule on the aromatic ring by the electrophilic fluorinating agent 1-fluoropyridinium trifluoromethane sulfonate (known as 1-fluoropyridinium triflate Fig. 1). The other drugs were used: cannabidiol (CBD; THC Pharm., Germany), WIN55,212-2 (CB1,2 receptor agonist; Tocris, USA), AM251 (CB1 receptor antagonist; Tocris, USA), AM630 (CB2 receptor antagonist; Tocris, USA). All drugs were dissolved in 2% Tween 80 in sterile saline, except AM251 and AM630, which were dissolved in 1% DMSO in sterile saline.

2.3. Experimental design

The antinociceptive effects of WIN55,212-2 (1, 3, and 5 mg/kg), CBD (10, 30, and 90 mg/kg), and HUF-101 (3, 10, and 30 mg/kg) were evaluated in the hot plate (n = 7–8/group), acetic-acid-induced writhing (n = 5–6/group), and carrageenan-induced mechanical hyperalgesia tests (n = 6–10/group). To investigate the involvement of the CB1 and CB2 receptors in the effects induced by effective doses of WIN55,212-2, CBD, and HUF-101 in these three test animals were pretreated (30 min before WIN55,212-2, CBD, or HUF-101) with the CB1 receptor antagonist AM251 (1 and 3 mg/kg; n = 5–10/group) or the CB2 receptor antagonist AM630 (1 and 3 mg/kg; n = 5–8/group). Finally, we evaluate if WIN55,212-2, CBD, or HUF-101 would induce the cannabinoid tetrad (hypolocomotion, catalepsy, hypothermia, and antinociception; n = 5–9/group). The doses were based on previous studies (Costis et al., 2004a). The drugs were injected intraperitoneally (I.P.) in a 10 mL/kg volume.

3. Procedures

3.1. Hot plate

Mice were placed in a 10-cm wide glass cylinder on a hot plate maintained at 56°C. The end-point (latency) was characterized by the removal of the paw followed by clear paw flinching or licking movements. A maximum latency (cut-off) was set at 30 s to avoid tissue damage (Kinsey et al., 2011). The latency before (baseline) and 45 min after the treatment were evaluated. This time-point was based on preliminary data from our group. The results were expressed by the delta of paw withdrawal latency (in seconds) calculated by subtracting the
3.3. Acetic acid-induced writhing response test

The abdominal writhing responses were induced by intraperitoneal (i.p.) injection of acetic acid 0.6% (100 μL/10 g of body weight; Merck, Germany). The treatment with drugs was performed 30 min before the acetic acid injection. After the acetic acid injection, each mouse was placed in a large glass cylinder, and the intensity of nociceptive behavior was quantified by counting the total number of writhes occurring between 0 and 20 min. The writhing response consists of a contraction of the abdominal muscle together with a stretching of hind limbs. The intensity of the writhing response was expressed as the total number of writhing responses during 20 min (Collier et al., 1969).

3.3. Evaluation of mechanical hyperalgesia: electronic von Frey test

In this study, we used the term mechanical hyperalgesia to describe the decrease in the mechanical nociceptive threshold of mice. Mechanical hyperalgesia was tested as previously reported (Cunha et al., 2004). Briefly, in a quiet room, mice were placed in acrylic cages (12 x 20 x 17 cm) with wire grid floors. The test consisted of evoking a hind paw flexion reflex with a hand-held force transducer adapted with a 0.5-mm² polypropylene tip for mice (Electronic von Frey, IITC Life Science, USA). The tip was applied in between the five distal footpads with a gradual increase in pressure, and the stimulus was
automatically discontinued and its intensity recorded when the paw was withdrawn. The end-point was characterized by the removal of the paw in a clear flinch response after paw withdrawal. A baseline value was obtained for each mouse. One day later, animals received the specific treatments 30 min before the intraplantar administration of carrageenan (0.1 mg/20 μL) (FMC Corp., USA; dissolved in sterile saline; in the right paw). Three hours after the carrageenan injection, the intensity of hyperalgesia was measured. The results were expressed as the delta of mechanical threshold (in grams, g), which was calculated by subtracting the average of three measurements after treatments from the average of three measurements before treatments (baseline).

3.4. Cannabinoid treads assay

First, baseline values (before treatment) of tail temperature and nociceptive response in the hot plate test were obtained. The tail temperature was recorded using a thermal camera Termovisor NV 384 model (THERMOCOM4, Nashua, NH 03062, USA) placed at a distance of 50 cm from the animal. Five pictures were taken from each animal from 5 different points of the animal’s tail. An average was calculated using the software Launch Guide iData (Wuhan Guide Infrared Technology Co., Ltd., China). The results were expressed by the delta of temperature (Celsius), which was calculated by subtracting the average of the measurements after the treatment from the baseline. 30 min after the treatment with WIN55,212-2, CBD, or HUF-101, the catalepsy time was evaluated. The catalepsy test consists of placing the animal in an unusual posture and record the time that it remains in this position. For this experiment, we used a glass bar (diameter = 0.5 cm) elevated 4 cm from the floor. The time that both forepaws remained on the apparatus was measured by an experimenter that was blind to the treatment conditions, with a maximum time of 300 s (Gomes et al., 2013). Immediately after the catalepsy test, we evaluate possible changes induced by the treatments in the tail temperature. 2 min later, to assess locomotor activity, mice were placed in the center of a Plegixas circular arena (40 cm diameter with 40 cm-high walls) and the total distance traveled was measured during 10 min using the Any-Maze software (Stoeling Co., Ireland). Finally, changes in the nociceptive responses induced by the treatments were evaluated.

3.5. Statistical analysis

Data were presented as the mean ± SEM and analyzed by student’s t-test, one or two-way ANOVA, according to the experimental design. Post-hoc analysis was performed using the Dunnett’s or Student Newman-Keuls (S-N-K) tests. The level of statistical significance was 95% (p < 0.05).

4. Results

4.1. Antinociceptive effects of HUF-101, CBD and WIN55,212-2

4.1.1. Hot plate test

Whereas only the highest dose of WIN55,212-2 (5 mg/kg) and HUF-101 (30 mg/kg) induced antinociceptive effects in the hot plate test (WIN55,212-2: F3,24 = 6.9; p < 0.05, one-way ANOVA; HUF-101: F3,24 = 8.3; p < 0.05, one-way ANOVA; p < 0.05 vs. vehicle, Dunnett’s test; Fig. 2A and C), CBD had no effect (F3,24 = 0.14; p > 0.05, one-way ANOVA; Fig. 2B).

4.1.2. Acetic acid-induced abdominal writhing test

WIN55,212-2, CBD, and HUF-101 induced antinociceptive effects in this test. WIN55,212-2, at the doses of 3 and 5 mg/kg, reduced the number of abdominal writhing responses induced by acetic acid (F3,24 = 18.4; p < 0.05, one-way ANOVA; p < 0.05 vs. vehicle; Dunnett’s test; Fig. 2D). Similarly, CBD (30 and 90 mg/kg) and HUF-101 (30 mg/kg) reduced the number of abdominal writhing responses (CBD: F3,24 = 16.3; p < 0.05, one-way ANOVA; HUF-101: F3,24 = 6.4; p < 0.05, one-way ANOVA; p < 0.05 vs. vehicle; Dunnett’s test; Fig. 2E and F).

4.1.3. Carrageenan-induced mechanical hyperalgesia

Similar to the acetic acid-induced writhing test, all compounds tested reduced the hyperalgesia induced by intraplantar carrageenan. WIN55,212-2 (1 mg/kg) decreased the intensity of mechanical hyperalgesia measured by the electronic von Frey method (F3,24 = 4.8; p < 0.05, one-way ANOVA; p < 0.05 vs. vehicle, Dunnett’s test; Fig. 2G). Interestingly, WIN55,212-2, at the doses of 3 and 5 mg/kg, did not induce any effect (Fig. 2G). Antinociceptive effects in this model were also observed after CBD (30 and 90 mg/kg) and HUF-101 (3, 10, and 30 mg/kg) administration (CBD: F3,24 = 26.5; p < 0.05, one-way ANOVA; HUF-101: F3,24 = 17.2; p < 0.05, one-way ANOVA; p < 0.05 vs. vehicle, Dunnett’s test; Fig. 2H and I).

4.2. Involvement of CB1 and CB2 receptors in the antinociceptive effects induced by WIN55,212-2, CBD, and HUF-101

4.2.1. Hot plate test

Given that CBD did not induce any effect in this model, only the possible involvement of CB1 and CB2 receptors in the WIN55,212-2 and HUF-101 effects was evaluated. Antinociceptive effects of WIN55,212-2 and HUF-101 in the hot plate test were mediated by the activation of both CB1 and CB2 receptors. Pre-treatment with the CB1 receptor antagonist AM251 (1 and 3 mg/kg) blocked the effects induced by WIN55,212-2 (interaction between first and second drug injection: F3,24 = 12.6; p < 0.05, two-way ANOVA; p < 0.05 vs. vehicle + WIN55,212-2, S-N-K test; Fig. 3A) as well as by HUF-101 (interaction between first and second drug injection F2,24 = 7.5; p < 0.05, two-way ANOVA; p < 0.05 vs. vehicle + HUF-101, followed by S-N-K test; Fig. 3B).

Regarding the involvement of CB2 receptors, the pre-treatment with the CB2 receptor antagonist AM650 (3 mg/kg) blocked the effects induced by HUF-101 (p < 0.05 vs. vehicle + HUF-101, S-N-K test; Fig. 3D). Additionally, animals that received a pre-treatment with AM650 (1 mg/kg) before WIN55,212-2 or HUF-101 were not significantly different when compared to controls (vehicle + vehicle) (p > 0.05 vs. S-N-K test; Fig. 3C and D), indicating that AM650 attenuated the antinociceptive effects induced by these drugs.

4.2.2. Acetic acid-induced writhing test

The WIN55,212-2 effects in reducing the number of writhing responses were blocked by the pre-treatment with the CB1 receptor antagonist AM251 (1 and 3 mg/kg) (interaction between first and second drug injection: F3,24 = 4.0; p < 0.05, two-way ANOVA, p < 0.05 vs. vehicle + WIN55,212-2, S-N-K test, Fig. 4A), but not by the pre-treatment with the CB2 receptor antagonist AM650 (Fig. 4D). Antinociceptive effects induced by CBD and HUF-101 in the acetic acid-induced writhing test do not seem to be mediated by CB1 and CB2 receptors since their effects were not attenuated by the pre-treatment with AM251 and AM650 (Fig. 4B-C and E-F).

4.2.3. Carrageenan-induced mechanical hyperalgesia

The antinociceptive effects induced by WIN55,212-2, CBD, and HUF-101 in this test were blocked by the pre-treatment with both the CB1 antagonist AM251 and the CB2 antagonist AM650. WIN55,212-2 effects were blocked by AM251, at the doses of 1 and 3 mg/kg (p < 0.05 vs. vehicle + WIN55,212-2, S-N-K, Fig. 5A), and by AM650 (3 mg/kg) (p < 0.05 vs. vehicle + WIN55,212-2, S-N-K, Fig. 5D). A similar finding was observed with the pre-treatment with AM251 and AM650 before CBD (p < 0.05 vs. vehicle + CBD, S-N-K test; Fig. 5B and E) and HUF-101 as well (p < 0.05 vs. vehicle + HUF-101, S-N-K test; Fig. 5C and F).
Fig. 3. Effect, in the hot plate test, of the pretreatment with vehicle (VEH) or the CB2 receptor antagonist AM251 (1 or 3 mg/kg) followed by (A) VEH or WIN55,212-2 (5 mg/kg, n = 6–8/group) or (B) VEH or HUF-101 (30 mg/kg, n = 6–7/group). Effect of the pretreatment with VEH or the CB2 receptor antagonist AM630 (1 or 3 mg/kg) followed by (C) VEH or WIN55,212-2 (5 mg/kg, n = 6–8/group) or (D) VEH or HUF-101 (30 mg/kg, n = 7–8/group) in the hot plate test. Data represent the mean ± SEM. *p < 0.05 from VEH-VEH group, †p < 0.05 from VEH-WIN55,212-2 or VEH-HUF-101 group.

Fig. 4. Effect, in the acid acetic-induced abdominal writhing test, of the pretreatment with vehicle (VEH) or the CB2 receptor antagonist AM251 (1 or 3 mg/kg) followed by (A) VEH or WIN55,212-2 (5 mg/kg, n = 6–8/group), (B) VEH or CBD (30 mg/kg, n = 5–7/group), or (C) VEH or HUF-101 (30 mg/kg, n = 6–7/group). Effect of the pretreatment with VEH or the CB2 receptor antagonist AM630 (1 or 3 mg/kg) followed by a second injection of (D) VEH or WIN55,212-2 (5 mg/kg, n = 6–8/group), (E) VEH or CBD (30 mg/kg, n = 6–8/group) or (F) VEH or HUF-101 (30 mg/kg, n = 5–6/group) in the acid acetic-induced abdominal writhing test. Data represent the mean ± SEM. *p < 0.05 from VEH-VEH group, †p < 0.05 from VEH-WIN55,212-2 group.
Fig. 5. Effect, in the carrageenan-induced mechanical hyperalgesia, of the pretreatment with (VEH) or the CB2 receptor antagonist AM281 (1 or 3 mg/kg) followed by (A) VEH or WIN55,212-2 (1 mg/kg, n = 6/group), (B) VEH or CBD (30 mg/kg, n = 6/group) or (C) VEH or HUF-101 (3 mg/kg, n = 6/group). Effect of the pretreatment with VEH or the CB2 receptor antagonist AM630 (1 or 3 mg/kg) followed by a second injection of (D) VEH or WIN55,212-2 (1 mg/kg, n = 6/group), (E) VEH or CBD (30 mg/kg, n = 6/group) or (F) VEH or HUF-101 (3 mg/kg, n = 6/group) in the inflammatory hyperalgesia induced by carrageenan. Data represent the mean ± SEM. *p < 0.05 from VEH-WINS,212-2, VEH-CBD or VEH-HUF-101 groups.

4.3. Effects of WIN55,212-2, CBD, and HUF-101 in the cannabinoid tetrad assay

Consistent with evidence from the literature (Fox et al., 2001), WIN55,212-2 (5 mg/kg) induced the typical cannabinoid tetrad (Fig. 5) which was indicated by catalepsy (t16 = 3.7; p < 0.05), hypothermia (t16 = 2.7; p < 0.05), hypolocomotion (t16 = 2.7; p < 0.05) and a tendency for antinociceptive effects in the hot plate test (t16 = 1.9; p = 0.1). HUF-101 (30 mg/kg), as expected, induced antinociceptive effect in the hot plate test (F2,13 = 6.5; p < 0.05, one-way ANOVA, p < 0.05 vs. vehicle, Dunnnett’s test; Fig. 5). But, unlike WIN55,212-2, CBD and HUF-101 did not induce the cannabinoid tetrad (Fig. 5).

5. Discussion

In the present study, the potential antinociceptive effects of the novel cannabinoid derivative HUF-101 were evaluated in three different tests: hot plate, acetic acid-induced writhing, and carrageenan-induced inflammatory hyperalgesia. Additionally, HUF-101 effects were compared to those induced by CBD and the synthetic mixed CB1/2 receptor agonist WIN55,212-2. Whereas HUF-101 and WIN55,212-2 were effective in all the tests, CBD induced antinociceptive effects only in the acetic acid-induced writhing and carrageenan-induced inflammatory hyperalgesia. Importantly, HUF-101 induced antinociceptive effects at lower doses than CBD. Furthermore, HUF-101 effects, at least in the hot plate and carrageenan-induced inflammatory hyperalgesia tests, seem to involve the activation of CB1 and CB2 receptors. However, unlike WIN55,212-2, HUF-101 did not induce the cannabinoid tetrad.

As mentioned in the introduction, there is a clear need to develop more efficient and safe drugs to treat pain, particularly chronic conditions (Stevens and Higgins, 2017). In this way, CBD could be a useful compound, since it has a positive safety profile (Bergamaschi et al., 2011) and produces antinociceptive effects (Costa et al., 2007; King et al., 2017; Ward et al., 2013). There are, however, inconsistent results regarding CBD effects in pain models (Booher et al., 2009; Costa et al., 2004; Neelakantan et al., 2015; Sofina et al., 1979; Varvel et al., 2006). Accordingly, similar to Neelakantan et al. (2015), CBD induced antinociceptive effects in the acetic acid-induced writhing, but not in the hot plate test. Additionally, it also attenuated the thermal hyperalgesia induced by intraplantar carrageenan (Costa et al., 2004b). Different of CBD, however, HUF-101 was effective in all tests employed. Additionally, in the carrageenan-induced hyperalgesia, HUF-101 induced an antinociceptive effect at a dose 10 times lower than CBD.

To investigate if HUF-101 antinociceptive effects depend on activation of the endocannabinoid system, we tested if CB1 or CB2 receptor antagonists would prevent them. Recently, the endocannabinoid system has emerged as an attractive therapeutic target for pain treatment, and the use of the cannabinoids with this aim is gradually increasing, particularly in patients where conventional treatments fail (Bagiás et al., 2014). This system plays a major role in the inhibitory control of nociceptive stimuli by acting at peripheral, spinal, and supraspinal levels. The CB1 receptor located in nociceptive terminals at the periphery, inhibits nociceptive transmission, whereas CB2 receptors found mainly in immune cells and keratinocytes decrease the release of pronociceptive substances. At the spinal cord level, CB2 receptors modulate the
immune responses, leading to neuronal sensitization during chronic pain. At the supraspinal level, CB1 receptors activate the descending inhibitory pathway and inhibit the ascending nociceptive transmission (Maldonado et al., 2016). The antinoceptive effect of cannabinoids can be mediated by CB1 and/or CB2 receptors depending on the type of pain (Bugidé et al., 2014; Cox and Welch, 2004; Desroches et al., 2014; Munawar et al., 2017; Vincenzi et al., 2013).

We found that the effects of HUF-101 in the hot plate test, those of HUF-101, and CBD in the carrageenan-induced inflammatory hyperalgesia, were attenuated by the CB1 antagonist AM251 and the CB2 antagonist AM650. Despite the low affinity of CBD for CB1 and CB2 receptors, this compound is able to inhibit the fatty acid amide hydrolase (FAAH) enzyme which hydrolyzes the endocannabinoid anandamide, increasing the levels of this endocannabinoid (Covey et al., 2017; Bisogno et al., 2001; De Filippis et al., 2008). Therefore, CBD can indirectly facilitate the endocannabinoid-mediated neurotransmission (Casarotto et al., 2010). If HUF-101 could produce a similar effect (a possibility that remains to be tested), part of its antinoceptive effects could be explained by facilitation of anandamide-mediated neurotransmission.

Consistent with the participation of cannabinoid receptors in pain modulation and previous results from the literature (Luszczki and Fiorek-Luszczki, 2012), the non-selective CB1/CB2 agonist WIN55,212-2 as well as antinoceptive effect. Interestingly, while WIN55,212-2 effects were attenuated by AM251 but not by AM650 in the acute acid-induced writhing test, the antinoceptive effects observed after HUF-101 and CBD administration in this test were not attenuated by the pretreatment with AM251 or AM650. Therefore, it is possible that other mechanisms are responsible for these effects in this test. CBD can also interfere with other mechanisms involved in pain control, such as the activation of 5-HT1A (Russo et al., 2005) and TRPV1 (Campos and Guimarães, 2009) receptors, enhancing of adenosine signaling through inhibition of its uptake (Carrier et al., 2006), and via its anti-inflammatory properties. The involvement of these mechanisms in the antinoceptive effects of HUF-101 remains to be tested.

The use of CB1 agonists, such as THC, in pain relief, is limited by their side effects, such as sedation, psychotic-like behavior, addiction, tolerance, and cognitive impairment (Luongo et al., 2017). Given that part of HUF-101 and CBD antinoceptive effects were mediated by the activation of CB1 receptors, we further evaluate if these compounds would cause the classical cannabinoid tetrad (hypolocomotion, anantinoception, hypothermia, and catalepsy) commonly induced by THC and other CB1 receptor agonists. As expected, (Wiley et al., 2014; El-Alfy et al., 2010), unlike the positive control WIN55,212-2, HUF-101 and CBD did not induce the cannabinoid tetrad. Given the low affinity of CBD for CB2 and CB3 receptors, these findings support the idea that its effects mediated by CB1 receptors are likely due to an increase in the levels of the endocannabinoid anandamide. In fact, drugs that inhibit anandamide hydrolysis produce antinoceptive effects without induction of the cannabinoid tetrad (Long et al., 2009; Ahn et al., 2011).

In conclusion, our findings show that HUF-101 induces antinoceptive effects at lower doses than CBD, indicating that the addition of fluorine to the CBD molecule may have improved its pharmacological profile. Part of HUF-101 antinoceptive effects involves the activation of CB1 and CB2 receptors. This activation, however, is probably indirect since it does not cause the typical tetrad effects induced by CB1 agonists. Thus, this new compound could be a therapeutic alternative to pain relief.
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Conflict of interest


References


