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Antinociceptive effects of JWH015 in female and male rats

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Abstract

Despite greater chronic pain prevalence in females compared to males, and the analgesic potential of cannabinoid receptor type 2 (CB2) agonists, CB2 agonists have rarely been tested in females. The aim of the present study was to compare the antinociceptive effects of a CB2-preferring agonist, (2-Methyl-1-propyl-1*H*-indol-3-yl)-1-naphthalenylmethanone (JWH015), in female and male rats, against acute pain and persistent inflammatory pain. JWH015 (5-20 mg/kg i.p.) produced dose- and time-dependent increases in latency to respond on the tail withdrawal and paw pressure tests that did not differ statistically between the sexes. JWH015 dose-dependently decreased locomotor activity in both sexes, but was more potent in females than males. JWH015 produced little catalepsy in either sex. In females, the antinociceptive effects of JWH015 against acute pain were blocked by rimonabant and SR144528, whereas locomotor suppression was antagonized by rimonabant. When given 3 days after intraplantar injection of complete Freund's adjuvant (CFA), JWH015 produced a significantly greater anti-allodynic effect in females at the highest dose tested (10 mg/kg i.p.). Anti-allodynic effects of JWH015 were antagonized by rimonabant and SR144528 in both sexes. These studies indicate that systemically administered JWH015 produced antinociception that was both CB1 and CB2 receptor-mediated in both sexes. Unlike THC and other non-selective cannabinoid agonists, the CB2-preferring agonist JWH015 may produce more equivalent antinociception in females and males.

Keywords

Sex differences; Gender; Cannabinoids; Inflammatory pain; rat

Introduction

Cannabinoid drugs may provide a useful alternative to opioids for the treatment of chronic pain. Clinical trials testing various cannabinoids indicate that they significantly reduce chronic pain associated with multiple sclerosis, fibromyalgia, diabetic or other neuropathy, and rheumatoid arthritis (for reviews see Lynch and Campbell, 2011; Lynch and Ware, 2015). Adverse effects are reported to be relatively mild and well-tolerated, and some studies also report improvements in sleep (Lynch and Ware, 2015). Thus, cannabinoids may be relatively safe for long-term use to alleviate chronic pain (Ware et al., 2015).

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One drawback to the use of cannabinoids for treating chronic pain is their central nervous system side-effects, including psychoactive effects. However, psychoactive effects such as reinforcing/rewarding effects of cannabinoids appear to be mediated exclusively by the cannabinoid 1 (CB1) receptor (Braida et al., 2004; Zangen et al., 2006). For this reason, it has been suggested that cannabinoids acting at cannabinoid 2 (CB2) receptors may be preferable for long-term use as pain therapeutics (Malan et al., 2003; Guindon and Hohmann, 2008). In animal studies, both CB1 and CB2 receptors have been shown to mediate the antinociceptive effects of a range of cannabinoids; however, CB2 receptors appear to be more involved in cannabinoid antinociception against chronic than acute pain, perhaps because CB2 receptors are upregulated during inflammation and nerve injury (Zhang et al., 2003; Hsieh et al., 2011).

In the present study, we examined the effects of (2-Methyl-1-propyl-1*H*-indol-3-yl)-1-naphthalenylmethanone (JWH015) in acute pain tests and in a persistent inflammatory pain model. JWH015 binds to both CB1 and CB2 receptors, but has approximately 10-30 times greater affinity for CB2 than CB1 receptors, compared to the approximately 1:1 CB1:CB2 binding ratio for delta-9-tetrahydrocannabinol (THC) (Huffman et al., 2005; Pertwee, 2008). JWH015 given intrathecally has been shown to decrease mechanical allodynia and/or thermal hyperalgesia that developed after hindpaw incision (Romero-Sandoval & Eisenach, 2007; Landry et al., 2012) or peripheral nerve injury (Romero-Sandoval et al., 2008) in male rats, and after bone cancer induction in male mice (Gu et al., 2011). To our knowledge, JWH015 has not been tested in females of any species. Given the greater prevalence of chronic pain in women compared to men (Unruh, 1996), it is important to characterize the therapeutic potential of CB2 agonists in both sexes.

Sex differences in the antinociceptive potency of non-selective cannabinoids have been observed. For example, THC and CP55,940 were more potent in female rats compared to males against acute thermal and/or mechanical pain (Tseng & Craft, 2001; Romero et al., 2002; Craft et al., 2012) and against persistent inflammatory pain (Craft et al., 2013). Thus, both females and males were tested in the present study, and we hypothesized that JWH015 would be more potent in females than in males. A previous study demonstrated that JWH015-induced antinociception was blocked by a CB2- but not CB1-receptor-selective antagonist in male rats (Romero-Sandoval & Eisenach, 2007), so we expected that JWH015-induced antinociception would be CB2 receptor-mediated in both sexes.

Methods

Subjects

Adult Sprague-Dawley rats were bred in-house from Harlan stock (Livermore, CA): they were 60-89 days old on the first day of testing (body weight 184-272 g females, 239-431 g males). Rats were housed under a 12:12 h light:dark cycle (lights on at 06.00 h) in a room maintained at 21±2°C, with TekFresh® bedding (Envigo, Denver, CO), with typically 2 same-sex rats/cage. Males and females were housed in the same room, but on different racks that were approximately 1.5 m apart, and rats had free access to food and water except during testing. Rats were randomly assigned to treatment groups with the exception that care was taken to avoid assigning same-sex siblings to the same treatment group. Rats were

tested during the light part of the cycle, typically between 0900-1500 h, and were treated in accordance with the National Research Council *Guide for the Care and Use of Laboratory Animals* (2011).

Apparatus

Tail withdrawal antinociception was assessed using a 2.5-L water bath (Precision Scientifics Inc., Winchester, VA) set to $50 \pm 0.5^\circ\text{C}$; latency to withdraw the tail was timed with a hand-held stopwatch to the nearest 0.01 s. Paw pressure antinociception was assessed using an Analgesy-meter (Ugo-Basile, Varese, Italy). The pressure on the paw began at 30 g and increased at a constant rate of 48 g/s to a maximum of 750 g (15 s). Horizontal locomotor activity was measured using a photobeam apparatus (Opto-varimex, Columbus Instruments, Columbus, OH), in which 15 photobeams cross the width of a 20 cm $\times$ 40 cm $\times$ 23 cm transparent rodent cage, with photobeams spaced 2.5 cm apart and 8 cm above the cage floor. Catalepsy was measured using a bar test (ring stand with a 1.5-cm diameter horizontal bar); time spent on the bar was measured with a hand-held stopwatch. An electronic von Frey anesthesiometer (IITC Life Science, Woodland Hills, CA) was used to measure mechanical allodynia. To measure thermal hyperalgesia, a plantar (Hargreaves) apparatus (Ugo Basile, Varese, Italy) was used. Tests were conducted on both the left and right hindpaws; half of the rats in each treatment group were tested on the left hindpaw first and the other half were tested on the right hindpaw first. An incapacitance meter (Stoelting, Wood Dale, IL) was used to record weight placed on each hindpaw in g. Calipers were used to assess, in mm, maximal dorsal-ventral hindpaw thickness, a measure of edema.

Behavioral procedures

Study 1: JWH015 Effects on Acute Pain and Motor Behaviors—Baseline nociception was assessed by testing each rat twice on the tail withdrawal and paw pressure tests, in that order. Thirty min later, vehicle or a single dose of JWH015 (5, 10 or 20 mg/kg) was injected i.p. (experimenter blinded to dose). Rats were then tested on tail withdrawal and paw pressure tests at 15, 30, 60 and 120 min post-injection. For the tail withdrawal test, the distal 5 cm of the tail was submerged in the warm water bath and latency to withdraw the tail was recorded; if the rat did not respond by the 12-s cutoff, the test was terminated and 12 s was recorded. For the paw pressure test, latency to withdraw or attempt to withdraw the hind paw was recorded; if the rat did not respond by the 15-s cutoff, the test was terminated and 15 s was recorded. Horizontal locomotor activity was measured as the number of photobeams broken in a 5-min period, beginning immediately after nociceptive testing at 30, 60 and 120 min post-injection. Catalepsy was assessed immediately after the locomotor test at the 60-min time point only, on the basis of a previous study demonstrating significant antinociceptive effects of JWH015 at 60 min post-injection in male rats (Landry et al., 2012). Latency to withdraw both paws from the bar or jump up on the bar was recorded; if the rat did not respond by 12 s, the test was terminated and 12 s was recorded. Rats were returned to their home cages between testing periods.

In a separate experiment, cannabinoid receptor mediation of the effects of JWH015 on acute pain and motor behaviors was examined in females only. After baseline testing as described above, vehicle or a single dose of rimonabant (0.1 mg/kg) or SR144528 (3.2 mg/kg) was

injected i.p.; these antagonist doses were the lowest effective doses in a previous study of cannabinoid receptor mediation of THC's acute antinociceptive effects in female rats (Craft et al., 2012). Thirty min later, vehicle or a single dose of JWH015 (5, 10, 20 or 40 mg/kg) was injected i.p., and rats were tested as described above.

Study 2: JWH015 Effects on Persistent Inflammatory Pain—Persistent paw inflammation and pain were induced with a 0.1-ml i.pl. injection of a 1 mg/ml complete Freund's adjuvant (CFA) solution (killed *Mycobacterium butyricum* suspended in mineral oil: Sigma-Aldrich, St. Louis, MO). On Day 0 (prior to CFA injection), rats were habituated to hanging wire cages for 15 min. Baseline sensitivity was determined on the von Frey test (force applied to induce hindpaw withdrawal, in g), the Hargreaves test (latency to withdraw the hindpaw from a heat stimulus, in s), and the incapacitance test (weight born on left vs. right hindpaws). Three measurements were taken per hindpaw on each of the tests, with approximately 30 s between trials. Maximal dorsal-ventral hindpaw thickness was measured. After baseline testing was completed, rats were briefly anesthetized with isoflurane, and CFA was injected into the right plantar hindpaw. Three days after CFA treatment, rats were injected i.p. with vehicle, 5 or 10 mg/kg JWH015 (experimenter blinded to dose), and placed into hanging wire cages for a 15-min habituation period. Then rats were assessed on the von Frey, Hargreaves, and incapacitance tests at 15, 30, 60, 120, 180 and 240 min post-injection. Given that no significant catalepsy was observed 60 min after JWH015 injection, and peak antinociception was observed at 15-30 min post-injection in Study 1, in Study 2 catalepsy was assessed at 15 and 30 min post-injection. Paw thickness was measured 60, 120 and 240 min after vehicle or JWH015 injection. Rats were returned to their home cages between testing periods.

To determine receptor mediation of the effects of JWH015 in the CFA model, a separate experiment was conducted. An i.pl. CFA injection was administered to the right hindpaw while rats were under brief isoflurane anesthesia. Three days after CFA injection, vehicle or antagonist (rimonabant or SR144528, 1.0 mg/kg) was injected i.p., then 30 min later vehicle or 10 mg/kg JWH015 was administered i.p. (experimenter blinded to antagonist condition). Antagonist doses were chosen on the basis of a previous study examining receptor mediation of the effects of THC in the CFA model in female and male rats (Craft et al., 2013). Mechanical allodynia was measured as described above, at 30, 60 and 120 min post-injection.

Drugs

Rimonabant (SR141716A) and SR144528 (National Institute on Drug Abuse, Bethesda, MD) were dissolved in a 1:1:18 (ethanol:cremaphor:saline) solution, which served as the vehicle. In cellular assays, these antagonists demonstrate binding selectivity for CB1 (rimonabant) or CB2 (SR144528) receptors, although they may act as inverse agonists at high concentrations (Govaerts et al., 2004). In male rats, rimonabant doses as low as 0.32-1 mg/kg i.p. antagonize THC's antinociceptive and motoric effects, whereas SR144528 doses as high as 10 mg/kg i.p. do not (Craft et al., 2012). JWH015 was purchased from Cayman Chemical (Ann Arbor, MI); it was dissolved in DMSO to which distilled water was added

for a final DMSO concentration of 75%. All drugs were administered i.p. in volumes of 1-2 ml/kg.

Data Analysis

Study 1—Baseline nociceptive latencies for each rat on the tail withdrawal and paw pressure tests were calculated as the mean of the two pre-injection trials. To adjust for individual differences in baseline latency to respond, response latencies following drug administration were converted to % Maximum Possible Effect (% MPE) in each rat: $[(\text{drug latency} - \text{mean baseline latency}) / (\text{cutoff latency} - \text{mean baseline latency})] \times 100$. Because female rats tend to locomote more than males (Craft, 2008), locomotor activity in drug-treated rats (# photobeam breaks) at each time point was converted to % control activity in the same-sex, vehicle control group, at each time point: $[\# \text{ photobeam breaks in drug-treated rat} / \text{mean } \# \text{ photobeam breaks in same-sex vehicle control group}] \times 100$. Catalepsy scores were dropped before analysis if the rat was noted to be moving its paws and/or displaying continuous sniffing/head movement along the bar during the test. Based on this criterion, the catalepsy score of 1 rat (a vehicle-treated female) was dropped.

Time course data (% MPE tail withdrawal and paw pressure scores, and % control locomotor scores for individual rats) were analyzed using ANOVA, with factors of Sex (2 levels), JWH015 dose (3-4 levels) and Time (3-4 levels, repeated). Catalepsy data (time spent on bar, in s) were analyzed using ANOVA, with factors of Sex (2 levels) and JWH015 dose (4 levels). In the first experiment, Dunnett's post-hoc test was used to compare each dose to vehicle; t-tests with a Bonferroni correction were used for other post-hoc comparisons (e.g., sex differences at single time points). The effects of JWH015 were compared in vehicle- vs. antagonist-pretreated rats by ANOVA, with factors of antagonist (3 levels: vehicle, rimonabant, SR144528), JWH015 dose (2 levels: 10 and 20 mg/kg); Tukey's post-hoc test was used to compare each antagonist to vehicle. Significance was set at $p = 0.05$ for all statistical tests; Greenhouse-Geisser-adjusted F , df and p values are only noted when the assumption of homogeneous variance was not met *and* the adjusted p value was >0.05 (i.e., if the adjusted p value was still 0.05 , the unadjusted F , df and p values are reported).

Study 2—At baseline and at each time point after drug injection, the mean of the 3 trials was calculated for each paw of each rat on the von Frey, Hargreaves and weight-bearing tests. A mean of the two catalepsy scores (taken at 15 and 30 min post-injection) was calculated for each rat, except in cases when the rat was noted to be moving its paws and/or displaying continuous sniffing/head movement along the bar during a test, in which case that catalepsy score was dropped. Based on this criterion, 2 rats (both females: 1 vehicle-treated, 1 treated with 10 mg/kg JWH015) had no usable catalepsy scores so they were not included in the analysis; 5 other rats had 1 usable catalepsy score (2 males treated with 5 mg/kg JWH015, 2 vehicle-treated females and 1 female treated with 5 mg/kg JWH015). For the von Frey and Hargreaves tests, baseline data were compared between males and females, and given the significant sex differences on all measures, vehicle and drug data for the right hindpaw were transformed to percent of the rat's own baseline score at each time point, before analysis of drug effects on the von Frey, Hargreaves and weight-bearing tests. For example, for the von Frey test, % of baseline = $[\text{mechanical threshold in right hindpaw after}$

drug injection, in g / baseline mechanical threshold in right hindpaw, in g] $\times$ 100. Paw thickness was converted to % of baseline paw thickness (baseline = 3 days after CFA, but before JWH015 injection), for each rat at each time point. The time course of drug effects on the von Frey, Hargreaves, weight-bearing and paw thickness tests were then analyzed by ANOVA, with variables Sex (2 levels), JWH015 dose (3 levels), and Time (3-6 levels, repeated). Because there were no interactions of sex with time (data not shown), area-under-the-curve (AUC) values were then calculated using the trapezoidal rule, to summarize the time course data for the von Frey, Hargreaves and weight-bearing tests. AUC data are shown graphically, and were analyzed by ANOVA with variables Sex (2 levels) and JWH015 dose (3 levels). No reduction in paw thickness was observed until the 120-min time point (and the 120-min data were not significantly different from 240-min data); thus, the mean of values at 120 and 240 min post-injection were calculated for each rat, and these means were analyzed by ANOVA, with variables Sex (2 levels) and JWH015 dose (3 levels).

For the antagonism experiment, AUC values were analyzed by ANOVA, with variables Antagonist (vehicle, rimonabant, SR144528), JWH015 dose (2 levels), and Sex (2 levels).

Results

Study 1: JWH015 Effects on Acute Pain and Motor Activity

Figure 1 shows the time course of antinociceptive effects of JWH015 on the tail withdrawal and paw pressure tests. JWH015 produced dose- and time-dependent increases in latency to respond on the tail withdrawal test in females and males, with peak effects at 15-30 min that declined to baseline by 60-120 min post-injection (JWH015 dose $\times$ Time: $F(9,189)=4.18$, $p<0.001$; Fig. 1, left panels). In females, JWH015 produced time-dependent increases in latency to respond (JWH015 dose $\times$ Time: $F(9,96)=4.37$, $p<0.001$); post-hoc tests indicated that these increases were significant at 20 mg/kg ($p=0.014$), but not at 10 mg/kg ($p=0.055$), or at 5 mg/kg. In males, JWH015 produced significant increases in latency to respond (JWH015 dose: $F(1,31)=5.14$, $p<0.005$); post-hoc tests indicated that these increases were significant at 5 mg/kg ($p=0.021$) and 20 mg/kg ($p=0.004$), but not at 10 mg/kg. Although the highest dose of JWH015 tested, 20 mg/kg, produced a greater peak effect in females compared to males, there were no significant sex differences in JWH015 effect. On the paw pressure test (Fig. 1 right panels), JWH015 also produced dose-dependent increases in latency to respond (JWH015 dose: $F(3,63)=10.77$, $p<0.001$) that was not statistically different between females and males. In females, JWH015 produced significant increases in latency to respond ($F(3,32)=7.97$, $p<0.001$); post-hoc tests indicated that these increases were significant at 20 mg/kg only ($p<0.001$). In males, JWH015 produced significant increases in latency to respond ($F(3,31)=4.04$, $p<0.02$); post-hoc tests indicated that these increases were significant at 20 mg/kg only ($p=0.025$).

Figure 2 shows the motoric effects of JWH015, as measured on locomotor activity and catalepsy tests. The left panel shows that JWH015 dose-dependently decreased horizontal locomotion in both sexes; however, drug effects were greater in females than males at some doses and time points (Sex $\times$ JWH015 dose $\times$ Time: $F(4,98)=2.87$, $p<0.05$). Specifically, whereas 5 mg/kg decreased locomotion in females at 30-60 min post-injection, this dose increased or did not alter locomotion in males (Sex $\times$ Time: $F(2,28)=5.69$, $p<0.01$). The 10

mg/kg dose of JWH015 decreased locomotion more in females than males at early time points (Sex $\times$ Time: $F(2,34)=7.13$, $p<0.005$), whereas the highest dose tested, 20 mg/kg, decreased locomotion in both sexes with no statistically significant differences. In contrast to the dose-dependent decreases in behavior on the locomotor activity test, JWH015 produced no significant catalepsy at any dose, in either sex (JWH015 dose: $F(3,63)=1.79$, NS; Fig. 2, right panel).

Figure 3 shows antagonism of JWH015 by rimonabant and SR144528 at peak times of JWH015 effect (15 min post-injection for paw pressure and tail withdrawal; 30 min post-injection for locomotor activity). This experiment was conducted in female rats only. On the tail withdrawal and paw pressure tests, the JWH015 dose-effect curve was shifted to the right or flattened by both rimonabant and SR144528. A statistical comparison of the effects produced by 10-20 mg/kg JWH015 between the vehicle- and antagonist-pretreated groups confirmed that both antagonists significantly reduced JWH015 effects, on the tail withdrawal test (Antagonist: $F(2,28)=6.69$, $p<0.005$; rimonabant vs. vehicle: $p<0.001$; SR144528 vs. vehicle: $p<0.01$) and on the paw pressure test (Antagonist: $F(2,28)=4.12$, $p<0.05$; rimonabant vs. vehicle: $p<0.053$; SR144528 vs. vehicle: $p=0.05$). Rimonabant also antagonized JWH015-induced decreases in locomotor activity, whereas the SR144528-induced rightward shift in the JWH015 dose-effect curve was minimal (Fig. 3, lower panel). A statistical comparison of the effect of 10-20 mg/kg JWH015 between the vehicle- and antagonist-pretreated groups confirmed that only rimonabant significantly blocked JWH015-induced decreases in locomotor activity (Antagonist: $F(2,28)=6.91$, $p<0.005$; rimonabant vs. vehicle: $p=0.002$; SR144528 vs. vehicle: $p=0.27$).

Study 2: JWH015 Effects on Persistent Inflammatory Pain

There were no sex differences in the time course of JWH015 effect on any nociceptive test in Study 2 (no significant Sex $\times$ Time or Sex $\times$ Dose $\times$ Time interactions; data not shown), so drug effects are summarized as AUC values. Figure 4 (upper left panel) shows that i.pl. CFA injection decreased mechanical threshold (von Frey test) similarly in both sexes; that is, there was a significant difference in mechanical threshold between the left, non-CFA-injected paw and the right, CFA-injected paw in vehicle-treated rats (compare first pair vs. second pair of bars over "0") (Paw: $F(1,18)=129.00$, $p<0.001$). When administered 3 days after i.pl. CFA, JWH015 produced dose-dependent increases in mechanical threshold in both sexes (Dose: $F(2,54)=29.06$, $p<0.001$), with greater effects in females compared to males (Sex: $F(1,54)=11.63$, $p<0.001$; upper left panel). Further analysis at each dose showed that males and females differed significantly only at the highest dose tested, 10 mg/kg ($p=0.025$).

Figure 4 (upper right panel) shows that i.pl. CFA injection also shortened latency to respond to noxious heat (Hargreaves test) similarly in both sexes; that is, heat sensitivity was significantly greater in the right, CFA-injected paw than in the left, non-CFA-injected paw (compare first pair vs. second pair of bars over "0") (Paw: $F(1,18)=32.97$, $p<0.001$). JWH015 dose-dependently attenuated CFA-induced heat hypersensitivity, with no sex difference (Dose: $F(2,54)=16.77$, $p<0.001$).

Figure 4 (lower left panel) shows that i.pl. CFA injection also decreased weight-bearing on the CFA-injected paw compared to the non-CFA-injected paw in both sexes (compare first

pair vs. second pair of bars over “0”) (Paw: $F(1,18)=60.60$, $p<0.001$). JWH015 slightly increased weight-bearing on the CFA-injected hindpaw in both sexes, but this effect was not statistically significant (Dose: $F(2,54)=1.34$, NS; Fig. 4). Maximal effects on this test were 25-30% MPE at the time of peak effect (data not shown).

CFA injection also increased paw thickness, an index of edema. The average increase in paw thickness from before to 3 days after CFA injection was approximately 0.63 mm to 1.12 mm in vehicle-treated males, and 0.54 mm to 1.0 mm in vehicle-treated females (data not shown). Figure 4 (lower right panel) shows that JWH015 significantly decreased paw thickness in the CFA-injected paw (Dose: $F(2,54)=14.43$, $p<0.001$). There was no significant main effect of sex or sex by dose interaction on paw thickness. Analysis of the JWH015 effect within sex showed a significant effect in females ($F(2,27)=35.36$, $p<0.001$) but not males ($F(2,27)=3.09$, $p=0.062$).

In this experiment, catalepsy was measured at 15 and 30 min instead of 60 min post-injection, to try to capture peak JWH015 effects on catalepsy; at these earlier time points JWH015 produced some catalepsy, but with no significant sex difference (Dose: $F(2,52)=4.85$, $p<0.02$; data not shown). For example, mean time spent on the bar was 4.1 ± 0.6 vs. 5.5 ± 1.1 s in females vs. males, respectively, at 10 mg/kg.

To determine CB1 and CB2 receptor mediation of JWH015 effect against persistent inflammatory pain, a separate experiment was conducted in which vehicle, rimonabant or SR144528 was administered 30 min before vehicle or 10 mg/kg JWH015. Figure 5 shows that the anti-allodynic effect of JWH015 was antagonized by both rimonabant and SR144528, with no sex difference (Antagonist $\times$ JWH015: $F(2,107)=6.76$, $p<0.002$).

Discussion

This study demonstrates that when administered systemically, the putative CB2-preferring agonist JWH015 produces antinociception in both female and male rats on tests of acute heat and pressure pain. Furthermore, in a model of persistent inflammatory pain, JWH015 attenuates mechanical allodynia, heat hyperalgesia, and edema. However, the antinociceptive effects of JWH015 were mediated not only by CB2 receptors, but also by CB1 receptors, as indicated by antagonism by both rimonabant and SR144528 on the tail withdrawal and paw pressure tests (Study 1, females), and on the von Frey test (Study 2, females and males). JWH015 also dose-dependently decreased locomotor activity but produced only mild catalepsy at doses that produced antinociception. JWH015 produced similar antinociception in females and males, but greater locomotor suppression in females.

The results of the present study agree with those of previous studies demonstrating that CB2-preferring agonists can produce antinociception, extending these findings to females. For example, when administered systemically to male mice, the CB2-preferring agonist AM1241 produced dose-dependent antinociception on hotplate and tail immersion tests (Ibrahim et al., 2006), and another CB2-preferring agonist, JWH133, decreased formalin-induced (phase I) pain-related behavior (Jafari et al., 2007). Intrathecally administered JWH015 decreased mechanical allodynia and/or thermal hyperalgesia that developed after

hindpaw incision (Romero-Sandoval & Eisenach, 2007; Landry et al., 2012) or peripheral nerve injury (Romero-Sandoval et al., 2008) in male rats, and after bone cancer induction in male mice (Gu et al., 2011). Several of these studies confirmed CB2 receptor involvement in JWH015-induced antinociception via AM630 antagonism, but only one study ruled out CB1 receptor involvement (no antagonism by AM281: Romero-Sandoval & Eisenach, 2007). Surprisingly, in the present study both CB2 and CB1 receptor-selective antagonists blocked JWH015-induced antinociception. JWH015 has been estimated to have 10-30 times greater affinity for CB2 than CB1 receptors (Huffman et al., 2005; Pertwee, 2008), and there is little previous evidence that it acts at CB1 receptors. The only study we could find showed that the electrophysiological effects of JWH015 in mouse hippocampal neurons were CB1 receptor-mediated (Murataeva et al., 2012). The CB1 receptor-mediated antinociceptive effects of JWH015 observed in the present study could be due to the fact that we administered the drug systemically rather than i.t., the route used in most previous studies examining receptor mediation of JWH015-induced antinociception (Gu et al., 2011; Landry et al., 2012; Romero-Sandoval et al., 2008; Romero-Sandoval & Eisenach, 2007). Systemically administered drugs may act at supraspinal, spinal and peripheral sites, all of which are known to be involved in cannabinoid antinociception, and all of which contain CB1 receptors (Tsou et al., 1998; Walker & Huang, 2002).

Significantly greater JWH015 effects were observed in females compared to males on the von Frey test of mechanical allodynia, in the persistent inflammatory pain model (Study 2). We and others have reported that non-selective cannabinoid agonists such as THC and CP55,940 are more potent in producing antinociception in female than in male rats (Tseng & Craft, 2001; Romero et al., 2002; Craft et al., 2012; Wakley et al., 2014a), including in this inflammatory pain model (Craft et al., 2013). However, in the present study, sex differences in JWH015-induced antinociception were observed only on one test, and only at 10 mg/kg. This dose also suppressed locomotor activity more in females than males (Study 1), so it is possible that the sex difference in the anti-allodynic effect of JWH015 is due to sex differences in its sedative effect. Given that the locomotor-suppressant effects of JWH015 were CB1 (and not CB2) receptor-mediated, the present results further suggest that sex differences in cannabinoid antinociception may be more likely to be observed with agonists that act at CB1 receptors, when drugs are administered systemically.

Sex differences in brain cannabinoid receptor mRNA or protein have been examined in a few studies, but appear to be highly region-dependent, and may also depend on the hormonal state of females (Castelli et al., 2014; Rodriguez de Fonseca et al., 1994; Xing et al., 2011). In particular, greater cannabinoid receptor mRNA and protein has been found in female Sprague-Dawley rats compared to males in cerebellum (CB1), and in brainstem (CB2) (Xing et al., 2011); cannabinoid actions in these brain areas may contribute to their motoric and antinociceptive effects, respectively. We are not aware of any published studies comparing spinal or peripheral cannabinoid receptor density or function in female vs. male rats, other than one study demonstrating greater CB1 receptor mRNA in the trigeminal ganglia of males compared to females when masseter muscle inflammation was induced by local CFA injection (Niu et al., 2012). Thus, it remains to be determined to what extent sex differences in cannabinoid antinociception after systemic drug administration are explained by sex

differences in CB1 and/or CB2 receptor pharmacology at supraspinal vs. spinal vs. peripheral levels.

We are aware of three published studies comparing cannabinoid analgesia in women vs. men. In one study, nabilone decreased temporal summation of heat hyperalgesia in healthy, non-cannabis-using women but not men (Redmond et al., 2008). In another study, conducted in healthy, daily cannabis users, dronabinol and smoked marijuana produced similar analgesic effects in women and men on the cold pressor test (Cooper et al., 2013). A subsequent retrospective analysis revealed greater analgesia from smoked cannabis in men compared to women daily cannabis users (Cooper & Haney, 2016). The extent to which participants were drug-tolerant in the latter two studies is not known; we have shown that female rats are more susceptible than males to the development of tolerance to THC-induced antinociception (Wakley et al., 2014b), suggesting that sex differences in cannabinoid potency and efficacy can change with repeated use. Clearly, additional studies are needed to determine whether sex differences observed in rodent studies of cannabinoid antinociception are reliable and translatable to humans, and to determine whether results from human laboratory studies generalize to clinical pain populations, so as to clarify whether the analgesic potency and efficacy of cannabinoids – particularly CB2-preferring agonists – are the same in women and men.

Conclusion

Unlike non-selective cannabinoid agonists such as THC, which tend to be more potent in producing antinociception in female compared to male rats, the CB2-preferring agonist JWH015 produced similar antinociception in female and male rats on tests of acute pain and persistent inflammatory pain, despite being more potent in suppressing locomotor activity in females than in males. JWH015-induced antinociception was CB1- and CB2-receptor-mediated, whereas its locomotor suppressant effect was CB1-receptor-mediated. Future studies examining a wider variety of cannabinoid agonists will be needed to determine whether sex differences in cannabinoid antinociception are consistent across drugs in this class, and whether they reflect sex differences in CB1 and/or CB2 receptor pharmacology.

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Figure 1. Time course of i.p. JWH015 effect on acute pain tests in female rats (upper panels) and males (lower panels). Each point is the mean $\pm$ S.E.M. of 8-10 rats. *Significantly different from same-sex vehicle group at same time point.

Figure 2. Time course of i.p. JWH015 effect on locomotor activity in female (F) and male (M) rats (left panel), and JWH015-induced catalepsy measured at 60 min post-injection (right panel). Each point/bar is the mean $\pm$ S.E.M. of 8-10 rats. #Significant sex difference at a single dose, $p < 0.05$.

Figure 3. Dose-effect curves for i.p. JWH015 alone and in combination with the CB1 antagonist rimonabant (0.1 mg/kg i.p.) or the CB2 antagonist SR144528 (3.2 mg/kg i.p.), on two acute pain tests (upper panels), and locomotor activity (lower panel), in female rats. Curves were constructed from time course data, using data at 15 min post-injection (paw pressure and tail withdrawal) or 30 min post-injection (locomotor activity). Each point is the mean $\pm$ S.E.M. of 5-7 rats.

Figure 4. JWH015-induced antinociception against persistent inflammatory pain. Three days after i.pl. CFA injection, vehicle or JWH015 (5 or 10 mg/kg) was injected i.p., and rats were tested on the von Frey test of mechanical allodynia (upper left panel), the Hargreaves test of thermal hyperalgesia (upper right panel), biased weight-bearing (lower left panel) and thickness of the CFA-injected paw, an index of edema (lower right panel). Area-under-the-curve values were calculated from time-effect determinations (see Methods). The first pair of bars over the “0” dose in each panel is the response in vehicle-treated rats' non-CFA (left) hindpaw, whereas the second pair of bars in each panel is the response in vehicle-treated rats' CFA-injected (right) hindpaw. The bars above “5” and “10” are the response in JWH015-treated rats' CFA-injected (right) hindpaw. Each bar is the mean + S.E.M. of 10 rats. +Significant effect of CFA in vehicle (0)-treated rats; *Significant effect of JWH015 compared to same-sex, vehicle-treated rats (second pair of bars over “0”); #Significant sex difference compared to males treated with same JWH015 dose, $p < 0.05$.

Figure 5.

CB1 vs. CB2 receptor mediation of JWH015's anti-allodynic effect against persistent inflammatory pain. Three days after i.p. hindpaw CFA injection, vehicle, rimonabant (1 mg/kg) or SR144528 (1 mg/kg) was injected i.p.; 30 min later vehicle or JWH015 (10 mg/kg) was injected i.p., and rats were tested for mechanical allodynia. Each point is the mean + S.E.M. of 8 rats/sex (vehicle or antagonist alone) or 12 rats/sex (JWH015-treated groups). *Significantly different from same-sex, vehicle+vehicle group; +Significant antagonism compared to same-sex, vehicle+JWH015 group, $p < 0.05$.