Sex Differences in Cannabinoid 1 vs. Cannabinoid 2 Receptor-Selective Antagonism of Antinociception Produced by $\Delta^9$-Tetrahydrocannabinol and CP55,940 in the Rat

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ABSTRACT

The purpose of this study was to determine whether sex differences in cannabinoid (CB)-induced antinociception and motoric effects can be attributed to differential activation of CB$_1$ or CB$_2$ receptors. Rats were injected intraperitoneally with vehicle, rimonabant [5-[(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-1-piperidinyl]-1H-pyrazole-3-carboxamide (SR141716A), a putative CB$_1$ receptor-selective antagonist; 0.1–10 mg/kg] or 5-(1,1-dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]phenol (CP55,940) (0.05–1.6 mg/kg). Thirty minutes later, $\Delta^9$-tetrahydrocannabinol (THC; 1.25–40 mg/kg) or 5-(1,1-dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]phenol (CP55,940) (0.05–1.6 mg/kg) was injected. Paw pressure and tail withdrawal antinociception, locomotor activity, and catalepsy were measured. Rimonabant dose-dependently antagonized THC and CP55,940 in each test, but was up to 10 times more potent in female than male rats on the nociceptive tests; estimates of rimonabant affinity (apparent pK$_{B}$) for the CB$_1$ receptor were approximately 0.5 to 1 mol/kg higher in female than male rats. SR144528 partially antagonized THC-induced tail withdrawal antinociception and locomotor activity in females, but this antagonism was not dose-dependent or consistent; no SR144528 antagonism was observed in either sex tested with CP55,940. Neither the time course of rimonabant antagonism nor the plasma levels of rimonabant differed between the sexes. Rimonabant and SR144528 did not antagonize morphine-induced antinociception, and naloxone did not antagonize THC-induced antinociception in either sex. These results suggest that THC produces acute antinociceptive and motoric effects via activation of CB$_1$, and perhaps under some conditions, CB$_2$ receptors, in female rats, whereas THC acts primarily at CB$_2$ receptors in male rats. Higher apparent pK$_{B}$ for rimonabant in female rats suggests that cannabinoid drugs bind with greater affinity to CB$_1$ receptors in female than male rats, probably contributing to greater antinociceptive effects observed in female compared with male rats.

Introduction

Sex differences in a variety of cannabinoid (CB) effects have been demonstrated in animals. For example, cannabinoids such as $\Delta^9$-tetrahydrocannabinol (THC) are more potent in female than male rodents in producing antinociception (Tseng and Craft, 2001; Romero et al., 2002), hypothermia (Borgen et al., 1973; Wiley et al., 2007), and motoric effects (Cohn et al, 1972; Tseng and Craft, 2001). Female rats also acquire cannabinoid self-administration faster than males and maintain higher rates of responding (Fattore et al., 2007). In contrast, male rodents are more sensitive than females to the hyperphagic effect of cannabinoid agonists (Diaz et al., 2009). Sex differences in cannabinoid effects also have been demonstrated in humans. For example, dronabinol (synthetic THC) retarded gastric emptying to a greater extent in women than men (Esfandyari et al., 2006), and women reported more dizziness than men after cannabinoid intake (Mathew et al., 2003). In contrast, men reported greater ratings of “high” as well as some other subjective effects after cannabinoid intake (Haney, 2007). Sex differences in cannabinoid analgesia have not yet been examined in humans, but a growing number of controlled clinical studies demonstrates that chronic pain is alleviated by cannabinoids (Russo, 2008).

Cannabinoids are known to produce antinociception via supraspinal, spinal, and peripheral mechanisms and by acting at CB$_1$ and CB$_2$ receptors (Anand et al., 2009). It is not known which cannabinoid receptor subtype(s) mediate antinociception in females, and it is possible that cannabinoid antinociceptive effects are mediated by CB$_1$ receptors in female than male rats, probably contributing to greater antinociceptive effects observed in female compared with male rats.

ABBREVIATIONS: CB, cannabinoid; THC, tetrahydrocannabinol; ANOVA, analysis of variance; %MPE, percentage of maximum possible effect; CP55,940, 5-(1,1-dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]phenol; SR141716A (rimonabant), 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3-carboxamide; SR144528, 5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-N-[(1S,2S,4R)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl]-1H-pyrazole-3-carboxamide; n.s., not significant; C.I., confidence interval.
of these mechanisms contribute to greater cannabinoid antinociception in females compared with males. Antinociception produced by systemically administered THC can be attenuated by the CB1 receptor-selective antagonist rimonabant [5-(4-chloro-3-methylphenyl)-1-(4-methylphenyl)methyl-N-[(1S,2S,4R)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl]-1H-pyrazole-3-carboxamide (SR144528), also shown to be an inverse agonist], administered either intracerebroventriculally or intrathecally, in both male and female rats (Tseng and Craft, 2004), demonstrating supraspinal and spinal CB2 receptor involvement in both sexes. In male rodents, antinociception produced by CB2 receptor activation can occur spinaly and peripherally (Gutierrez et al., 2007; Romero-Sandoval and Eisenach, 2007). Although antinociception via supraspinal CB2 receptor activation has yet to be demonstrated, CB2 receptors have been detected in pain-relevant brain areas such as the periaqueductal gray, albeit at a lower density than CB1 receptors (Gong et al., 2006).

The purpose of the present study was to test the hypothesis that sex differences in cannabinoid antinociception are mediated by both CB1 and CB2 receptors. Gonadally intact male and female rats were pretreated with the putative CB1 or CB2 receptor-selective antagonist rimonabant or 5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-N-[(1S,2S,4R)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl]-1H-pyrazole-3-carboxamide (SR144528), respectively (Shire et al., 1999), in combination with vehicle or a cannabinoid agonist, and then tested over a 4-h period on two nociceptive and two motor activity assays. Time-course analyses were conducted for the primary psychoactive compound in cannabis, THC, and the synthetic cannabinoid agonist 5-(1,1-dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]phenol (CP55,940). Both of these cannabinoids are generally characterized as non-selective, mixed CB1/CB2 agonists; however, CP55,940 has significantly more efficacy than THC at CB1 receptors (Govaerts et al., 2004). The acute antinociceptive effects of both agonists seem to be mediated by CB1 receptors in male rats (Lichtman and Martin, 1997) and mice (Varvel et al., 2005). To investigate a pharmacodynamic mechanism underlying sex differences in agonist/antagonist interaction in the present study, complete agonist dose-effect curves were obtained, alone and in combination with the most effective doses of each antagonist. In vivo apparent \( p[K] \) values (Negus et al., 1993; Rowlett and Woolverton, 1996) were calculated from these curves to test the hypothesis that sex differences in behavioral effects of cannabinoid drugs are caused by sex differences in the affinity of cannabinoid drugs for cannabinoid receptors. The time course of antagonist effect as well as plasma levels of antagonist were examined to determine whether sex differences in antagonism of cannabinoid agonist-induced behaviors could be attributed to sex differences in agonist duration of action or absorption. Finally, potential sex differences in the actions of THC at opioid receptors (naloxone antagonism), and in the actions of morphine at cannabinoid receptors, were examined to determine the specificity of sex differences in cannabinoid pharmacology.

Materials and Methods

Subjects. Adult (60–85 days old) male and female Sprague-Dawley rats were used (bred in-house from stock from Taconic Farms, Germantown, NY). Rats were housed under a 12-h light/dark cycle (lights on at 6:00 AM), in a room maintained at 21 ± 2°C. Rats were treated in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996). Rats were assigned randomly to treatment groups, with the exception that we avoided assigning same-sex siblings to any group that had six or fewer rats. Each rat was tested with a single drug combination.

Apparatus. Tail withdrawal antinociception was assessed by using a 2.5-liter water bath (Precision Scientific, Winchester, VA) set to 50 ± 0.5°C; latency to withdraw the tail was timed with a hand-held stopwatch, and cutoff was 12 s. Paw pressure antinociception was assessed by 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 cutoff). Horizontal locomotor activity was measured by using a photobeam apparatus (Opto-vaximex; Columbus Instruments, Columbus, OH): 15 photobeams crossed the width of a 20 × 40 × 23-cm clear Plexiglas rodent cage, with photobeams spaced 2.5 cm apart and 8 cm above the cage floor. Catalepsy was measured by using a bar test: a ring stand with a 1.5-cm diameter horizontal bar set at 13 cm (for females) or 15.5 cm (for males) above the countertop.

Drugs. Rimonabant, SR144528, THC, and CP55,940 (National Institute on Drug Abuse, Bethesda, MD) were dissolved in a 1:1:18 ethanol/cremophor/saline solution, which served as the vehicle. Naloxone hydrochloride and morphine sulfate (Sigma-Aldrich, St. Louis, MO) were dissolved in physiological saline. All cannabinoid drugs were administered intraperitoneally, and opioids were administered subcutaneously, all in volumes of 1 ml/kg, except for the highest doses of the cannabinoid agonists (20–40 mg/kg THC and 0.4–1.6 mg/kg CP55,940), which were administered in larger volumes (2–8 ml/kg) because of solubility limitations. The 8 ml/kg volume of the 1:1:18 vehicle was tested in two female and two male rats to determine whether the greater amount of ethanol (approximately 0.35 g/kg) would produce significant antinociceptive or motoric effects on any of the four tests, and it did not (data not shown). Finally, two different batches of SR144528 were used during the course of these experiments, and the older batch (kept more than 10 years in a freezer) used in initial experiments partially antagonized THC on some measures in female rats, whereas the newer batch did not. We hypothesized that the older batch had degraded such that it was no longer CB2 receptor-selective; however, analysis of the two batches showed that they were identical and purely SR144528 (Dr. H. Seltzman, Research Triangle Institute, Research Triangle Park, NC, personal communication).

Behavioral Procedure. Baseline nociception was measured by testing each rat on the tail withdrawal and paw pressure tests, in that order, twice. After baseline testing, vehicle or a single dose of rimonabant (0.1–10 mg/kg), SR144528 (1.0–10 mg/kg), or naloxone (1.0 mg/kg) was injected. In most cases, 30 min later (rimonabant, SR144528 pretreatment) or 5 min later (naloxone pretreatment), vehicle or agonist (THC, CP55,940, morphine) was injected. In most experiments, rats were then tested on tail withdrawal and paw pressure tests at 15, 30, 60, 120, and 240 min postinjection. For the tail withdrawal assay, 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 hindpaw 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 photobeam breaks in a 5-min period, beginning immediately after nociceptive testing at 30, 60, 120, and 240 min postinjection. Catalepsy was assessed immediately after the locomotor test, at the 60-min time point only. Latency to withdraw both forepaws from the bar or jump onto the bar was recorded; if the rat did not respond by 12 s, the test was terminated and 12 s was recorded. If a rat moved its forepaws across the bar (approximately 1% of all rats tested), it was not considered cataleptic and its score was dropped from the dataset before analysis. Rats were returned to their home cages between testing periods.
Exceptions to the above method were: 1) morphine dose-effect curves were obtained by using a cumulative dosing procedure with 15-min injection/test intervals; and 2) for the antagonist time-course determination, vehicle, rimonabant, or SR144528 was injected 5, 30, 60, or 90 min before THC.

The experimenter who collected approximately 50% of the THC data (J.D.L.) was blinded to treatment assignment: A.A.W. prepared drugs and filled syringes, which were labeled only with a rat number, and J.D.L. injected and tested rats. Most other testing was conducted without blinding. THC data collected by J.D.L. and A.A.W. were not significantly different, so those data were pooled.

**Determination of Plasma Rimonabant Levels.** To determine whether sex differences in rimonabant antagonism could be caused by sex differences in drug absorption, a separate group of female and male rats was injected with 1.0 mg/kg rimonabant; rats were euthanized 60 min postinjection, and plasma levels of rimonabant were determined by high-performance liquid chromatography. The method of Hurtado et al. (2010) was followed, with minor changes: a mobile phase of 75% acetonitrile and 25% water (v/v) was used, with a flow rate of 1.0 ml/min; pterostilbene was used as the internal standard.

**Results**

**Sex Differences in Antagonism of Cannabinoid Antinociception.**

**Sex Differences in Baseline Measurements.** Sex differences in baseline latencies to respond on the nociceptive tests were examined in rats in the cannabinoid antagonist + THC time-course experiment, because this was the largest dataset. Nociceptive latencies were significantly shorter in females than males: 3.73 ± 0.08 versus 4.09 ± 0.09 s in females versus males, respectively, on the tail withdrawal test (t_{306} = −2.87; p = 0.004) and 3.90 ± 0.09 versus 4.90 ± 0.11 s, respectively, on the paw pressure test (t_{306} = −7.15; p < 0.001). On the locomotor activity test, vehicle-treated females were slightly, but not significantly, more active than vehicle-treated males (F_{1,26} = 2.25; p = 0.15; Table 1). On the catalepsy test, there were no sex differences in vehicle-treated rats: mean latency to remove both paws from the bar (or jump up on the bar) was approximately 1.0 to 1.5 s in both sexes (see Fig. 2, right).

**Sex Differences in Behavioral Effects of THC.** Figure 1 shows time-effect curves for THC alone (vehicle + THC) in female versus male rats on the two nociceptive tests. THC produced dose- and time-dependent antinociception in both sexes on both tests, but it was more potent in females than males. For example, whereas 10 mg/kg THC produced near-maximal paw pressure antinociception in females, 20 mg/kg was required to produce a similar effect in males (Fig. 1, left).

Statistical comparison of the THC doses that were tested in both sexes (1.25–10 mg/kg) yielded a significant sex difference on the paw pressure test (F_{1,108} = 6.27; p = 0.014) and the tail withdrawal test (F_{1,108} = 29.58; p < 0.001). THC’s antinociceptive effects generally peaked at 30 to 60 min postinjection in both sexes on both tests. Estrous stage accounted for a significant portion of the variance in response to THC on the tail withdrawal but not the paw pressure test, with females in estrus showing the greatest THC-induced antinociception (F_{1,95} = 5.06; p = 0.027; data not shown).

Figure 2 shows THC-induced suppression of locomotor activity (left) and catalepsy (right). Statistical comparison of the THC doses that were tested in both sexes (1.25–10 mg/kg) indicated greater locomotor suppression in female than male rats at some doses and time points (e.g., at 5 mg/kg, 120–240 min postinjection; sex × time × THC dose: F_{9,244} = 2.62; p = 0.007), and greater catalepsy in females at 5 and 10 mg/kg (sex × THC dose: F_{4,108} = 4.45; p = 0.002). Estrous stage did not significantly influence females’ response to THC on tests of motor activity (data not shown).
CB₁ versus CB₂ Antagonism of THC-Induced Antinociception and Sedation: Time-Effect Curves. Figure 3 shows antagonism of 5 mg/kg THC on the paw pressure test, by rimonabant (top) and SR144528 (bottom), in female versus male rats. Rimonabant dose-dependently antagonized THC-induced antinociception in both sexes (females: $F_{3,37} = 7.99, p < 0.001$; males: $F_{3,34} = 5.21, p = 0.005$); however, nearly complete antagonism was observed at 1.0 mg/kg in females versus 10 mg/kg in males. SR144528 (1–10 mg/kg) did not antagonize THC-induced paw pressure antinociception in males ($F_{3,37} = 0.44$; n.s.); in females, antagonism by SR144528 was not statistically significant ($F_{3,38} = 2.59; p = 0.07$).

Figure 4 shows antagonism of 5 mg/kg THC by rimonabant and SR144528 on the tail withdrawal test in female versus male rats. Rimonabant dose-dependently antagonized THC-induced tail withdrawal antinociception in females at relatively low doses ($F_{3,37} = 17.19; p < 0.001$), with nearly complete antagonism at 0.32 mg/kg. In males, antagonism was also dose-dependent and essentially complete at 3.2 mg/kg ($F_{3,34} = 3.12; p = 0.039$). SR144528 partially antagonized THC-induced tail withdrawal antinociception in fe-
males ($F_{3,38} = 3.63; p = 0.021$), but not in males ($F_{3,37} = 0.59$; n.s.).

Figure 5 shows antagonism of 5 mg/kg THC-induced suppression of locomotor activity. Rimonabant antagonized THC-induced decreases in locomotor activity in both sexes; however, the potency and time course of antagonism differed between the sexes. In female rats, 0.1 to 1.0 mg/kg rimonabant dose-dependently antagonized THC’s effect ($F_{3,37} = 7.02; p = 0.001$), and there was no dose by time interaction. In males, rimonabant antagonized THC’s locomotor-suppressant effect only at 30 to 60 min post-THC injection (rimonabant dose $\times$ time: $F_{9,102} = 2.64; p = 0.009$). SR144528 attenuated THC’s locomotor-suppressant effect in females ($F_{3,38} = 3.99; p = 0.015$) but only partially and only at the intermediate dose, 3.2 mg/kg. In contrast, SR144528 tended to exacerbate THC-induced locomotor suppression in males ($F_{3,37} = 3.08; p = 0.039$).
Figure 6 shows antagonism of catalepsy produced by 5 mg/kg THC, which was measured 60 min post-THC injection. In female rats, 0.1 to 10 mg/kg rimonabant dose-dependently antagonized THC-induced catalepsy ($F_{4,40} = 6.73; p < 0.001$). In males, rimonabant antagonism was not significant ($F_{3,34} = 0.67$; n.s.). SR144528 (1.0–10 mg/kg) did not antagonize THC-induced catalepsy in rats of either sex (Fig. 6).

CB1 Antagonism of THC-Induced Antinociception and Sedation: THC Dose-Effect Curves. To better quantify apparent sex differences in CB1 receptor-selective antagonism of THC’s behavioral effects, doses of THC higher and lower than 5 mg/kg were examined in combination with the antagonist doses that were the most effective in each sex (determined from the first antagonist experiment; Fig. 3). Agonist-antagonist interactions were graphed and statistically compared only at the time of peak agonist/antagonist effect (i.e., 30–60 min post-THC injection; see Materials and Methods). Figure 7 shows THC dose-effect curves alone and in combination with two doses of rimonabant on the two nociceptive tests. The ED$_{50}$ values derived from each dose-effect curve and relative potencies of antagonist + THC versus vehicle + THC are shown in Table 2. In female rats, 0.32
and 1.0 mg/kg rimonabant shifted the THC dose-effect curve to the right on both nociceptive tests (Fig. 7), with 1.0 mg/kg increasing the ED50 for THC approximately 6- to 7-fold (Table 2). In males, 1.0 and 10 mg/kg rimonabant shifted the THC dose-effect curve farther to the right, to nearly the same extent that 1.0 mg/kg did in females (Fig. 7; Table 2).

Figure 8 shows THC dose-effect curves on the two tests of motor function, alone and in combination with the two doses of rimonabant in each sex. On the locomotor activity test, the THC dose-effect curve was shifted rightward by rimonabant (0.32 and 1.0 mg/kg in female rats; 1.0 and 10 mg/kg in male rats). The magnitude of the shift produced by 1.0 mg/kg rimonabant did not differ between females and males (see potency ratios in Table 2). On the catalepsy test, rimonabant produced dose-dependent rightward shifts in the THC dose-effect curve that looked very similar to those observed on the nociceptive tests: whereas 1.0 mg/kg rimonabant produced a 4.5-fold increase the THC ED50 in females, it produced essentially no change in THC potency in males (Fig. 8). Although ED50 values for THC could not be calculated for the catalepsy measure in males because of the shallowness of the dose-effect curve, the higher dose of rimonabant tested in males, 10 mg/kg, seemed to shift the THC dose effect to the right (Fig. 8).

Apparent pK_B values, calculated in all cases in which potency ratios could be calculated, were higher in female than in male rats. The apparent pK_B values for rimonabant calculated from paw pressure data were 6.40 to 6.58 mol/kg (1.0 and 0.32 mg/kg rimonabant, respectively) in females versus 5.31 to 5.98 mol/kg (10 and 1.0 mg/kg rimonabant, respectively) in males. A similar sex difference was obtained from tail withdrawal data: apparent pK_B values were 6.44 to 6.70 mol/kg in females versus 5.92 mol/kg in males. In contrast, sex differences in apparent pK_B values were smaller using locomotor activity and catalepsy data: pK_B estimates ranged from 6.08 to 6.32 versus 5.80 mol/kg in females versus males, respectively.
CB1 versus CB2 Antagonism of CP55,940-Induced Antinociception and Sedation. We next compared the ability of rimonabant and SR144528 to antagonize the antinociceptive and motoric effects of another mixed CB1/CB2 (but higher potency and efficacy) cannabinoid agonist, CP55,940. Similar to THCa, CP55,940’s antinociceptive effects peaked at approximately 30 to 60 min postinjection in both sexes on both tests (data not shown). Figure 9 shows antagonism of CP55,940-induced antinociception by 1.0 and 10 mg/kg rimonabant in female versus male rats. The \( ED_{50} \) values derived from each dose-effect curve and potency ratios of antagonist + CP55,940 versus vehicle + CP55,940 are shown in Table 3. On the paw pressure test (Fig. 9, left), rimonabant shifted the CP55,940 dose-effect curve to the right in both sexes; however, the rightward shifts were greater in females than males (see Table 3). Rimonabant also tended to flatten the CP55,940 curves in females but not males. Similar effects were observed on the tail withdrawal test (Fig. 9, right): rimonabant shifted the CP55,940 dose-effect curve approximately 4- and 12-fold to

### Table 2

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<th>( ED_{50} ) (mg/kg)</th>
<th>Potency Ratio ( \alpha )</th>
<th>( ED_{50} ) (mg/kg)</th>
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<td>6.87 (4.55, 10.37)</td>
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- Relative potency of antagonist + agonist vs. vehicle + agonist.
- \( ED_{50} \) could not be estimated (mean maximum effect ≤ 35%MPE at highest dose tested).
- Slopes significantly different, \( P < 0.05 \), so potency ratio could not be estimated.
- Significant sex difference (mean for males lies outside of the C.I. around the mean for females).
- \* Significant sex difference (mean for males lies outside of the C.I. around the mean for females).

Fig. 8. Rightward shifts of THC dose-effect curve produced by the CB1 receptor-selective antagonist rimonabant on the locomotor activity (left) and catalepsy tests (right) in female (top) versus male (bottom) rats. Vehicle or a single dose of antagonist was administered 30 min before THC; THC dose-effect curves were constructed from data obtained at 30 to 60 min (locomotion) or 60 min (catalepsy) after THC injection. Locomotor data are presented as the percentage of same-sex, vehicle-treated controls; the mean number of photobeam breaks in vehicle-treated rats was 532 for females and 452 for males. Points above V are the effects of vehicle or antagonist administered alone (in combination with vehicle). Each point is the mean ± 1 S.E.M. (n = 8–12 rats).
the right in males (Table 3), whereas rimonabant flattened the CP55,940 curve in females such that ED50 values could not be calculated (slopes of CP55,940 curves were significantly different between vehicle- and rimonabant-treated females; p < 0.05). SR144528 did not shift the CP55,940 curves to the right in either sex on either nociceptive test (data not shown).

Figure 10 shows antagonism of CP55,940 by rimonabant on the two tests of motor function. The 1.0 and 10 mg/kg doses of rimonabant produced dose-dependent rightward shifts in the CP55,940 dose-effect curves in both sexes, and the shifts were comparable in magnitude (see Table 3). On the catalepsy test, rimonabant seemed to shift the CP55,940 curve farther to the right in female than male rats (Fig. 10), but the potency ratios could not be compared quantitatively because of the low efficacy of CP55,940 in males (and given the limitations on dose ranges that could be tested because of drug insolubility). Estrous stage did not significantly influence females’ responses to CP55,940 on any of the four behavioral tests (data not shown).

APPARENT pK
B values, calculated in all cases in which potency ratios could be calculated, tended to be higher in female than in male rats. For example, the apparent pK
B for rimonabant (1.0 mg/kg dose) on the paw pressure test was 6.02 versus 5.46 mol/kg in females versus males, respectively. In contrast, sex differences in apparent pK
B were very small on

TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>Female Rats</th>
<th>Male Rats</th>
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<tbody>
<tr>
<td></td>
<td>ED50 (mg/kg)</td>
<td>Potency Ratioα</td>
</tr>
<tr>
<td>Vehicle + CP55,940</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paw pressure</td>
<td>0.09 (0.07, 0.11)</td>
<td>0.12 (0.06, 0.18)*</td>
</tr>
<tr>
<td>Tail withdrawal</td>
<td>0.13 (0.09, 0.24)</td>
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<tr>
<td>Locomotor</td>
<td>0.10 (0.04, 0.20)</td>
<td></td>
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<tr>
<td>Catalepsy</td>
<td>0.19 (0.15, 0.49)</td>
<td></td>
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<tr>
<td>Rimonabant 1.0 + CP55,940</td>
<td></td>
<td></td>
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<tr>
<td>Paw pressure</td>
<td>0.32 (0.18, 4.05)</td>
<td>3.26 (1.95, 5.45)</td>
</tr>
<tr>
<td>Tail withdrawal</td>
<td>–b</td>
<td>–c</td>
</tr>
<tr>
<td>Locomotor</td>
<td>0.40 (0.17, 1.00)</td>
<td>3.74 (1.34, 10.47)</td>
</tr>
<tr>
<td>Catalepsy</td>
<td>0.94 (0.64, 1.92)</td>
<td>3.49 (2.00, 6.06)</td>
</tr>
<tr>
<td>Rimonabant 10 + CP55,940</td>
<td></td>
<td></td>
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<tr>
<td>Paw pressure</td>
<td>0.87 (0.51, 9.14)</td>
<td>8.48 (4.96, 14.49)</td>
</tr>
<tr>
<td>Tail withdrawal</td>
<td>–b</td>
<td>–c</td>
</tr>
<tr>
<td>Locomotor</td>
<td>0.80 (0.35, 59.70)</td>
<td>7.36 (2.58, 20.99)</td>
</tr>
<tr>
<td>Catalepsy</td>
<td>–b</td>
<td>–c</td>
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</table>

α Relative potency of antagonist + agonist vs. vehicle + agonist.

β ED50 could not be estimated (mean maximum effect <35%MPE at highest dose tested).

γ Slopes significantly different, P < 0.05, so potency ratio could not be estimated.

* Significant sex difference (mean for males lies outside of the C.I. around the mean for females).
the locomotor and catalepsy tests: \( pK_B \) estimates (1.0 mg/kg rimonabant dose) were 6.06 to 6.10 versus 5.92 to 5.98 mol/kg in females versus males, respectively.

**Time Course of Rimonabant and SR144528 Antagonism.** To test whether sex differences in the antagonism of THC could be caused by a different time course of antagonist effect in female versus male rats, separate rats were pretreated with vehicle, rimonabant (1.0 mg/kg), or SR144528 (3.2 mg/kg) either 5, 30, 60, or 90 min before THC (5 mg/kg) was administered. Figures 11 and 12 show the time course of antagonism of the antinociceptive and motoric effects of THC, respectively, at the time of peak agonist effect (30–60 min after THC injection; see Materials and Methods). There were no significant sex differences in the time course of antagonism of THC-induced antinociception (Fig. 11; sex \( \times \) antagonist \( \times \) pretreatment time: \( F_{6,155} = 0.95 \) (paw pressure), \( F_{6,155} = 1.43 \) (tail withdrawal); n.s.). In both sexes, rimonabant was maximally effective when given 5 to 30 min before THC.
before THC, that is, when antinociceptive effects were assessed 35 to 90 min after antagonist administration, and was clearly waning by the 90-min pretreatment time (i.e., when behavioral effects were assessed 120–150 min after antagonist administration). The time course of rimonabant antagonism of THC’s motoric effects was very similar, although again, antagonism of catalepsy was not statistically significant in males. SR144528 did not significantly antagonize any behavioral effect of THC at any pretreatment time in either sex.

Plasma Levels of Rimonabant. When measured at 60 min postinjection, there were no sex differences in plasma levels of rimonabant: mean \( \pm \) S.E.M. levels were 4.40 \( \pm \) 0.50 µg/ml in female rats versus 4.91 \( \pm \) 0.47 µg/ml in males (t \(_{18} = 0.79\); n.s.).

Effects of CB\(_1\) and CB\(_2\) Antagonists Alone. Neither rimonabant nor SR144528 given alone (in combination with vehicle) produced antinociception or hyperalgesia on the paw pressure or tail withdrawal tests, and neither antagonist produced catalepsy in either sex (see points over V on Figs. 7 and 8). However, Table 1 shows that both antagonists decreased locomotor activity to some extent, particularly in male rats. Rimonabant decreased locomotor activity more in males than females (F \(_{1,43} = 4.96\); p = 0.031); this sex difference was significant at the highest antagonist dose tested, 10 mg/kg (p = 0.024). SR144528 also decreased locomotor activity more in males than females (F \(_{1,53} = 10.67\); p = 0.002); this sex difference was significant at 1.0 mg/kg (p = 0.024) and 3.2 mg/kg (p = 0.039). The fact that both antagonists decreased locomotor activity to some extent when given alone would presumably interfere with their ability to antagonize the locomotor-s suppressant effects of the cannabinoid agonists.

**CB\(_1\) Antagonism of Morphine-Induced Antinociception.** Greater potency of rimonabant in female than male rats was not expected. Therefore, rimonabant was examined in combination with the \( \mu \)-opioid agonist morphine to test whether sex differences in cannabinoid antagonism were specific to the cannabinoid system. Table 4 shows that morphine by itself was more potent in males than females on the paw pressure and tail withdrawal tests. Rimonabant (1.0 mg/kg) failed to significantly antagonize morphine-induced antinociception on either the tail withdrawal or paw pressure tests in either sex (Table 4). SR144528 (3.2 mg/kg) also failed to

### Table 4

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<tr>
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<th>Female Rats</th>
<th>Male Rats</th>
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<tbody>
<tr>
<td></td>
<td>ED(_{50})</td>
<td>Potency Ratio(^a)</td>
</tr>
<tr>
<td></td>
<td>mg/kg</td>
<td></td>
</tr>
<tr>
<td>Vehicle + morphine</td>
<td></td>
<td></td>
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<tr>
<td>Paw pressure</td>
<td>7.80 (6.34, 9.66)</td>
<td></td>
</tr>
<tr>
<td>Tail withdrawal</td>
<td>6.58 (5.40, 8.15)</td>
<td></td>
</tr>
<tr>
<td>Rimonabant 1.0 + morphine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paw pressure</td>
<td>6.08 (5.13, 7.26)</td>
<td>0.77 (0.60, 1.00)</td>
</tr>
<tr>
<td>Tail withdrawal</td>
<td>5.93 (5.15, 6.85)</td>
<td>0.91 (0.72, 1.14)</td>
</tr>
</tbody>
</table>

\(^a\)Relative potency of antagonist + agonist vs. vehicle + agonist.

\(^b\)Significant sex difference (mean for males lies outside of the C.I. around the mean for females).
antagonize morphine-induced antinociception in rats of either sex (data not shown).

**Naloxone Antagonism of THC-Induced Antinociception.** To further test whether sex differences in the antagonism of THC were confined to the cannabinoid system, the ability of an opioid antagonist to block the effects of 5 mg/kg THC was examined in female versus male rats. Naloxone (1.0 mg/kg) did not significantly alter THC-induced paw pressure ($F_{1,24} = 1.28$; n.s.) or tail withdrawal ($F_{1,24} = 0.47$; n.s.) antinociception in either sex (data not shown). Naloxone also did not alter THC-induced suppression of locomotor activity in either sex ($F_{1,24} = 0.37$; n.s.; data not shown). Naloxone attenuated the catalepsy produced by 5 mg/kg THC, but this effect was not statistically significant ($F_{1,24} = 3.36$; $p = 0.08$; data not shown).

**Discussion**

The main finding of this study is that the CB$_1$ receptor-selective antagonist rimonabant was up to 10 times more potent in female than male rats in blocking the antinociceptive effects of THC and CP55,940. Estimates of rimonabant affinity (apparent $pK_i$) for the CB$_1$ receptor calculated from the behavioral data were approximately 0.5 to 1 mol/kg higher in females than males. Neither the time course of rimonabant antagonism of THC nor plasma levels of rimonabant differed between the sexes, suggesting that the sex difference in antagonism is not caused by peripheral pharmacokinetic factors. The sex difference in rimonabant antagonism did not extend to the opioid agonist morphine, and the opioid antagonist naloxone did not significantly attenuate THC’s effects in either sex, indicating that sex differences in antagonism are specific to the cannabinoid system. Taken together, these results suggest that cannabinoid drugs bind with greater affinity to CB$_1$ receptors in females than males, which may contribute to greater cannabinoid agonist effects observed in female compared with male rats.

**Sex Differences in Cannabinoid Agonist Effects.** THC and CP55,940 alone were more potent in producing behavioral effects in female compared with male rats. Specifically, $ED_{50}$ values for THC (Table 2) were significantly lower in females than males on paw pressure, tail withdrawal, and locomotor tests, and the THC dose-effect curve for catalepsy was clearly steeper in females than males (Fig. 2), suggesting greater efficacy in females. For CP55,940, sex differences were smaller, but potency was still significantly greater in females than males on the paw pressure and catalepsy tests (Table 3). Similar sex differences in cannabinoid agonist potency have been reported previously by our laboratory (Tseng and Craft, 2001, 2004) and others (Cohn et al., 1972; Romero et al., 2002; also see Wiley et al., 2007; Wiley and Evans, 2009). The antinociceptive tests in this and previous studies all used a delayed or absent withdrawal response as the antinociceptive endpoint. Thus it is possible that greater antinociception in females results from greater motor impairment in females compared with males: in females, each agonist was nearly equi potent in producing catalepsy and antinociception, whereas in males, each agonist was significantly more potent in producing antinociception than catalepsy (Tables 2 and 3). If “antinociception” simply results from motor impairment in females, nociceptive and motor measures would be expected to change in tandem. One finding in the present study argues against this: rimonabant antagonism of THC-induced antinociception was greater than its antagonism of motor impairment (Table 2). Furthermore, we reported previously that ovarian hormones modulate antinociceptive but not motoric effects of THC, using the same behavioral tests (Craft and Leitl, 2008; Wakley and Craft, 2011). Although antinociception and catalepsy do not always change in tandem, motor impairment may still contribute to longer latencies on the nociceptive tests. To determine whether sex differences exist in cannabinoid antinociception per se, antinociception produced by local cannabinoid administration in a peripheral pain assay (e.g., Ko and Woods, 1999), or by a peripherally restricted cannabinoid agonist (Yu et al., 2010), could be compared in female and male rats, because cannabinoid-induced motor impairment does not typically occur using these approaches. We are pursuing such strategies to better distinguish sex differences in cannabinoid-induced antinociception versus motoric effects.

**Sex Differences in Antagonism of Cannabinoid Agonist Effects.** As expected, rimonabant dose-dependently antagonized nearly all behavioral effects of THC and CP55,940 in both sexes. The only exception was THC-induced catalepsy in male rats; however, the failure to observe significant antagonism of this behavior is probably because THC alone produced only minimal catalepsy in males. The effective dose range of rimonabant against other agonist-behavior changes in males, 1.0 to 10 mg/kg, agrees with previous reports of systemic rimonabant potency against a range of behavioral effects produced by THC and other cannabinoid agonists in male rats (e.g., Lichtman and Martin, 1997; Järbe et al., 2010). The greater potency of rimonabant (higher apparent $pK_i$) observed in female rats was unexpected. We are aware of only one previous study comparing the potency of rimonabant between females and males. In that study, intracerebroventricular or intrathecal rimonabant (1–1000 μg) antagonized paw pressure antinociception and catalepsy produced by 10 mg/kg i.p. THC in both sexes to approximately the same extent and with similar potency in most cases (Tseng and Craft, 2004). The fact that sex differences in rimonabant potency were observed when rimonabant was administered systemically but not when it was administered centrally suggests that peripheral cannabinoid pharmacology may differ between males and females. Peripheral mechanisms may be particularly vital to antinociception produced by cannabinoids (Agarwal et al., 2007; Kunos et al., 2009). We are currently examining sex differences in peripheral cannabinoid antinociception to determine to what extent these may contribute to sex differences in antinociception after systemic cannabinoid administration.

Another unexpected finding in the present study was the attenuation of THC’s effects by the putative CB$_2$ receptor-selective antagonist SR144528. SR144528 antagonism was not dose-dependent and was observed only inconsistently: in the first experiment (Figs. 3–6), the intermediate dose of SR144528, 3.2 mg/kg, partially but significantly antagonized the tail withdrawal antinociception and depressed locomotion produced by THC in female rats only. In contrast, in the antagonist time-course experiment, 3.2 mg/kg SR144528 did not antagonize any effects of THC in either sex. At present we cannot explain the inconsistent antagonism. Although THC is known to bind to CB$_1$ and CB$_2$ receptors with similar affinity (Govaerts et al., 2004), its acute antinociceptive and
motoric effects, as characterized previously in males, seem to be exclusively CB₁ receptor-mediated (for review, see Pertwee, 2008). The present results suggest that THC acts at CB₁ receptors, and sometimes CB₂ receptors, in females; however, given the inconsistency of the SR144528 antagonism, this result must be interpreted with caution.

Pharmacokinetic versus Pharmacodynamic Mechanisms. One possible explanation for the greater potency of rimonabant observed in female compared with male rats is a pharmacokinetic difference. For example, perhaps rimonabant is absorbed or transported to target receptor sites more readily or it is metabolized or excreted more slowly in females than males. To address some of these possibilities, we first examined the time course of rimonabant antagonism of THC and found no sex difference. In all cases, rimonabant was maximally effective in both sexes when injected 5 or 30 min before THC (behavioral effects being measured 35–65 or 60–90 min, respectively, after rimonabant injection; Figs. 11 and 12). Antagonism waned steadily thereafter, such that in most cases it was no longer significant at the 90-min pretreatment time (i.e., when behavior was measured 120–150 min after rimonabant injection: Figs. 11 and 12). This time course agrees with a recent study in which rimonabant antagonism of THC’s discriminative stimulus effects was maximal at 20 to 60 min postinjection and almost completely gone by 4 h postinjection in male rats (Järbe et al., 2010), as well as a study in male mice showing that [³H]rimonabant binding site displacement was greatest at 30 to 60 min postinjection and waned by 3 to 4 h postinjection (Rinaldi-Carmona et al., 1996).

Plasma rimonabant levels also were compared between female and male rats. There was no sex difference, suggesting again that sex differences in rimonabant antagonism of THC and CP55,940 were not caused by greater antagonist absorption in females. However, it is possible that transport differences in rimonabant pharmacokinetics in any species, so this possibility remains to be addressed.

Alternatively, sex differences in the endogenous cannabinoid system may explain the present results. Endocannabinoid production is greater in female than male rats, in brain regions such as the pituitary, hypothalamus, striatum, and midbrain (González et al., 2000; Bradshaw et al., 2006). In addition, brain CB₁ receptor density and affinity may differ between female and male rats, although the direction of these differences seems to be brain site-specific. For example, cannabinoid receptor density is greater in females than males in the amygdala (Riebe et al., 2010) but lower in females than males in the hypothalamus (Riebe et al., 2010) and mesencephalon (Rodríguez de Fonseca et al., 1994). Higher binding affinity also has been observed in females in striatum, limbic forebrain, and mesencephalon (Rodríguez de Fonseca et al., 1994) and in males in hypothalamus (Riebe et al., 2010). One study in humans also reported greater peripheral cannabinoid receptor expression in women than men (Onai et al., 1999), although these were presumably CB₂ receptors given that they were on leukocytes.

In conclusion, sex differences in vivo apparent pKᵦₐ values calculated from the rimonabant + cannabinoid agonist dose-effect curves support the hypothesis that sex differences in cannabinoid antinociceptive potency are pharmacodynamic in nature. Specifically, the results suggest that one mechanism underlying greater cannabinoid antinociception in females compared with males is greater CB₁ receptor affinity in females compared with males. Given the potential of supraspinal, spinal, and peripheral cannabinoid receptor involvement in systemic cannabinoid antinociception, in the future it will be important to compare CB₁ receptor affinity and density in females versus males at all levels of the neuraxis. In addition, sex differences in the actions of cannabinoids at receptors other than CB₁ and CB₂ (e.g., G protein-coupled receptor 55; Ryberg et al., 2007) may contribute to sex differences in cannabinoid antinociception.

Acknowledgments
We thank Dr. Neal Davies and Stephanie Martinez for extensive assistance determining plasma rimonabant levels.

Authorship Contributions
Participated in research design: Craft and Laggart.
Conducted experiments: Wakley, Tsutsui, and Laggart.
Performed data analysis: Craft.
Wrote or contributed to the writing of the manuscript: Craft, Wakley, Tsutsui, and Laggart.

References
Järbe TU, Gifford RS, and Makiyanius A (2010) Antagonism of 5-HT1C/5-HT1D receptors given that they were on leukocytes.


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