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JWH-018 in rhesus monkeys: differential antagonism of discriminative stimulus, rate-decreasing, and hypothermic effects

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SUMMARY

Several effects of the abused synthetic cannabinoid JWH-018 were compared to those of Δ^9 -tetrahydrocannabinol (Δ^9 -THC) in rhesus monkeys. JWH-018 (0.1 mg/kg i.v.) was established as a discriminative stimulus and rimonabant was used to examine mechanisms responsible for discrimination as well as operant response rate-decreasing and hypothermic effects. JWH-018 dose-dependently increased drug-lever responding ($ED_{50} = 0.01$ mg/kg) and decreased response rate ($ED_{50} = 0.064$ mg/kg). Among various cannabinoids, the relative potency for producing discriminative stimulus and rate-decreasing effects was the same: CP-55940 = JWH-018 > Δ^9 -THC = WIN-55212-2 = JWH-073. The benzodiazepine agonist midazolam and the NMDA antagonist ketamine did not exert JWH-018 like discriminative stimulus effects up to doses that disrupted responding. JWH-018 and Δ^9 -THC decreased rectal temperature by 2.2 and 2.8 °C, respectively; the doses decreasing temperature by 2 °C were 0.21 and 1.14 mg/kg, respectively. Antagonism did not differ between JWH-018 and Δ^9 -THC, but did differ among effects. The apparent affinities of rimonabant calculated in the presence of JWH-018 and Δ^9 -THC were not different from each other for antagonism of discriminative stimulus effects (6.58 and 6.59, respectively) or hypothermic effects (7.08 and 7.19, respectively). Apparent affinity estimates are consistent with the same receptors mediating the discriminative stimulus and hypothermic effects of both JWH-018 and Δ^9 -THC. However, there was more limited and less orderly antagonism of rate-decreasing effects, suggesting that an additional receptor mechanism is involved in mediating the effects of cannabinoid on response rate. Overall, these results strongly suggest that JWH-018 and Δ^9 -THC act at the same receptors to produce several of their shared psychopharmacological effects.

Keywords

apparent affinity; cannabinoid; Δ^9 -tetrahydrocannabinol; drug discrimination; hypothermia; JWH-018; Schild analysis

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1. Introduction

The widespread use of cannabis and the emerging use of synthetic cannabinoids underscore a need for comparing receptor mechanisms underlying in vivo effects. JWH-018 is one of the first synthetic cannabinoids identified in various abused products (Seeley et al., 2013) and has higher intrinsic activity (i.e., efficacy) than 9 -THC at the cannabinoid CB₁ receptor subtype, evidenced by 5-fold greater stimulation of guanosine 5'-O-(3-[35S]thio)triphosphate (GTP γ S) binding in rodent brain tissue (Brents et al., 2011). Such a marked difference in cannabinoid CB₁ receptor efficacy in vitro could be an important determinant of the relative abuse and dependence liability of synthetic cannabinoids and Cannabis, but relationships between cannabinoid CB₁ receptor efficacy and in vivo effects have not been fully established. One goal of this study was to examine cannabinoid CB₁ receptor involvement in several different effects of JWH-018 and 9 -THC in rhesus monkeys. A second goal of this study was to examine the extent to which the in vivo effects of JWH-018 and 9 -THC differ as a function of cannabinoid CB₁ receptor efficacy. In an attempt to yield conditions of high cannabinoid CB₁ receptor efficacy demand, a relatively large dose of JWH-018 was established as a discriminative stimulus. The training dose (0.1 mg/kg i.v.) was one-half log unit smaller than a dose of JWH-018 that suppressed operant responding under the fixed ratio 5 schedule of stimulus-shock termination used here. For other drug classes, such as P opioids, drug discrimination assays have been used to demonstrate that a relatively large training dose of a high efficacy agonist yielded conditions of high efficacy demand, as evidenced by partial or no substitution of low efficacy agonists (Young et al., 1992).

Drug discrimination appears to be an ideal assay for examining relationships between cannabinoid CB₁ receptor efficacy and in vivo effects inasmuch as cannabinoid CB₁ receptors are solely responsible for mediating the discriminative stimulus effects of cannabinoids. When 9 -THC is the training drug, various cannabinoid CB₁ receptor agonists produce high levels of drug-appropriate responding, i.e., substitute for the 9 -THC discriminative stimulus (Balster and Prescott, 1992). Cannabinoid CB₁ receptor agonists aside from 9 -THC have been used less frequently as training drugs; however, the results are similar to those obtained when 9 -THC is trained, i.e., cannabinoid CB₁ receptor agonists share effects with each other regardless of the training drug (De Vry and Jentzsch, 2004; Järbe et al., 2010; Kangas et al., 2013), with a few exceptions noted for endogenous cannabinoid-based drugs that are rapidly metabolized (McMahon et al., 2008; Wiley et al., 2011). The cannabinoid CB₁ receptor-selective antagonist rimonabant has been shown to antagonize the discriminative stimulus effects of 9 -THC and other cannabinoid agonists. In some assays, Schild analysis was used to estimate the apparent affinity of rimonabant. The slope of the Schild plot was not different from unity and the apparent affinity or pA₂ for rimonabant was not different when determined in the presence of various cannabinoid CB₁ receptor agonists (McMahon, 2006; 2009; Ginsburg et al., 2012; Kangas et al., 2013). In contrast, antagonists at non-cannabinoid CB₁ receptors (e.g., CB₂ receptors; McMahon, 2006) did not attenuate the discriminative stimulus effects of cannabinoids. Taken together, these results strongly suggest that cannabinoid CB₁ receptors exclusively mediate the discriminative stimulus effects of cannabinoid agonists.

Various adverse effects (e.g., hypertension, seizures, and panic attacks) often requiring immediate medical care have been reported after use of products containing synthetic cannabinoid agonists (Müller et al., 2010; Vearrier and Osterhoudt, 2010; Simmons et al., 2011; Schneir and Baumbacher, 2012). The extent to which adverse effects are due to synthetic cannabinoids, as opposed to other material contained in synthetic cannabinoid-based products, is unresolved. If synthetic cannabinoids are responsible for the adverse effects, it is not clear to what extent high cannabinoid CB₁ receptor efficacy is responsible, as opposed to actions at some other receptor type. Here, hypothermia and disruption of responding under the schedule of stimulus-shock termination were measured in an attempt to provide added sensitivity to differences in cannabinoid CB₁ receptor efficacy as well as actions at other receptor types. The hypothermic effects of cannabinoid agonists are mediated by cannabinoid CB₁ receptors (e.g., McMahon et al., 2005) and generally require larger doses than the smallest doses of cannabinoid agonist producing discriminative stimulus effects. As the dose of agonist required for an effect increases, the efficacy demand at the receptors mediating that effect also increases, thereby increasing the likelihood of detecting a difference between low and high efficacy agonists. For example, cannabinoid receptor agonists can differ in maximum hypothermia, and these differences appear to be due to differences in cannabinoid CB₁ receptor efficacy (Paronis et al., 2012). As compared with discriminative stimulus effects, decreases in operant response rate are less pharmacologically selective, i.e., any number of receptor mechanisms can lead to disruption of operant responding. The effects of JWH-018 to decrease body temperature and to disrupt operant responding have been less well studied. Moreover, quantitative analysis of antagonism of effects of cannabinoids aside from discriminative stimulus effects has been infrequently studied. Therefore, antagonism of rate-decreasing and hypothermic effects by rimonabant was quantified and compared to antagonism of discriminative stimulus effects.

2. Material and Methods

2.1 Subjects

Five pharmacologically and experimentally naïve male adolescent rhesus monkeys (*Macaca mulatta*) were housed individually on a 14-h light/10-h dark schedule and maintained at 95% free-feeding weight (range 5.5–6.5 kg) with a diet consisting of primate chow (High Protein Monkey Diet; Harlan Teklad, Madison, WI), fresh fruit, and peanuts. Water was provided in the home cage. The Institutional Animal Care and Use Committee of The University of Texas Health Science Center at San Antonio approved the experimental protocols, and studies were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 2011).

2.2 Surgery

Monkeys were anesthetized with ketamine (10 mg/kg i.m.) followed by isoflurane (1.5–3.0% inhaled via facemask). A catheter (heparin-coated polyurethane; o.d. = 1.68 mm; i.d. = 1.02 mm; Instech Laboratories, Plymouth Meeting, PA) was inserted into a subclavian or femoral vein and secured to the vessel with suture silk (coated vicryl; Ethicon Inc., Somerville, NJ). The catheter extended from the vessel to the midscapular region of the back and was attached to a vascular access port located s.c. (Mida-cbas-c50; Instech

Laboratories). Monkeys received meloxicam and penicillin daily for a minimum of three days after surgery.

2.3 Apparatus

Monkeys were seated in chairs (model R001; Primate Products, Miami, FL) and were fitted with shoes containing brass electrodes through which a brief electric stimulus (3 mA, 250 ms) could be delivered from an A/C generator. Experiments were conducted in ventilated, sound-attenuating chambers equipped with two levers; a light was positioned above each lever. The chambers were connected to a computer with an interface (MED Associates, St. Albans, VT); experimental events were controlled and recorded with Med-PC software (MED Associates).

2.4 Drugs

⁹-THC (100 mg/ml in absolute ethanol) and rimonabant (Research Technology Branch of the National Institute on Drug Abuse, Rockville, MD), JWH-018 (IU Chem Holding Co, Ltd., Shanghai, China), CP-55940 (Tocris Bioscience, Ellisville, MO), JWH-073 (Research Chemical Supplier, Scottsdale, AZ), and WIN-55212-2 (Sigma-Aldrich, St. Louis, MO) were dissolved in a mixture of 1 part absolute ethanol, 1 part Emulphor-620 (Rhodia Inc., Cranbury, NJ), and 18 parts physiologic saline. Midazolam hydrochloride (Roche Pharma Inc., Manati, Puerto Rico) and ketamine hydrochloride (Fort Dodge Laboratories, Fort Dodge, IA) were purchased as commercially prepared solutions and were diluted with sterile saline. Doses were expressed as the weight of the forms listed above in milligrams per kilogram of body weight. Drugs were administered intravenously in a volume of 0.1 to 1 ml/kg.

2.5 Drug discrimination

A two-lever drug versus no-drug operant conditioning-based procedure was used to train monkeys to discriminate between JWH-018 (0.1 mg/kg; i.v.) from vehicle during once daily sessions 7 days per week. Responding was maintained under a fixed ratio 5 (FR5) schedule of stimulus-shock termination using the same experimental parameters used previously to establish other cannabinoids as discriminative stimuli (Ginsburg et al., 2012). Experimental sessions were divided into multiple 10-min cycles; each cycle consisted of a 5-min timeout immediately followed by a 5-min schedule of stimulus-shock termination. During the timeout, red lights were not illuminated in the operant chamber and responses had no programmed consequence. Illumination of two red lights, one above each lever, signaled the beginning of the stimulus-shock termination schedule. Five consecutive responses on the correct lever extinguished the red lights for 30 s and prevented delivery of shock for 40 s. The correct lever was determined by administration of the training dose of JWH-018 (0.1 mg/kg i.v.) or vehicle within the first min of a cycle. Determination of correct levers varied among monkeys (i.e., left lever associated with 0.1 mg/kg of JWH-018; right lever associated with vehicle) and remained the same for that monkey for the duration of the study. Responding on the incorrect lever reset the response requirement on the correct lever.

The training sequence generally included two consecutive sessions of training with JWH-018 followed by two consecutive sessions of training with vehicle (i.e., over 4

consecutive days the training sequence was drug-drug-vehicle-vehicle). Once every 2 weeks on average, training alternated every day between drug and vehicle (i.e., over 4 consecutive days the training sequence was drug-vehicle-drug-vehicle). The training dose (0.1 mg/kg i.v.) of JWH-018 or vehicle was administered in the first min of a cycle followed in the first min of subsequent cycles by either vehicle or sham (i.e., dull pressure applied to the skin overlying the vascular access port). Drug training consisted of no more than three cycles, or a maximum of 30 min after JWH-018 administration, to ensure a relatively stable drug effect. Drug training was preceded by 0-3 vehicle-training cycles. Vehicle training consisted of up to 6 cycles. The criteria for testing required at least 80% of the total responses on the correct lever and fewer than five responses on the incorrect lever before completion of the first FR on the correct lever for every cycle during five consecutive or six out of seven training sessions. Between each test, monkeys satisfied the test criteria for both a vehicle and drug training session.

Test sessions were identical to training sessions except that five consecutive responses on either lever postponed the shock schedule and various doses of JWH-018 or other drugs were administered. Dose-effect functions for JWH-018, Δ^9 -THC, WIN-55212, CP-55940, JWH-073, midazolam, and ketamine were determined by administering vehicle in the first cycle followed by doses increasing by 0.5 log unit in subsequent cycles. The dose-effect function included ineffective doses (i.e., doses producing responses predominantly on the vehicle lever) up to doses that either produced more than 80% of responses on the JWH-018 lever or decreased response rate to less than 20% of control. Tests with rimonabant were conducted by administering an intravenous dose in the first cycle followed by cumulative doses of JWH-018 or Δ^9 -THC in subsequent cycles. The effects of drugs were determined in each of five monkeys, except midazolam and ketamine, which were tested in four out of the five monkeys due to the loss of a catheter in monkey.

2.6 Hypothermia

Rectal temperature was measured in the same monkeys used in the discrimination assay but on those days discrimination sessions were not conducted. Monkeys were seated in chairs and a probe (RET-1; Physitemp Instruments, Inc., NJ, USA) was inserted 15 cm into the rectum. Temperature in °C was registered by a Microcomputer Thermometer (7001H; Physitemp Instruments, Inc.) attached to the probe. Temperature was measured immediately before and at various times after drug administration. On separate days, a time course was determined for the effects of vehicle, JWH-018 (0.1 and 0.32 mg/kg), or Δ^9 -THC (1 and 3.2 mg/kg). Temperature was measured at 3, 10, 30, 60, 90, 120, 180, 240, 300 and 360 min after drug administration.

The delayed onset to maximum hypothermia precluded use of cumulative dosing. To establish dose-effect functions for JWH-018 and Δ^9 -THC alone and in combination with rimonabant (1 mg/kg), a single dose or dose combination was administered on different days. Rectal temperature was measured 30 min after JWH-018 and Δ^9 -THC; vehicle or rimonabant (1 mg/kg) were administered 10 min prior to a dose of JWH-018 or Δ^9 -THC. These time parameters were chosen to approximate the interval between administration of rimonabant and either JWH-018 or Δ^9 -THC in the discrimination assay. The order of testing

with drugs and doses was non-systematic and drugs were not administered for at least one day in between drug tests.

2.7 Data analyses

Discrimination data were expressed as a percentage of responses on the drug lever divided by responses on both the drug and vehicle levers. Rate of responding on both levers (i.e., drug and vehicle) was calculated as responses per s excluding responses during timeouts. Rate of responding during a test was expressed as the percentage of the control response rate, defined as the average response rate for all cycles during the five previous vehicle training sessions excluding sessions during which the test criteria were not satisfied.

Doses corresponding to the 50% level of effect (ED_{50} values) were estimated with linear regression (Prism version 5.0 for Windows; GraphPad Software Inc., San Diego, CA) of the individual dose-effect data. Data included in the analysis spanned the linear portion of the dose-response function and a common best-fitting slope was used for further analyses (Kenakin, 1997). ED_{50} values, potency ratios, and 95% confidence limits were calculated by parallel line analyses (Tallarida and Raffa, 1992). Potencies were considered significantly different when the 95% confidence limits of the potency ratio did not include 1. The effects of drugs on response rate were examined with linear regression; a significant dose-dependent decrease in responding was evidenced by the slope being significantly different from 0 ($P < 0.05$). ED_{50} values and potency ratios were calculated when response rate was decreased to below 50%.

Rectal temperature was expressed as a difference from baseline (i.e. temperature measured immediately before drug administration) and was plotted as a function of dose or time. For time course data, significant changes in temperature were assessed with one-way repeated measures ANOVA for each dose separately using time as the main effect ($P < 0.05$). To calculate the relative potency of JWH-018 and 9 -THC, the dose decreasing temperature by 2 °C was estimated by interpolation of data at the time of maximum hypothermia, which differed between JWH-018 and 9 -THC. To calculate the magnitude of antagonism by rimonabant, the dose of agonist alone or in combination with rimonabant decreasing temperature by 1 °C was estimated by interpolation and a potency ratio was calculated by dividing the interpolated values. One-way ANOVA was used to analyze differences in maximum effect.

Schild plots expressed the logarithm of the dose ratio -1 on the ordinate and the negative logarithm of the molar dose of rimonabant on the abscissa (Arunlakshana and Schild, 1959). Straight lines were simultaneously fit to individual Schild plots with the equation $\log(\text{dose ratio} - 1) = -\log(\text{molar dose of rimonabant}) \times \text{slope} + \text{intercept}$. The Schild plot for rimonabant was examined with two mathematical models: a simpler model (i.e., slope constrained to unity or -1) and a more complex model that allowed slope to vary. The two models were compared with an F -ratio test. If the calculated F value was not significant, then the pA_2 value was calculated with both the constrained and unconstrained slope. When only one dose of rimonabant was administered in combination with JWH-018 and 9 -THC, a single-dose apparent affinity estimate was calculated for individual monkeys with the

following equation: $pK_B = -\log(B/\text{dose ratio} - 1)$, with B expressed in moles per kilogram of body weight.

3. Results

3.1 Effects of JWH-018, cannabinoid agonists and noncannabinoids: discrimination and response rate

The criteria for discrimination testing were satisfied in 60, 61, 71, 105, and 134 sessions (including both vehicle and JWH-018 training sessions) per individual monkey. Absolute rate of responding in responses per s for each respective monkey was 2.6, 2.0, 3.2, 3.2, and 2.5. Vehicle produced 3% responses on the drug-associated lever, whereas JWH-018 dose-dependently increased the percentage of drug-lever responses up to 100% at the training dose (0.1 mg/kg); drug-lever responding was also relatively high (98%) at a dose 0.5 log unit smaller than the training dose (Fig. 1, top left). The ED_{50} value (95% confidence limits) of JWH-018 was 0.0097 (0.0072-0.015) mg/kg. CP-55940, Δ^9 -THC, WIN-55212-2, and JWH-073 also dose-dependently increased JWH-018 lever responding (Fig. 1, top left). The slopes of the dose-effect functions for all five cannabinoids were not significantly different from each other ($F_{4,54}=0.30$, $P=0.87$). The ED_{50} values (95% confidence limits) were 0.005 (0.003-0.009) mg/kg for CP-55940, 0.041 (0.027-0.06) mg/kg for Δ^9 -THC, 0.039 (0.017-0.09) mg/kg for WIN-55212, and 0.071 (0.041-0.13) mg/kg for JWH-073. CP 55940 was significantly more potent than JWH-018, which in turn was significantly more potent than Δ^9 -THC, WIN 55212-2, and JWH-073. The latter three drugs did not significantly differ in potency.

The cannabinoids dose-dependently decreased rate of responding (Fig. 1, bottom left). The ED_{50} values (95% confidence limits) were 0.021 (0.005-0.076) mg/kg for CP55940, 0.064 (0.024-0.17) mg/kg for JWH-018, 0.12 (0.074-0.19) mg/kg for Δ^9 -THC, and 0.17 (0.067-0.42) mg/kg for JWH-073. The rank order potency for decreasing response rate was CP 55940 = JWH-018 > Δ^9 -THC = JWH-073. The slope of the WIN-55212-2 dose-effect function was not significantly different from 0 ($F_{1,13}=3.67$, $P=0.07$) and response rate was not decreased to less than 50% of control up to a dose of 0.1 mg/kg.

Midazolam and ketamine produced significantly less drug-lever responding, i.e., 28% and 13%, respectively, than the training dose of JWH-018 ($F_{3,6} = 42.2$, $P<0.001$) up to doses that significantly decreased response rate (Fig. 1, right). The ED_{50} values (95% confidence limits) for decreasing response rate were 0.21 (0.08-0.57) mg/kg for midazolam and 0.88 (0.27-2.84) mg/kg for ketamine.

At the training dose (0.1 mg/kg) of JWH-018, discriminative stimulus and rate-decreasing effects varied significantly as a function of time ($F_{5,15} = 6.16$, $P<0.01$ and $F_{3,15} = 5.25$, $P<0.01$, respectively) and the time courses were similar to each other (Fig. 2, top and bottom, respectively). When measuring the effects of JWH-018 every 10 min up to 60 min after administration, JWH-018 lever responding was 97-100% and response rate was 13-24% of control. In Fig. 2, these data were averaged and plotted as a single value above 60 min. Discriminative stimulus effects decreased systematically from 86% at 90 min to 7%

at 300 min. Over the same time period, effects on response rate also returned to control, i.e., response rate was 41% of control at 90 min and 80% of control at 300 min.

3.2 Antagonism of the discriminative stimulus and rate-decreasing effects of JWH-018 and Δ^9 -THC

Up to a dose of 3.2 mg/kg, rimonabant produced predominantly vehicle-lever responding and did not modify response rate (Fig. 3, top, VEH). Rimonabant surmountably and dose-dependently antagonized the discriminative stimulus effects of JWH-018 (Fig. 3, top left). The ED₅₀ values (95% confidence limits) of the discriminative stimulus effects of JWH-018 in combination with rimonabant at doses of 0.32, 1, and 3.2 mg/kg were 0.045 (0.028-0.070), 0.14 (0.10-0.20), and 0.44 (0.30-0.65) mg/kg, respectively. The respective dose ratios were 4.6-, 15-, and 46-fold. A single dose (1 mg/kg) of rimonabant antagonized the discriminative stimulus effects of Δ^9 -THC, as evidenced by a rightward shift in the Δ^9 -THC dose-response function and a significant 11-fold increase in the ED₅₀ value to 0.46 (0.26-0.82) mg/kg (Fig. 3, top right).

Rimonabant antagonized the effects of JWH-018 to decrease response rate; however, antagonism of rate-decreasing effects differed quantitatively from antagonism of discriminative stimulus effects (Fig. 3, bottom left). Whereas the smallest dose (0.32 mg/kg) of rimonabant significantly antagonized the JWH-018 discriminative stimulus, there was no significant antagonism of rate-decreasing effects, as evidenced by no significant difference in the ED₅₀ value determined in the presence of rimonabant (0.10 mg/kg) as compared with control (0.064 mg/kg). Significant antagonism was obtained with both 1 and 3.2 mg/kg of rimonabant as evidenced by increases in the ED₅₀ values (95% confidence limits) to 0.20 (0.10-0.39) and 0.31 (0.18-0.54) mg/kg, respectively, which correspond to 3.0- and 4.8-fold increases. The magnitude of antagonism of the rate-decreasing effects of JWH-018 at 1 and 3.2 mg/kg of rimonabant was markedly less than antagonism of the discriminative stimulus effects of JWH-018 at the same doses of rimonabant. Rimonabant (1 mg/kg) also significantly increased the ED₅₀ value of Δ^9 -THC to produce rate-decreasing effects from the control of 0.12 mg/kg to 0.44 mg/kg, i.e., 3.7-fold. This was less than antagonism of the discriminative stimulus effects of Δ^9 -THC at the same dose (1 mg/kg) of rimonabant (Fig. 3, right).

The Schild plot for rimonabant in combination with JWH-018 for discriminative stimulus effects is shown in Fig. 4 (top). The coefficient of determination (r^2) was 0.84 and the unconstrained slope (95% confidence limits) was -1.19 (-1.56 to -0.83), which was not significantly different from unity (i.e., -1; $P=0.26$). The apparent pA₂ value of rimonabant calculated from the unconstrained slope was 6.45 (4.74-8.17) in the presence of JWH-018. When the slope was constrained to -1, the apparent pA₂ value (95% confidence limits) was 6.58 (6.43-6.73) in the presence of JWH-018. The single-dose apparent affinity (pK_B) value (95% confidence limits) of rimonabant (1 mg/kg) determined in the presence of Δ^9 -THC was 6.59 (6.22-6.97) (Fig. 4, bottom).

3.3 Hypothermic effects of JWH-018 and Δ^9 -THC and antagonism by rimonabant

JWH-018 significantly decreased rectal temperature as a function of time at doses of 0.1 and 0.32 mg/kg ($F_{2,7}=4.36$, $P<0.01$ and $F_{2,7}=11.1$, $P<0.001$, respectively) (Fig. 5, top). At doses of 0.1 and 0.32 mg/kg, the largest decrease in rectal temperature was 1.6 and 2.2 °C, respectively, at 90 min. By 240 min, the hypothermic effects began to subside. Δ^9 -THC also significantly decreased temperature as a function of time at doses of 1 and 3.2 mg/kg ($P<0.0001$) (Fig. 5, bottom). The largest decrease in rectal temperature after a dose of 1 mg/kg of Δ^9 -THC was 1.5 °C at 180 min. A larger dose (3.2 mg/kg) decreased temperature by 2.8 °C at 240 min. Hypothermia began to subside at 300 and 360 min for 1 and 3.2 mg/kg of Δ^9 -THC, respectively. The doses producing a 2°C decrease in rectal temperature ($ED_{-2^\circ C}$ values) for JWH-018 and Δ^9 THC were estimated to be 0.21 and 1.14 mg/kg, respectively; this difference in potency was statistically significant ($P<0.05$).

In separate experiments assessing rectal temperature 30 min after JWH-018 or Δ^9 -THC and 40 min after vehicle or rimonabant, the $ED_{-1^\circ C}$ (95% confidence limits) values for JWH-018 alone and in combination with 1 mg/kg rimonabant were calculated to be 0.035 (0.02-0.07) and 1.35 (0.52-3.5), respectively (Fig. 6, top left), a difference of 38-fold. The single-dose apparent affinity estimated (95% confidence limits) from this rightward shift in the JWH-018 dose-response function was 7.08 (6.91-7.25) (Fig. 6, top right). The $ED_{-1^\circ C}$ (95% confidence limits) values for Δ^9 -THC alone and in combination with 1 mg/kg rimonabant were 0.30 (0.04-2.1) and 13.0 (1.67-101), respectively (Fig. 6, bottom left); the magnitude of antagonism of the hypothermic effects of Δ^9 -THC was 43-fold. The single-dose apparent affinity estimate (95% confidence limits) determined in the presence of Δ^9 -THC was 7.19 (5.25-9.14) (Fig. 6, bottom right).

4. Discussion

JWH-018 was readily established as a discriminative stimulus in rhesus monkeys and the profile of drugs sharing effects with JWH-018 was strikingly similar to that described previously in rhesus monkeys trained to discriminate Δ^9 -THC (McMahon, 2006; Ginsburg et al., 2012). Other cannabinoid agonists with higher efficacy than Δ^9 -THC, including CP 55940, WIN 55212-2, and JWH-073, substituted for both JWH-018 (current study) and Δ^9 -THC (McMahon, 2006; Ginsburg et al., 2012). According to receptor theory, if the cannabinoid CB_1 receptor efficacy demand of the JWH-018 discrimination exceeded that of the Δ^9 -THC discrimination, then any changes in the relative potencies of the cannabinoid CB_1 receptor agonists would be related to efficacy, i.e., greatest for the lowest efficacy cannabinoid CB_1 receptor agonist (Δ^9 -THC). However, the relative potency of the cannabinoid agonists did not vary as a function of training drug, thereby not indicative of a difference in the efficacy demand of the two discrimination assays. The benzodiazepine agonist midazolam and the NMDA antagonist ketamine did not substitute for JWH-018, demonstrating some degree of pharmacological selectivity of the JWH-018 discriminative stimulus for cannabinoid receptor agonism. The apparent affinity of rimonabant did not vary when calculated in monkeys discriminating JWH-018 (current study) or Δ^9 -THC (Ginsburg et al., 2012), nor did the apparent affinity of rimonabant differ with respect to antagonism of the hypothermic effects of JWH-018 and Δ^9 -THC. In contrast, rimonabant was less potent

and effective at antagonizing rate-decreasing effects as compared with discriminative stimulus and hypothermic effects, although antagonism of the rate-decreasing effects did not differ between JWH-018 and Δ^9 -THC. Overall, these results demonstrate striking overlap in the receptor pharmacology of JWH-018 and Δ^9 -THC on the one hand, yet divergence in the receptor pharmacology mediating various effects of cannabinoids on the other.

Herbal blends containing synthetic cannabinoids are popular alternatives to cannabis. The products are assumed to share effects with cannabis, they are not always regulated or illegal, and routine screens of illicit drug use do not detect the presence of synthetic cannabinoids (Seeley et al., 2013). In non-humans, synthetic cannabinoids detected in herbal blends generally overlap pharmacologically and share behavioral effects with Δ^9 -THC, including discriminative stimulus effects (Wiley et al., 1998; Huffman et al., 2005; Ginsburg et al., 2012). However, relative to cannabis use, there are numerous reports of more severe adverse events following use of products containing synthetic cannabinoids (Spaderna et al., 2013). In the current study, even when relatively large doses of JWH-018 were studied in rhesus monkeys, there was no evidence that JWH-018 differed from Δ^9 -THC with respect to discrimination, operant response-rate disruption, and hypothermic effects. Overall, there appears to be striking overlap in the effects of JWH-018 and Δ^9 -THC in non-human primates.

Cross-substitution among drugs, i.e., two drugs that are trained in separate groups of animals and that substitute for each other when tested, as demonstrated for JWH-018 and Δ^9 -THC here and previously (Ginsburg et al., 2012), provides strong evidence for a shared receptor mechanism of action. There is, however, some evidence suggesting that rimonabant can produce quantitatively different antagonism of JWH-018 as a function of training drug (Δ^9 -THC versus methanandamide; Järbe et al., 2010), suggesting that JWH-018 acts at different receptors depending on the training drug. In separate groups of rhesus monkeys trained to discriminate either JWH-018 or Δ^9 -THC (Ginsburg et al., 2012), the apparent affinity values of rimonabant not only did not differ when determined in the presence of JWH-018 and Δ^9 -THC, but also did not differ between training drugs (Ginsburg et al., 2012; current results). Collectively, these results underscore the utility of using Schild analysis to compare receptor mechanisms of drug action across different assay conditions. Moreover, these data strongly suggest that the same receptor type(s) mediate the discriminative stimulus effects of cannabinoids.

If, as suggested by apparent affinity analyses, the discriminative stimulus effects of JWH-018 and Δ^9 -THC are mediated solely by cannabinoid CB₁ receptors, then drug discrimination in rhesus monkeys appears to be ideally suited for examining potential differences in cannabinoid CB₁ receptor signaling (e.g., efficacy) among agonists. In assays measuring maximum stimulation of G_i-proteins in isolated tissue, JWH-018 produces 5-fold greater stimulation than Δ^9 -THC (Brents et al., 2011). However, in the current study, there was no evidence that discriminative stimulus effects varied as a function of cannabinoid CB₁ receptor efficacy even when a relatively large dose (0.1 mg/kg i.v.) of JWH-018 was trained; a larger dose (0.32 mg/kg i.v.) suppressed responding and therefore could not be easily trained. For P opioids, a large training dose of a high efficacy agonist resulted in assay conditions requiring high efficacy, as evidenced by failure of a low efficacy agonist to

substitute for the discriminative stimulus effects of the higher efficacy agonist (Young et al., 1992). However, Δ^9 -THC was able to fully substitute for JWH-018. Furthermore, while the relative potencies of agonists depend in part on differences in efficacy among the agonists as well as the efficacy demand of the particular experimental conditions under which they are studied, evidence for a difference in efficacy demand between the JWH-018 and Δ^9 -THC discrimination assays (Ginsburg et al., 2012) was not observed here inasmuch as the relative potency among cannabinoid CB₁ receptor agonists varying in efficacy did not differ between assays. The failure of a relatively large training dose of JWH-018 to create a high efficacy-demand assay is consistent with the larger reserve of cannabinoid CB₁ receptors (Gifford et al., 1999) as compared with μ opioid receptors (e.g., opioids; Childers, 2006). Depletion of receptor reserve, such as that resulting from chronic cannabinoid treatment, has provided evidence for an apparent relationship between cannabinoid CB₁ receptor efficacy and behavioral effects (Hruba et al., 2012).

The hypothermic effects of cannabinoids are mediated by cannabinoid CB₁ receptors (Martin et al., 1991; Compton et al., 1996; McMahon et al., 2005) and are, under some conditions, sensitive to differences in efficacy as evidenced by differences in maximum effect (Paronis et al., 2012). Here, the magnitude of hypothermia did not differ between JWH-018 and Δ^9 -THC at the largest doses tested, i.e., 0.32 and 3.2 mg/kg i.v., respectively. Maximum hypothermia is sometimes difficult to determine safely given that doses often produce adverse effects. The time to peak effect was much longer for hypothermic as compared with discriminative stimulus effects, thereby decreasing the feasibility of using cumulative dosing to examine antagonism of cannabinoid-induced hypothermia. The different methods of dosing could have been responsible for the different apparent affinities calculated for rimonabant as an antagonist of discrimination versus hypothermia, as opposed to the alternative explanation of a different receptor type underlying the two effects. However, with identical methods of dosing in the hypothermia experiment, the apparent pK_B values of rimonabant did not differ between JWH-018 and Δ^9 THC, further underscoring a common mechanism underlying in vivo effects of these two drugs.

Antagonism of discriminative stimulus and rate-decreasing effects by rimonabant was assessed simultaneously. There was at least a ½ log unit difference in the potency of rimonabant to antagonize the discriminative stimulus versus rate-decreasing effects of JWH-018 and the antagonism of the rate-decreasing effects of JWH-018 occurred within a limited dose range of rimonabant. Rimonabant also produced differential antagonism of the discriminative stimulus and rate-decreasing effects of Δ^9 -THC. Given that the relationship between antagonist dose and the magnitude of antagonism of rate-decreasing effects was non-linear, Schild analysis was not feasible. Collectively, these results suggest that cannabinoids alter operant responding through multiple receptor mechanisms including, but not limited to, cannabinoid CB₁ receptors that mediate the discriminative stimulus and hypothermic effects of cannabinoid agonists. Rimonabant is relatively selective for cannabinoid CB₁ receptors (Rinaldi-Carmona et al., 1994), but also binds to α_2 -adrenergic and μ opioid receptors; the latter is suggested to mediate the inverse agonism obtained at relatively large concentrations of rimonabant in vitro (Cinar and Scucz, 2009; Pertwee et al., 2010). The cannabinoid agonists included for study are relatively non-selective for cannabinoid CB₁ and CB₂ receptors and some of the agonists appear to produce

behavioral effects through non-cannabinoid CB₁ and non-CB₂ receptor mechanisms (Wiley et al., 2012), with GPR55 identified as a potential site of action for ⁹-THC in particular. Relatively large doses of rimonabant decrease operant response rate (McMahon, 2011), potentially through actions at non-cannabinoid CB₁ receptors, which in turn could yield less orderly antagonism than that of other effects mediated solely by cannabinoid CB₁ receptors. In addition or alternatively, involvement of non-cannabinoid CB₁ (i.e., rimonabant-insensitive) receptors in the rate-decreasing effects of ⁹-THC and JWH-018 cannot be excluded.

In summary, this study demonstrates striking overlap in the pharmacological, physiological, and behavioral effects of JWH-018 and ⁹-THC in primates. Toxicity reported following consumption of products containing mixtures of synthetic cannabinoids and other material, as well as the abuse and dependence risk of those products, has raised questions about potential differences underlying pharmacological mechanisms. Cannabinoid CB₁ receptors are clearly involved; however, the extent to which toxicity, abuse, and dependence vary as function of cannabinoid CB₁ receptor efficacy is not clear. There was no evidence that cannabinoid CB₁ receptor efficacy differentiated the effects of JWH-018 and ⁹-THC, at least with respect to the effects measured in this study. On the other hand, the effects of cannabinoids to disrupt operant responding appear to be mediated in part by a receptor type/and or mechanism that does not mediate the discriminative stimulus and hypothermic effects of cannabinoids.

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Abbreviations

ANOVA	analysis of variance
⁹-THC	⁹ -tetrahydrocannabinol
JWH-018	naphthalen-1-yl-(1-pentylindol-3-yl) methanone
JWH-073	naphthalen-1-yl-(1-butyldol-3-yl) methanone
WIN-55,212-2	4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1-i,j]quinolin-6-one
CP-55,940	(1R,3R,4R)-3-[2-hydroxy-4-(1,1-dimethylheptyl) phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol

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Fig. 1.

Discriminative stimulus effects and response rate after cannabinoid agonists (left) and noncannabinoids (right) in monkeys discriminating JWH-018 (0.1 mg/kg i.v.). Abscissae: vehicle (VEH) and dose of drug in mg/kg body weight. Ordinates: mean ($\pm$ S.E.M.) percentage of responses on the JWH-018 lever (top) and mean ($\pm$ S.E.M.) response rate expressed as a percentage of control (VEH training days) rate [Rate (% Control)] (bottom). Symbols represent the mean ($\pm$ S.E.M.) of values from 5 monkeys (left) or 4 monkeys (right).

Fig. 2. Time course of the discriminative stimulus (top) and rate- decreasing effects (bottom) of JHW-018 (0.1 mg/kg i.v.). Abscissae: time in minutes. Ordinates: mean ($\pm$ S.E.M.) percentage of responses on the JWH-018 lever (top) and mean ($\pm$ S.E.M.) response rate expressed as a percentage of control (VEH training days) rate [Rate (% Control)] (bottom). Symbols represent the mean ($\pm$ S.E.M.) of values from 4 monkeys.

Fig. 3.

Antagonism of the discriminative stimulus effects of JWH-018 (left) and Δ^9 -THC (right) by the cannabinoid CB₁ receptor antagonist rimonabant. Abscissae: vehicle (VEH) and dose in milligram per kilogram of body weight. Ordinates: mean ($\pm$ S.E.M.) percentage of responses on the JWH-018 lever (top) and mean ($\pm$ S.E.M.) response rate expressed as a percentage of control (VEH training days) rate [Rate (% Control)] (bottom). The control dose-response curves for JWH-018 and Δ^9 -THC are re-plotted from Fig. 1. Symbols represent the mean ($\pm$ S.E.M.) of values from 5 monkeys.

Fig. 4.

Schild plot for JWH-018 constructed from mean data shown in Fig. 3 (top) and log (dose ratio - 1) values with associated single dose apparent affinity estimate (pK_B) for rimonabant in combination with Δ^9 -THC (bottom). Abscissae: negative logarithm of the dose in moles per kilogram. Ordinate: mean ($\pm$ S.E.M.) logarithm of the dose ratio - 1. Top panel: Schild plot constructed from the unconstrained slope (dashed line) and by constraining the slope to -1 (solid line). Symbols represent the mean ($\pm$ S.E.M.) of values from 5 monkeys. Values in parentheses are 95% confidence limits.

Fig. 5.

Time course for the hypothermic effects of JWH-018 (top) and Δ^9 -THC (bottom).

Abscissae: time in minutes. Ordinates: mean ($\pm$ S.E.M.) change in body temperature from baseline. Symbols represent the mean ($\pm$ S.E.M.) of values from 4 monkeys.

Fig. 6.

Hypothermic effects of JWH-018 and Δ^9 -THC alone and in combination with rimonabant (1.0 mg/kg) (left) and log (dose ratio - 1) values with associated single-dose apparent affinity estimates (right). Abscissae: vehicle (VEH) and dose in milligram per kilogram of body weight (left) and negative logarithm of the dose in moles per kilogram (right).

Ordinates: mean ($\pm$ S.E.M.) change in body temperature from baseline (right) and mean ($\pm$ S.E.M.) logarithm of the dose ratio - 1 (left). Symbols represent the mean ($\pm$ S.E.M.) of values from 4 monkeys. Values in parentheses are 95% confidence limits.