0022-3565/06/3193-1211–1218$20.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 319:1211–1218, 2006

Vol. 319, No. 3
107110/3151548
*Printed in U.S.A.*

# Characterization of Cannabinoid Agonists and Apparent p$A_2$ Analysis of Cannabinoid Antagonists in Rhesus Monkeys Discriminating $\Delta^9$-Tetrahydrocannabinol

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Received May 8, 2006; accepted August 28, 2006

**ABSTRACT**

Cannabinoid $CB_1$ receptors are hypothesized to mediate the discriminative stimulus effects of cannabinoids. This study characterized a $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC; 0.1 mg/kg i.v.) discriminative stimulus and examined antagonism of cannabinoid agonists in rhesus monkeys. High levels of responding on the $\Delta^9$-THC lever were produced by cannabinoid agonists with the following rank order potency: CP 55940 [(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol] > $\Delta^9$-THC = WIN 55212-2 [(+)-[2,3-dihydro-5-methyl-3[(4-morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate salt] > arachidonylcyclopropylamide = (*R*)-methanandamide. A $CB_2$-selective agonist, AM 1241 [(*R*)-3-(2-iodo-5-nitrobenzoyl)-1-(1-methyl-2-piperidinylmethyl)-1*H*-indole], and noncannabinoids (cocaine, ketamine, midazolam, and morphine) did not produce high levels of $\Delta^9$-THC lever responding. The $CB_1$-selective antagonist SR 141716A [*N*-(piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-(4-chlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide] surmountably antagonized the discriminative stimulus effects of $\Delta^9$-THC and CP 55940, and Schild analysis was consistent with a simple, competitive interaction (apparent p$A_2$ values were 6.1 and 6.7, respectively). SR 141716A surmountably antagonized WIN 55212-2; however, larger doses disrupted responding, precluding Schild analysis. The $CB_1$-selective antagonist AM 251 surmountably antagonized $\Delta^9$-THC, CP 55940, and WIN 55212-2, and Schild analysis was consistent with a simple, competitive interaction (apparent p$A_2$ values were 6.3, 6.1, and 6.2, respectively). The $CB_2$-selective antagonist SR 144528 [*N*-[(1*S*)-*endo*-1,3,3-trimethylbicyclo(2.2.1)heptan-2-yl]5-(4-chloro-3-methyl-phenyl)-1-(4-methylbenzyl)pyrazole-3-carboxamide] did not modify the $\Delta^9$-THC discriminative stimulus. These results demonstrate that the discriminative stimulus effects of $\Delta^9$-THC are selective for cannabinoid activity, and the results of Schild analysis suggest that the same receptors mediate the discriminative stimulus effects of $\Delta^9$-THC, CP 55940, and WIN 55212-2. $CB_2$ receptors do not seem to mediate the discriminative stimulus effects of cannabinoid agonists. Schild analysis has the potential for identifying receptor subtypes that mediate the in vivo effects of cannabinoid agonists.

Cannabinoids, including exogenous compounds contained in *Cannabis sativa* ($\Delta^9$-THC) (Mechoulam et al., 1970), and endogenous cannabinoids (e.g., anandamide; Devane et al., 1992), can exert their activity by acting at two inhibitory G-protein-coupled receptors ($CB_1$ and $CB_2$; for review, see Devane et al., 1988; Munro et al., 1993; Howlett et al., 2002). With the availability of receptor-selective antagonists (Rinaldi-Carmona et al., 1994, 1998; Lan et al., 1999), it has been possible to define a role for $CB_1$ and $CB_2$ receptors in the effects of cannabinoids. The behavioral effects of $\Delta^9$-THC seem to be mediated by $CB_1$ receptors inasmuch as the $CB_1$ receptor-selective antagonist SR 141716A blocks the cataleptic, antinociceptive, and discriminative stimulus effects of $\Delta^9$-THC (Wiley et al., 1995b; Compton et al., 1996; Järbe et al., 2001; McMahon et al., 2005), self-administration of $\Delta^9$-THC (Tanda et al., 2000), and impairments of learning and memory induced by $\Delta^9$-THC (Lichtman and Martin, 1996; Winsauer et al., 1999). CP 55940 and WIN 55212-2 share behavioral effects

This work was supported by United States Public Health Service Grants DA15468 and DA19222.
Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.
doi:10.1124/jpet.106.107110.

**ABBREVIATIONS:** $\Delta^9$-THC, $\Delta^9$-tetrahydrocannabinol; CP 55940, (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)-cyclohexanol; WIN 55212-2, (+)-[2,3-dihydro-5-methyl-3[(4-morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate salt; SR 141716A, *N*-(piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-(4-chlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide; ACPA, arachidonylcyclopropylamide; AM 251, *N*-(piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide; AM 1241, (*R*)-3-(2-iodo-5-nitrobenzoyl)-1-(1-methyl-2-piperidinylmethyl)-1*H*-indole; SR 144528, *N*-[(1*S*)-*endo*-1,3,3-trimethylbicyclo(2.2.1)heptan-2-yl]5-(4-chloro-3-methyl-phenyl)-1-(4-methylbenzyl)pyrazole-3-carboxamide; FR, fixed ratio; CL, confidence limit.

with $\Delta^9$-THC, and at least some effects (e.g., discriminative stimulus effects) of these agonists are attenuated by SR 141716A (Pèrio et al., 1996).

To compare the mechanism of action of cannabinoids, receptor-selective antagonists have been combined with various agonists. In animals trained to discriminate $\Delta^9$-THC, for example, the potency of SR 141716A to antagonize equieffective doses of $\Delta^9$-THC and (*R*)-methanandamide (metabolically stable analog of anandamide) was the same (Järbe et al., 2006), whereas the potency of SR 141716A to antagonize equieffective doses of WIN 55212-2 and $\Delta^9$-THC was different (Pèrio et al., 1996). Differences in the potency of SR 141716A to antagonize cannabinoids might reflect differences in mechanism of action. SR 141716A also surmountably antagonized the discriminative stimulus effects of $\Delta^9$-THC (Järbe et al., 2001; McMahon et al., 2005). When surmountable antagonism can be obtained in vivo with at least three doses of an antagonist, and when the slope of a Schild plot is not different from unity (i.e., −1), the apparent affinity (potency) of an antagonist can be estimated (Arunlakshana and Schild, 1959). This approach can be used to examine whether the same receptors mediate the in vivo effects of agonists, as evidenced by equivalent apparent p$A_2$ values for an antagonist in the presence of different agonists.

The goal of the present study was to characterize a $\Delta^9$-THC (0.1 mg/kg i.v.) discriminative stimulus in rhesus monkeys with nonselective cannabinoid agonists (CP 55940 and WIN 55212-2) and agonists that are 325- and 41-fold selective for $CB_1$ relative to $CB_2$ receptors [ACPA and (*R*)-methanandamide, respectively; Khanolkar et al., 1996; Hillard et al., 1999]. The pharmacologic selectivity of the assay was examined with the noncannabinoids cocaine, midazolam, ketamine, and morphine. This study also examined whether $\Delta^9$-THC, CP 55940, and WIN 55212-2 are surmountably antagonized by SR 141716A and AM 251, antagonists that are 143- and 306-fold selective, respectively, for $CB_1$ relative to $CB_2$ receptors (Lan et al., 1999). When appropriate, Schild analysis was used to calculate apparent p$A_2$ values for SR 1417167A and AM 251. To evaluate a role for $CB_2$ receptors in the $\Delta^9$-THC discriminative stimulus, AM 1241 (100-fold selective for $CB_2$ relative to $CB_1$ receptors; Malan et al., 2001) was studied alone, and SR 144528 (700-fold selective for $CB_2$ relative to $CB_1$ receptors; Rinaldi-Carmona et al., 1998) was studied alone and in combination with $\Delta^9$-THC.

## Materials and Methods

**Subjects.** Two female and two male rhesus monkeys (*Macaca mulatta*), housed individually on a 14-/10-h light/dark schedule, were maintained at 95% free-feeding weight (range, 5.7–7.9 kg) with a diet comprising primate chow (High Protein Monkey Diet; Harlan Teklad, Madison, WI), fresh fruit, and peanuts, and were provided water in the home cage. All monkeys were pharmacologically and experimentally naive before being trained to discriminate 0.1 mg/kg i.v. $\Delta^9$-THC (McMahon et al., 2005). Monkeys were maintained in accordance with the Institutional Animal Care and Use Committee, The University of Texas Health Science Center at San Antonio, and the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

**Surgery.** Monkeys were prepared with chronic in-dwelling catheters (heparin-coated polyurethane; o.d., 1.68 mm; i.d., 1.02 mm; Instech Solomon, Plymouth Meeting, PA). Upon anesthesia with ketamine (10 mg/kg i.m.) and isoflurane (1.5–3.0%, inhaled via face mask), a catheter was inserted and advanced 5 cm into a subclavian vein to the level of the vena cava or into a femoral vein. Suture silk (coated Vicryl; Ethicon Inc., Somerville, NJ) was used to anchor the catheter to the vessel and to ligate the section of the vessel proximal to the catheter insertion. The other end of the catheter passed s.c. to the midscapular region of the back, where it was attached to a vascular access port (Mida-cbas-c50; Instech Solomon).

**Apparatus.** During experimental sessions, monkeys were seated in chairs (model R001; Primate Products, Miami, FL) that provided restraint and were placed in ventilated, sound-attenuating chambers equipped with two response levers and stimulus lights. Feet were placed in shoes containing brass electrodes through which a brief electric stimulus (3 mA, 250 ms) could be delivered from an A/C generator. An interface (MedAssociates, St. Albans, VT) connected the chambers to a computer, which controlled and recorded experimental events.

**Discrimination Procedure.** Three monkeys had been trained to discriminate $\Delta^9$-THC (0.1 mg/kg i.v.) from vehicle (McMahon et al., 2005), and a fourth monkey was trained for the current study. Monkeys responded under a fixed ratio 5 (FR5) schedule of stimulus-shock termination in a multiple-cycle procedure. Each cycle began with a 15-min timeout, during which responses had no programmed consequence, followed by a 5-min response period, during which illumination of red lights (one positioned above each of the two levers) signaled a pending electric stimulus (every 40 s). The correct lever was determined by an infusion of vehicle or $\Delta^9$-THC before the session; determination of correct levers (e.g., left, vehicle; right, $\Delta^9$-THC) varied among monkeys and remained the same for an individual throughout the study. Five consecutive responses on the correct lever extinguished the red lights and postponed the schedule for 30 s. Responding on the incorrect lever reset the response requirement on the correct lever. Response periods ended after 5 min or the delivery of four electric stimuli, whichever occurred first.

During training, $\Delta^9$-THC (0.1 mg/kg) or vehicle was administered 15 min before sessions during which additional vehicle infusions or sham (dull pressure applied to the skin overlying the vascular access port) were administered nonsystematically at the beginning of subsequent cycles. $\Delta^9$-THC training consisted of two to three cycles, and vehicle training consisted of two to six cycles; completion of the FR on the correct lever was required for a reinforcer during each training cycle. The first test was conducted when, for 5 consecutive or for 6 of 7 days, at least 80% of the total responses occurred on the correct lever, and fewer than five responses (one FR) occurred on the incorrect lever before completion of the FR on the correct lever. Tests were conducted at least 3 days apart and only when performance for consecutive training sessions, including both vehicle and $\Delta^9$-THC training sessions, satisfied the same criteria described above. The type of training session preceding test sessions varied nonsystematically.

Test sessions were identical to training sessions except that five consecutive responses on either lever postponed the shock schedule and animals received vehicle or sham 15 min before sessions followed by vehicle at the beginning of the first cycle and cumulative doses of $\Delta^9$-THC, CP 55940, WIN 55212-2, ACPA, (*R*)-methanandamide, AM 1241, cocaine, ketamine, midazolam, or morphine in subsequent cycles with doses increasing by 0.25 or 0.5 log unit per cycle. Additional tests were conducted by administering SR 141716A (0.32–3.2 mg/kg) or AM 251 (0.32–3.2 mg/kg) 15 min before sessions in which vehicle was administered in the first cycle followed by cumulative doses of $\Delta^9$-THC, CP 55940, or WIN 55212-2 in subsequent cycles. A similar test was conducted with SR 144528 (3.2 mg/kg) followed by cumulative doses of $\Delta^9$-THC. Tests ended when greater than 80% of the total responses occurred on the $\Delta^9$-THC lever or when electric stimuli were delivered; tests with cocaine and morphine ended at doses of 1.0 and 3.2 mg/kg, respectively. The duration of action of the training dose (0.1 mg/kg i.v.) of $\Delta^9$-THC was determined by conducting six-cycle test sessions in 2-h increments after administration.

**Drugs.** SR 141716A base, SR 144528 base, the levo enantiomer of $\Delta^9$-THC (100 mg/ml in absolute ethanol) (The Research Technology Branch, National Institute on Drug Abuse, Rockville, MD), ACPA, WIN 55212-2 (Sigma, St. Louis, MO), AM 251, and CP 55940 (Tocris, Ellisville, MO) were dissolved in a 1:1:18 mixture of absolute ethanol, Emulphor-620 (Rhone-Poulenc Inc., Princeton, NJ), and physiologic saline. AM 1241 (Sigma) and (*R*)-methanandamide (5 mg/ml in absolute ethanol; Tocris) were diluted to a 1:6:13 mixture of Emulphor-620, absolute ethanol, and physiologic saline. Cocaine hydrochloride and morphine sulfate (The Research Technology Branch, National Institute on Drug Abuse) were dissolved in saline. Midazolam hydrochloride (5 mg/ml in physiologic saline; Roche Pharma Inc., Manati, Puerto Rico) and ketamine hydrochloride (100 mg/ml in physiologic saline; Fort Dodge Laboratories, Fort Dodge, IA) were diluted with physiologic saline. Drugs were administered i.v. in a volume of 0.03 to 3 ml/kg, and doses (milligrams per kilogram) were expressed as the weight of the forms listed above.

**Data Analyses.** Discrimination data were expressed as a percentage of the total responses occurring on the $\Delta^9$-THC lever averaged among monkeys (±S.E.M.) and were plotted as a function of dose. The potencies of cannabinoid agonists for producing discriminative stimulus effects were estimated by analyzing the dose-effect curves by simultaneously fitting straight lines to the individual dose-response data by means of GraphPad Prism version 4.02 for Windows (GraphPad, San Diego, CA), using the following equation: effect = slope × log(dose) + intercept. Straight lines were fitted to the linear portion of dose-effect curves, defined by doses producing 20 to 80% of the maximum effect, including not more than one dose producing less than 20% of the maximal effect. Other doses were excluded from the analyses. The same approach was used to establish the potency of an agonist alone and in combination with various doses of an antagonist.

The slopes of dose-effect curves were compared with an *F* ratio test using GraphPad. If the slopes were not significantly different, then a common, best-fitting slope was used for further analyses (for detailed examples of this approach, see Kenakin, 1997). Doses corresponding to $ED_{50}$, potency ratios, and their 95% confidence limits (CL) were calculated by parallel line analyses of data from individual subjects (Tallarida, 2000). The potencies of different agonists were considered significantly different when the 95% CL of their potency ratio did not include 1.

For antagonism of $\Delta^9$-THC, CP 55940, and WIN 55212-2, linear regression was used to examine whether the slope of the Schild plot was significantly different from unity. If the slope of the Schild plot was not different from unity (i.e., when the 95% CL included 1 and not 0), then the apparent p$A_2$ and corresponding 95% CL were calculated. For SR 141716A (0.32 and 1.0 mg/kg) in combination with WIN 55212-2, a single-dose apparent affinity estimate was calculated with the following equation: $pK_B = -\log[B/\text{dose ratio} - 1]$, with B expressed in moles per kilogram of body weight.

Control response rate represents the average of the five vehicle training sessions before the test. Response rate was calculated as a percentage of control for individual animals and was then averaged among subjects (±S.E.M.) and plotted as a function of dose. Discrimination data were not included for analysis when response rate for an individual was less than 20% of control for that monkey; however, response rate data were included in the group average. ACPA, AM 1241, and SR 144528 were studied in three monkeys; all other drug treatments were studied in four monkeys.

## Results

**Control Performance.** Three monkeys had previously satisfied the criteria for testing in 39, 44, and 48 training

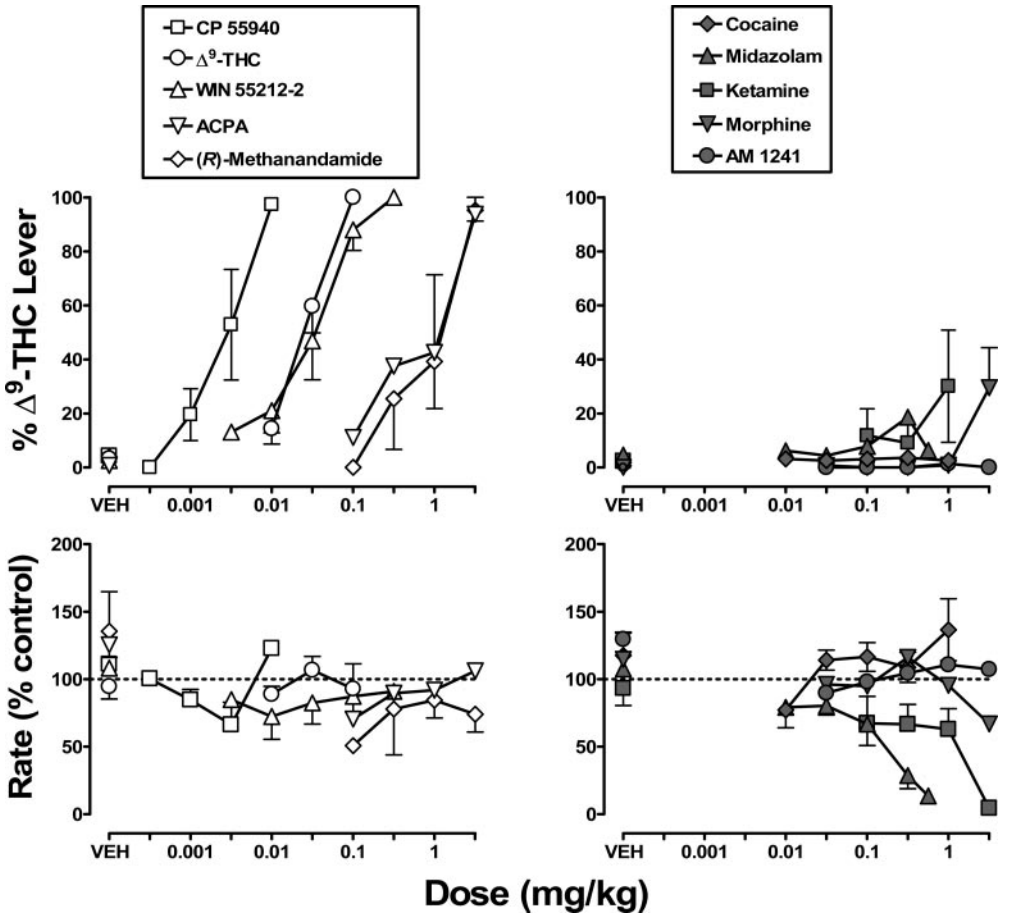


**Fig. 1.** Discriminative stimulus and rate effects of cannabinoid agonists and noncannabinoids in rhesus monkeys discriminating $\Delta^9$-THC (0.1 mg/kg i.v.). Abscissae, dose in milligrams per kilogram of body weight; VEH, vehicle. Ordinates, mean (±S.E.M.) percentage of responding on the $\Delta^9$-THC Lever (top) and mean (±S.E.M.) response rate expressed as a percentage of control (VEH training days) rate [Rate (percent control), bottom]. Data for ACPA and AM 1241 were averaged from three monkeys, and all other data were averaged from four monkeys.

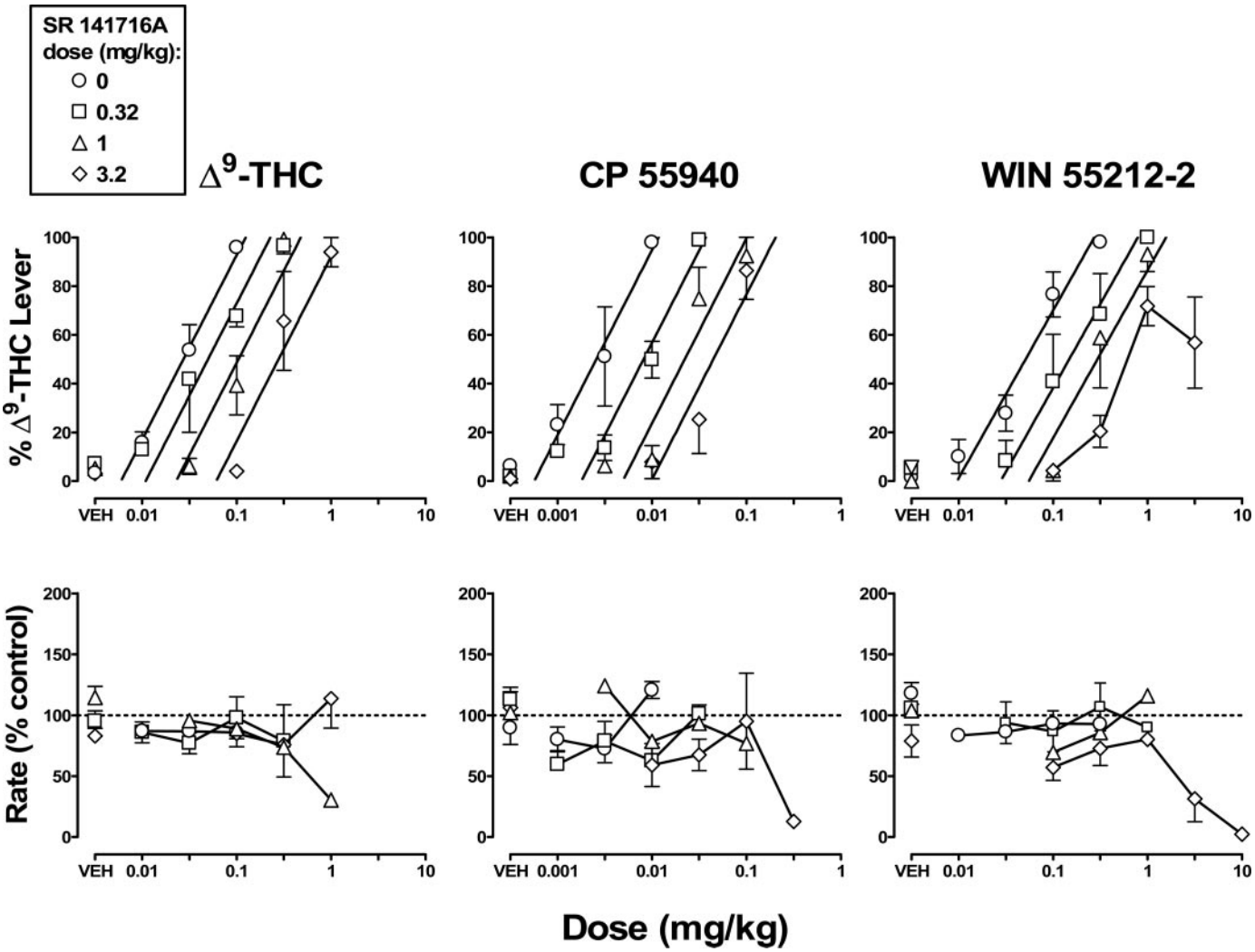


**Fig. 2.** Discriminative stimulus and rate effects of $\Delta^9$-THC (left), CP 55940 (middle), and WIN 55212-2 (right), alone and in combination with SR 141716A. Top, regression lines that were used to calculate $ED_{50}$s and 95% CL. SR 141716A was administered 15 min before the first cycle and 35 min before the first dose of agonist. Drug treatments were determined in four monkeys. Data for 1 mg/kg SR 141716A in combination with 1 mg/kg $\Delta^9$-THC and for 0.32 mg/kg CP 55940 and 10 mg/kg WIN 55212-2 were from one monkey. For other details, see Fig. 1.

sessions (including both vehicle and $\Delta^9$-THC training sessions) (McMahon et al., 2005). A fourth monkey added to this study satisfied the criteria for testing in 101 training sessions. Over 10 saline training sessions in which the criteria for testing were satisfied, average (±S.E.M.) rates of lever pressing for individual monkeys were 1.27 ± 0.09, 1.27 ± 0.08, 1.48 ± 0.10, and 0.65 ± 0.04 responses/s. Response rates averaged over the same number of $\Delta^9$-THC training sessions were 1.15 ± 0.11, 1.15 ± 0.05, 1.20 ± 0.04, and 0.69 ± 0.04 responses/s for the same monkeys, respectively.

**Discriminative Stimulus and Rate Effects of $\Delta^9$-THC, Other Cannabinoid Agonists, and Noncannabinoids.** The time course of the $\Delta^9$-THC discriminative stimulus was established by administering the training dose (0.1 mg/kg i.v.) in 2-h increments before 2-h test sessions. $\Delta^9$-THC (0.1 mg/kg i.v.) produced greater than 80% of responses on the drug-appropriate lever within 20 min and continued to do so for up to 4 h; responding was less than 20% on the drug-appropriate lever within 8 h (data not shown). To establish the potency of $\Delta^9$-THC, cumulative doses were administered within a single session; under these conditions, increasing doses of $\Delta^9$-THC increased drug lever responding such that the training dose (0.1 mg/kg) produced 100% of responses on the $\Delta^9$-THC lever (Fig. 1, top left, circles). Cumulative doses of CP 55940, WIN 55212-2, ACPA, and (*R*)-methanandamide also produced high levels of responding on the $\Delta^9$-THC lever (Fig. 1, top left). A test for parallel lines revealed that the dose-effect curves for discriminative stimulus effects were parallel for CP 55940, $\Delta^9$-THC, WIN 55212-2, ACPA, and (*R*)-methanandamide. $ED_{50}$s (95% CL) were 0.0025 (0.0014–0.0045) mg/kg for CP 55940, 0.025 (0.018–0.034) mg/kg for $\Delta^9$-THC, 0.035 (0.021–0.058) mg/kg for WIN 55212-2, 0.79 (0.24–2.6) mg/kg for ACPA, and 0.89 (0.47–1.7) mg/kg for (*R*)-methanandamide. CP 55940 was relatively more potent than $\Delta^9$-THC and WIN 55212-2, which were equipotent; each of these cannabinoid agonists was significantly more potent than ACPA and (*R*)-methanandamide, which were equipotent. Up to the smallest doses that produced greater than 80% of responses on the $\Delta^9$-THC lever, the cannabinoid agonists did not systematically modify response rate (Fig. 1, bottom left).

Up to a dose of 3.2 mg/kg AM 1241 produced minimal responding on the $\Delta^9$-THC lever; similar results were obtained with the noncannabinoids cocaine, midazolam, ket-

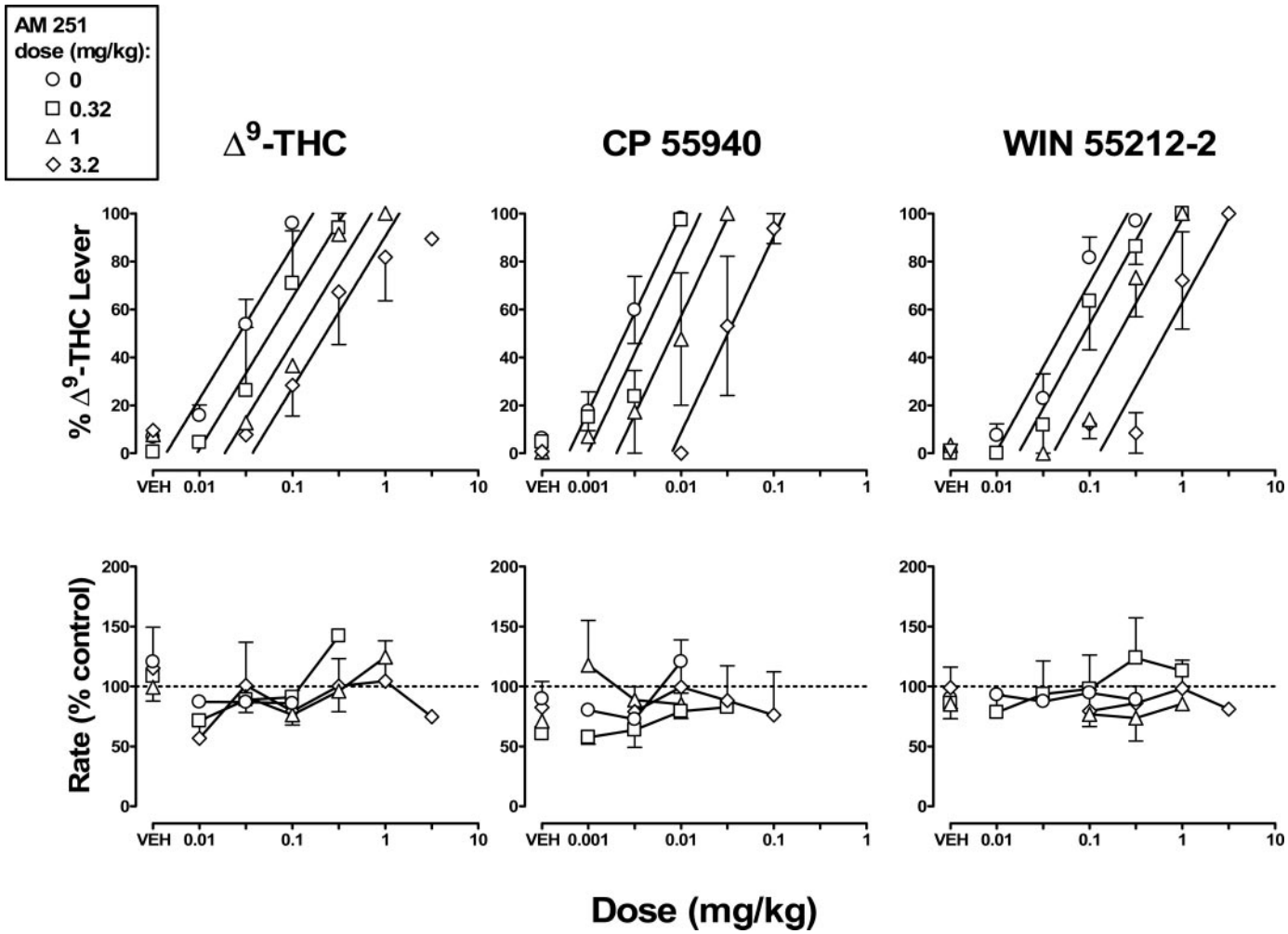


**Fig. 3.** Discriminative stimulus and rate effects of $\Delta^9$-THC (left), CP 55940 (middle), and WIN 55212-2 (right), alone and in combination with AM 251. Top, regression lines that were used to calculate $ED_{50}$s and 95% CL. AM 251 was administered 15 min before the first cycle and 35 min before the first dose of agonist. Drug treatments were determined in four monkeys. Data for 3.2 mg/kg $\Delta^9$-THC and WIN 55212-2 were from one monkey. For other details, see Fig. 1.

amine, and morphine (Fig. 1, top right). AM 1241 was studied up to the largest dose that could be administered i.v., and noncannabinoids were studied up to doses that decreased response rate to less than 20% of the control response rate (midazolam and ketamine; Fig. 1, bottom right), that slightly increased response rate (cocaine; Fig. 1, bottom right), or that were previously demonstrated to exert discriminative stimulus effects in rhesus monkeys (cocaine and morphine; e.g., McMahon et al., 2004).

**Antagonism of the Discriminative Stimulus Effects of $\Delta^9$-THC, CP 55940, and WIN 55212-2.** Up to a dose of 3.2 mg/kg, SR 141716A resulted in predominantly vehicle lever responding and did not modify response rate (Fig. 2, top and bottom, VEH). SR 141716A surmountably and dose-dependently antagonized the discriminative stimulus effects of $\Delta^9$-THC and CP 55940 (Fig. 2, top left and middle). Doses of 0.32, 1.0, and 3.2 mg/kg SR 141716A increased the $ED_{50}$ of $\Delta^9$-THC by 1.8-, 3.8-, and 10-fold, respectively, and increased the $ED_{50}$ of CP 55940 by 3.2-, 8.6-, and 17-fold, respectively. On average, response rate was not systematically altered by SR 141716A in combination with $\Delta^9$-THC or CP 55940, with the exception that response rate was decreased in one monkey at a dose of 1 mg/kg SR 141716A in combination with 1 mg/kg $\Delta^9$-THC (Fig. 2, bottom left, right-most triangle) and in the same monkey at a dose of 3.2 mg/kg SR 141716A in combination with 0.32 mg/kg CP 55940 (Fig. 2, bottom middle, right-most diamond).

SR 141716A dose-dependently antagonized the discriminative effects of WIN 55212-2 (Fig. 2, top right); however, surmountable antagonism was observed only at 0.32 and 1.0 mg/kg SR 141716A (i.e., the $ED_{50}$ of WIN 55212-2 was increased 2.9- and 4.3-fold, respectively). After administration of 3.2 mg/kg SR 141716A, maximal levels of $\Delta^9$-THC-lever responding were 72% at 1 mg/kg WIN 55212-2, and response rate was markedly decreased at 3.2 and 10 mg/kg WIN 55212-2 (Fig. 2, bottom right, diamonds).

Up to a dose of 3.2 mg/kg, AM 251 resulted in predominantly vehicle lever responding and did not modify response rate (Fig. 3, top and bottom, VEH). AM 251 surmountably and dose-dependently antagonized the discriminative stimulus effects of $\Delta^9$-THC, CP 55940, and WIN 55212-2 (Fig. 3, top). Doses of 0.32, 1.0, and 3.2 mg/kg AM 251 increased the $ED_{50}$ of $\Delta^9$-THC by 2.1-, 4.2-, and 8.6-fold, respectively, increased the $ED_{50}$ of CP 55940 by 1.7-, 3.3-, and 7.0-fold, respectively, and increased the $ED_{50}$ of WIN 55212-2 by 1.9-, 4.2-, and 11-fold, respectively. At the doses studied, response

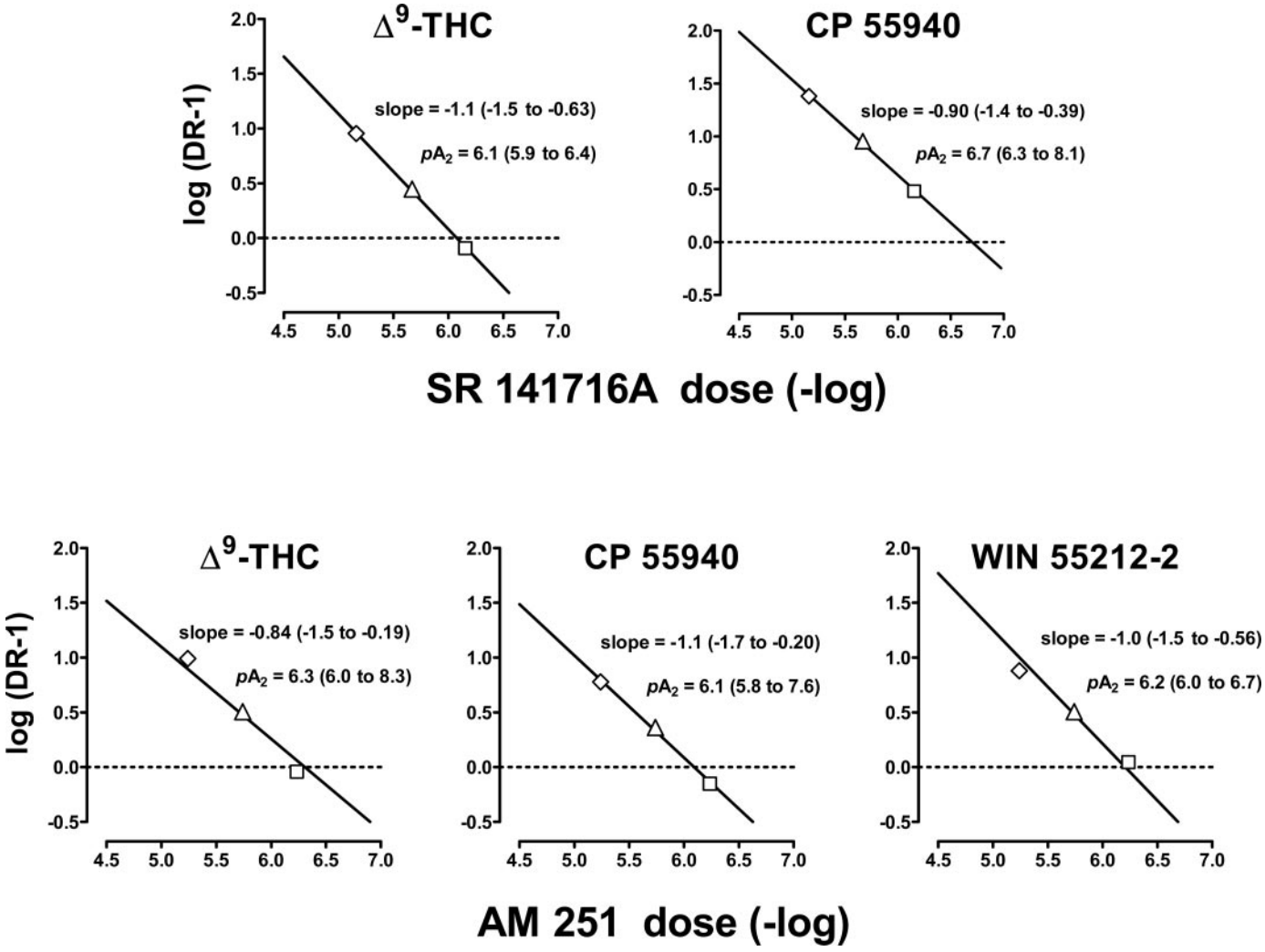


**Fig. 4.** Schild plots constructed from the mean data shown in Fig. 2 (top) and Fig. 3 (bottom). Abscissae, negative logarithm of the dose in mol/kg. Ordinates, logarithm of the dose ratio −1. Values in parentheses represent the 95% CL.

rate was not systematically altered by AM 251 in combination with $\Delta^9$-THC, CP 55940, or WIN 55212-2 (Fig. 3, bottom).

Figure 4 shows the Schild plots for SR 141716A antagonism of $\Delta^9$-THC and CP 55940 (top) and for AM 251 antagonism of $\Delta^9$-THC, CP 55940, and WIN 55212-2 (bottom). The coefficient of determination ($r^2$) was 0.99 for each line, and the slopes were not significantly different from unity (i.e., −1). For SR 141716A, apparent p$A_2$ values (95% CL) were 6.1 (5.9–6.4) in the presence of $\Delta^9$-THC and 6.7 (6.3–8.1) in the presence of CP 55940. Surmountable antagonism of WIN 55212-2 was obtained with only two doses of SR 141716A; therefore, Schild analysis could not be conducted. Single-dose apparent affinity analysis for 0.32 and 1.0 mg/kg SR 141716A yielded p$K_B$ values of 6.4 and 6.2, respectively, in combination with WIN 55212-2. For AM 251, apparent p$A_2$ values (95% CL) were 6.3 (6.0–8.3) in the presence of $\Delta^9$-THC, 6.1 (5.8–7.6) in the presence of CP 55940, and 6.2 (6.0–6.7) in the presence of WIN 55212-2.

SR 144528, at the largest dose (3.2 mg/kg) that could be administered i.v., did not significantly modify the discriminative stimulus effects of $\Delta^9$-THC (Fig. 5, top). Thus, the $ED_{50}$ of $\Delta^9$-THC (0.035 mg/kg) determined in the presence of SR 144528 was not significantly different from the control $ED_{50}$ (0.028 mg/kg), i.e., the 95% CL (0.6–2.7) of the potency ratio (1.2) included 1. Response rate was slightly increased after administration of SR 144528 (3.2 mg/kg) in two of the three monkeys tested (Fig. 5, bottom).

## Discussion

Cannabinoid agonists [CP 55940, WIN 55212-2, ACPA, and (*R*)-methanandamide] substituted for the discriminative stimulus effects of $\Delta^9$-THC (0.1 mg/kg i.v.) in rhesus monkeys. The $\Delta^9$-THC discriminative stimulus was pharmacologically selective insofar as noncannabinoids (cocaine, ketamine, midazolam, and morphine) did not mimic $\Delta^9$-THC. The $CB_1$ antagonists SR 141716A and AM 251 surmountably antagonized the discriminative stimulus effects of $\Delta^9$-THC, CP 55940, and WIN 55212-2, and Schild analysis was consistent with simple, competitive interactions (i.e., slopes of the Schild plots were not different from unity). SR 141716A and AM 251 had similar apparent p$A_2$ values (i.e., were equipotent), and their apparent p$A_2$ values were similar in the presence of $\Delta^9$-THC, CP 55940, and WIN 55212-2. The selective $CB_2$ agonist AM 1241 did not mimic $\Delta^9$-THC, and

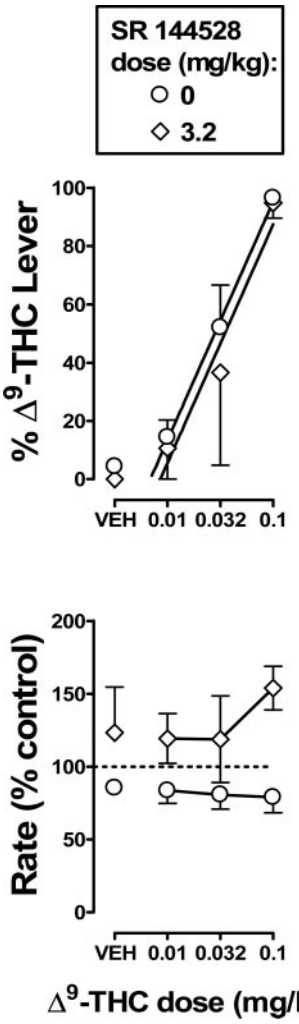


**Fig. 5.** Discriminative stimulus and rate effects of $\Delta^9$-THC in combination with SR 144528. Top, regression lines that were used to calculate $ED_{50}$s and 95% CL. SR 145228 was administered 15 min before the first cycle and 35 min before the first dose of agonist. Data were averaged from three monkeys. For other details, see Fig. 1.

the $CB_2$ antagonist SR 144528 (3.2 mg/kg) did not antagonize $\Delta^9$-THC. The results suggest that the same receptors, probably $CB_1$, mediate the discriminative stimulus effects of $\Delta^9$-THC, CP 55940, and WIN 55212-2.

Relative to other behavioral assays, drug discrimination possesses a high degree of pharmacologic selectivity, i.e., only cannabinoid agonists share discriminative stimulus effects with $\Delta^9$-THC (Balster and Prescott, 1992; Wiley et al., 1995a). Diazepam was one exception (Wiley and Martin, 1999); in the current study, however, midazolam did not mimic $\Delta^9$-THC. Midazolam and diazepam seem to have the same mechanism of action at benzodiazepine receptors (Lelas et al., 2000); therefore, differences across studies might reflect different species (monkeys versus rats) or routes of drug administration (i.v. versus i.p.). Failure of noncannabinoids to substitute for the $\Delta^9$-THC discriminative stimulus further underscores the pharmacologic selectivity of drug discrimination relative to other behavioral assays (e.g., Wiley and Martin, 2003).

The cannabinoid agonists chosen for study vary in their selectivity for cannabinoid receptor subtypes. CP 55940 and WIN 55212-2 are relatively nonselective for $CB_1$ and $CB_2$ receptors (e.g., Hillard et al., 1999), and they substituted for the discriminative stimulus effects of $\Delta^9$-THC with a relative potency similar to that reported previously (Gold et al., 1992; Wiley et al., 1995a). (*R*)-Methanandamide and ACPA are analogs of anandamide that have 41- and 325-fold selectivity for $CB_1$ relative to $CB_2$ receptors (Khanolkar et al., 1996; Hillard et al., 1999), and both compounds substituted for the discriminative stimulus effects of $\Delta^9$-THC, suggesting that endocannabinoid-related agonists share a mechanism of action with $\Delta^9$-THC. However, failure of (*R*)-methanandamide to substitute for the discriminative stimulus effects of $\Delta^9$-THC under some conditions suggests that the mechanism of action of these agonists is not identical (Järbe et al., 1998).

By comparing the potency of an antagonist to block the effects of different agonists, Schild analysis can be used to examine whether the same receptors mediate the behavioral effects of agonists (Woods et al., 1992; Paronis and Bergman, 1999). If the slope of a Schild plot is not different from unity (i.e., consistent with simple, competitive antagonism), then the apparent affinity (p$A_2$) of an antagonist can be calculated, providing an estimate of the $K_B$ of the antagonist. Moreover, if the apparent p$A_2$ of an antagonist is the same in the presence of different agonists, then the same receptors are concluded to mediate the effects of the agonists. SR 141716A and AM 251 surmountably antagonized the discriminative stimulus of $\Delta^9$-THC, CP 55940, and WIN 55212-2; the slopes of the Schild plots were not significantly different from $-1$, consistent with a significant, competitive interaction. SR 141716A and AM 251 were equipotent (e.g., apparent p$A_2$ values of 6.1 and 6.3, respectively, in the presence of $\Delta^9$-THC), and the apparent p$A_2$ values of an antagonist were similar in the presence of different agonists, suggesting that the same receptors mediate the discriminative stimulus effects of $\Delta^9$-THC, CP 55940, and WIN 55212-2. $CB_2$ receptors do not seem to mediate the $\Delta^9$-THC discriminative stimulus inasmuch as AM 1241 did not mimic $\Delta^9$-THC, and SR 144528 (3.2 mg/kg) did not antagonize $\Delta^9$-THC (also Järbe et al., 2006). However, i.v. administration of AM 1241 and SR 144528 failed to exert prominent behavioral effects (i.e., alterations in rates of lever pressing), and studies with larger doses of these compounds could potentially establish a role for $CB_2$ receptors in the discriminative stimulus effects of $\Delta^9$-THC.

Schild analysis could not be conducted with SR 141716A in combination with WIN 55212-2 because responding was markedly decreased at relatively large doses. Single-dose apparent affinity estimates (p$K_B$ values) were 6.4 and 6.2 for SR 141716A at doses of 0.32 and 1 mg/kg, respectively, in combination with WIN 55212-2; these values were similar to the apparent p$A_2$ values calculated for SR 141716A. Previous studies noted that SR 141716A did not consistently antagonize the effects of cannabinoid agonists on rate of responding (Järbe et al., 2003; McMahon et al., 2005), and these limitations might reflect intrinsic activity of SR 141716A (e.g., inverse agonism or blockade of endogenous tone at cannabinoid or other receptors; for review, see Pertwee, 2006). That similar disruptions in responding were not obtained with AM 251 in combination with WIN 55212-2 might reflect differences in the mechanism of action between SR 141716A and AM 251.

In summary, this study demonstrates the utility of Schild analysis for establishing similarities in the mechanism of

action by which cannabinoids exert their behavioral activity and further demonstrates the pharmacologic selectivity of cannabinoid agonist discriminations. The apparent $pA_2$ value of SR 141716A was similar in the presence of $\Delta^9$-THC and CP 55940, and the apparent $pA_2$ value of AM 251 was similar in the presence of $\Delta^9$-THC, CP 55940, and WIN 55212-2, suggesting that the same cannabinoid receptors mediate the discriminative stimulus effects of these cannabinoid agonists. These results are consistent with the general view that a single receptor subtype ($CB_1$) mediates many of the behavioral effects of cannabinoids, a view that probably underlies the infrequent use of Schild analysis to quantify cannabinoid antagonism in vivo. Recent studies with $CB_2$ receptor-selective ligands, however, suggest that $CB_2$ receptors mediate some behavioral effects of cannabinoids (antinociception; Ibrahim et al., 2006), and Schild analysis could be used to examine receptor subtypes that mediate antinociceptive and, perhaps, other behavioral effects of cannabinoids.

**Acknowledgments**

I thank B. Harrington, M. Hernandez, D. Logan, and R. Luna for providing technical assistance. L. R. Gerak provided valuable comments on the interpretation of Schild analysis.

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