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A Comparison of the Discriminative Stimulus Properties of Δ^9-Tetrahydrocannabinol and CP 55,940 in Rats and Rhesus Monkeys[1]

LISA H. GOLD, ROBERT L. BALSTER, RITA L. BARRETT, DARRELL T. BRITT and BILLY R. MARTIN
Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia
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ABSTRACT

CP 55,940 {(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol} is a potent bicyclic analog of Δ^9-tetrahydrocannabinol (THC) which has been used as a probe for a cannabinoid recognition site in neural tissue. In the present study, CP 55,490 was evaluated for Δ^9-THC-like effects in rats and rhesus monkeys trained to discriminate Δ^9-THC from vehicle. Rats trained to discriminate Δ^9-THC (3.0 mg/kg i.p.) from vehicle were tested with various doses of Δ^9-THC and CP 55,940 at both 30 and 90 min postinjection. Catalepsy was measured immediately after these operant tests using an adaptation of the mouse ring-test. In rats, CP 55,940 substituted for Δ^9-THC at both 30 and 90 min postinjection at a dose of 0.1 mg/kg that had minimal effects on rates of responding. Doses of Δ^9-THC (>3.0 mg/kg) and CP 55,940 (>0.1 mg/kg) that reduced response rates by greater than 50% also produced substantial increases in catalepsy. CP 55,940 and Δ^9-THC had a similar time course for discriminative stimulus effects, but CP 55,940 was about 30 times more potent. In monkeys, the training dose of Δ^9-THC ranged from 0.04 to 0.16 mg/kg i.m., adjusted individually to minimize response-rate disruption. After training, monkeys were tested with various doses of Δ^9-THC and CP 55,940 at 30 min postinjection. CP 55,940 fully substituted for Δ^9-THC in all four monkeys; the lowest dose needed to produce greater than 80% drug-lever responding ranged from 0.1 to 17 μg/kg. Marked ataxia, sedation and ptosis were observed in the monkeys after the highest doses of both cannabinoids. In summary, CP 55,940 produced discriminative stimulus effects similar to those of Δ^9-THC in both rats and rhesus monkeys with a potency consistent with its high affinity for a cannabinoid recognition site in brain tissue. These results support the use of CP 55,940 as a probe for the neural substrates mediating the behavioral effects of Δ^9-THC.

Δ^9-THC is the principle active ingredient in marijuana (Gaoni and Mechoulam, 1964). It follows that, in animals, Δ^9-THC produces many of the same pharmacological and behavioral effects associated with marijuana intoxication in humans (Edery *et al.*, 1971; Schulze *et al.*, 1988). CP 55,940 {(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol} is a bicyclic cannabinoid which was originally synthesized for its potent analgesic properties (Johnson and Melvin, 1986), and is the ligand currently being used to label (Devane *et al.*, 1988), characterize (Howlett *et al.*, 1988) and localize (Herkenham *et al.*, 1990) a cannabinoid recognition site in neural tissue and to evaluate the recently cloned receptor (Matsuda *et al.*, 1990). CP 55,940 has been shown to produce some biochemical and behavioral effects in common with Δ^9-THC (Howlett *et al.*, 1990a; Little *et al.*, 1988), although it has not been tested in humans.

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If CP 55,940 is to be used to probe molecular and biochemical actions of cannabinoids that are relevant to their abuse, it is important to establish that it produces behavioral effects similar to Δ^9-THC in models which assess the psychoactive properties of drugs. The discriminative stimulus properties of drugs in animals are often predictive of their subjective effects in humans (Balster, 1990; Schuster and Johanson, 1988), and studies of the discriminative stimulus properties of drugs are useful for predicting their abuse liability (Balster, 1990, 1991; Brady *et al.*, 1990; Holtzman, 1990). A number of drug discrimination studies have been carried out with cannabinoids showing that Δ^9-THC can serve as an effective discriminative stimulus in rats and pigeons (Balster and Ford, 1978; Järbe and McMillan, 1980; Krimmer and Barry, 1977). The ability of cannabinoids to produce Δ^9-THC-like discriminative stimulus effects correlates well with their ability to produce marijuana-like subjective effects in humans and, furthermore, drugs which do not produce marijuana-like effects in humans fail to substitute for Δ^9-THC in drug discrimination studies (Balster and

ABBREVIATIONS: THC, tetrahydrocannabinol; VEH, vehicle; FR, fixed-ratio; %DLR, percentage of drug-lever responding; MED, minimally effective dose.

Prescott, 1992). This general concordance between results in animal and human testing supports the use of Δ^9-THC discrimination in animals as a measure of cannabinoid effects relevant to cannabis abuse.

The purpose of the present study was to evaluate the efficacy and potency of CP 55,940 to produce Δ^9-THC-like discriminative stimulus effects in rats and rhesus monkeys. Although rats have been used extensively in cannabinoid discrimination research (Balster and Prescott, 1992), the effects of Δ^9-THC or other cannabinoids in monkeys have not been well characterized using drug discrimination procedures. Only one previous study in monkeys has established a discrimination based on Δ^9-THC (Ferraro *et al.*, 1974), and in another study the discriminative stimulus effects of the cannabinoid levonantradol were evaluated in rhesus monkeys trained on opioid discriminations (Young *et al.*, 1981). Thus, a second aim of the present study was to confirm the results of Ferraro *et al.* (1974) that Δ^9-THC discrimination can be established in monkeys and to explore further the conditions suitable for training. Finally, in a previous study (Prescott *et al.*, 1992) it was found that Δ^9-THC-like discriminative stimulus effects were accompanied by catalepsy at higher doses. A final aim of this study was to compare the specificity of both Δ^9-THC and CP 55,940 for producing discriminative stimulus and cataleptic effects in rats.

Materials and Methods

Subjects. Sixteen male Sprague-Dawley rats (Dominion Laboratories, Dublin, VA) were housed individually in a temperature-controlled environment under a normal 12-hr light/dark cycle. They had free access to water and were maintained at approximately 300 g by controlled feeding. Four adult male rhesus monkeys (7.3–11.4 kg) were housed individually in a temperature-controlled environment under a normal 12-hr light/dark cycle. Two monkeys had prior drug histories in i.v. drug self-administration research, one monkey was only modestly drug experienced and one monkey was drug naive. Monkeys were 20-hr meal-deprived before each daily experimental session (Monday-Friday) and received 130 to 200 g of supplemental food plus a multivitamin after daily sessions. Water was available at all times.

Drugs. Δ^9-THC was obtained from the National Institute on Drug Abuse (Rockville, MD) and dissolved in a 1:1 (v:v) mixture of absolute ethanol and Emulphor-620 (Rhône-Poulenc Inc., Paris, France) in order to make a stock solution of 100 mg/ml. Final drug concentrations were prepared by adding saline to this stock to form a clear, homogeneous suspension which consisted of emulphor-ethanol-saline (1:1:18). Further dilutions were made with the emulphor-ethanol-saline (1:1:18) VEH. Fresh solutions of the training drug were made at a minimum of once per week. CP 55,940 {(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol}, obtained from Dr. Lawrence Melvin (Pfizer Inc., Groton, CT), was also dissolved in a 1:1 (v:v) mixture of ethanol-emulphor to a concentration of 10 mg/ml. Final test concentrations were prepared by adding saline to this stock to form a suspension of emulphor-ethanol-saline (1:1:18) and diluting further with VEH. The molecular weights of Δ^9-THC and CP 55,940 are 314.5 and 376.7, respectively.

Rat drug discrimination procedure. Rats were trained to discriminate 3.0 mg/kg i.p. of Δ^9-THC from VEH as described previously (Prescott *et al.*, 1992). Briefly, rats were trained in a 2-lever operant procedure under a FR-10 schedule of food reinforcement during daily 10-min sessions. Δ^9-THC and VEH were administered 30 min presession and were given according to a double alternation sequence. During training sessions, responding on the injection-appropriate lever was reinforced, whereas responding on the incorrect lever reset the FR response requirement. Responding on both levers was reinforced during the 2-min test sessions. Rats were tested at both 30- and 90-min postinjection by using the repeat testing method (Järbe *et al.*, 1981).

Rats were trained in approximately 25 to 30 sessions and had been used to determine the discriminative stimulus properties of a number of test compounds before being entered into the experiments presented here. Test sessions were typically conducted on Tuesdays and Fridays with a double alternation sequence of Δ^9-THC and VEH continuing on Mondays, Wednesdays and Thursdays. Tests were conducted initially with a number of doses of Δ^9-THC (0.01–10.0 mg/kg i.p.). Next, CP 55,940 was tested for stimulus generalization (0.003–0.3 mg/kg) and a time course for CP 55,940 was determined by administering 0.1 mg/kg 90, 180, 360 and 600 min before separate test sessions. Then, the duration of stimulus effects of Δ^9-THC (3.0 mg/kg) was assessed. Control tests with each of the training conditions both preceded and followed each experimental sequence.

Data for drug discrimination are presented as the %DLR and overall rates of responding (responses/second) during control and generalization tests. The data are averages of results in all subjects except when response rates were below 0.05 responses/second or the rats failed to demonstrate appropriate stimulus control on the previous training day. In those cases, the %DLR for that test was not included in the group average. The reliability of response-rate suppression relative to rates after VEH administration was determined by paired t tests. ED_{50} values (and 95% CL) were also obtained from the group data by least-squares linear regression of the linear portion of the dose-effect curves for both %DLR and response-rate effects after $\log_{10}$ transformation of dose. To determine doses estimated to reduce response rates by 50%, the response rates for each subject after drug administration were expressed as the percentage of the average of the response rates on VEH tests conducted before and after the dose-effect curve determination for that subject.

Rat catalepsy procedure. Catalepsy was quantified by an adaptation of the mouse ring-test (Pertwee, 1972). Rats were placed on a wire ring (13 cm in diameter) with a wooden backboard (40 cm in diameter) attached to a ring-stand approximately 60 cm above the bench-top. Five minutes of behavior were recorded using a video camera. The time during the 5-min period in which the animal remained motionless was measured by computer-based image analysis of the videotapes. The catalepsy tests were conducted on test sessions immediately after the rats were removed from the operant chambers. Results were recorded as seconds of immobility (maximum of 300) and reported as group mean (±S.E.M.) of the percentage of the 300-sec observation period that subjects were immobile.

Rhesus monkey drug discrimination. Monkeys remained in their home cages for training and testing. Removable panels equipped with two response levers and associated stimulus lights were constructed using standard operant components (Coulbourn Instruments, Lehigh Valley, PA) and 1-g pellet dispensers (BRS/LVE, Laurel, MD). A light was located above each lever and a food receptacle was located between the levers. Panels were attached to the cages of the monkeys during experimental sessions. Data were collected using an IBM computer through a Logic "1" interface (MED Associates, East Fairfield, VT) utilizing MED-PC software (MED Associates).

Monkeys were trained initially to lever press on both levers for food pellet reinforcement during daily 30-min sessions. Availability of food was signaled by illumination of the stimulus lights above the levers and pellet delivery was associated with illumination of the food receptacle. Drug discrimination training began when the monkeys responded at approximately equivalent rates on both levers. Monkeys were injected with Δ^9-THC or VEH, i.m., 30 min before each session. Of several different formulations and routes of administration tested in rhesus monkeys, i.m. injection of Δ^9-THC in emulphor-ethanol-saline VEH has been determined to yield the greatest bioavailability and shortest time to peak effect (Perlin *et al.*, 1985). All monkeys were started on a training dose of 0.2 mg/kg, but this dose had to be lowered individually in order to minimize response-rate disruption. Monkeys were trained according to a double alternation schedule (*i.e.*, Δ^9-THC, Δ^9-THC, VEH, VEH, Δ^9-THC, etc.). The FR for food reinforcement was raised gradually to either 100 (n = 3) or 50 (n = 1). For two monkeys, responding on the left lever was reinforced after Δ^9-THC injections

and responding on the right lever was reinforced after VEH injections. The contingencies were reversed for the other two monkeys. Responses on the incorrect lever reset the FR requirement on the correct lever.

Acquisition of the discrimination was evaluated by determining if the first FR of each session was completed on the correct (injection-appropriate) lever. When 8 of 10 sessions occurred with correct first FRs, subjects were switched to a trials procedure. One monkey was moved into the trials procedure before reaching this criterion in order to facilitate acquisition of the discrimination. Daily training sessions were increased to a final 65-min session duration and consisted of four 5-min trials alternating with three 15-min timeout periods. The final individual training doses of Δ⁹-THC were 0.1 (n = 2), 0.16 (n = 1) and 0.04 mg/kg (n = 1). The concentration of the drug solution was held constant at 1.2 mg/ml resulting in an injection volume ranging from 0.3 to 1.4 ml. Monkeys that achieved the correct first FR in the first trial for four consecutive training days were moved into the testing phase. The entire training phase took between 11 and 13 months.

In order to be tested, monkeys had to complete the first FR on the correct lever during the first trial of the training day before testing. If monkeys did not meet this criterion, they were provided with continued training until they did. Test sessions were conducted on Tuesdays and Fridays and lasted for 5 min. Completed FRs on either lever were reinforced whereas responses on one lever reset the FR requirement on the other lever. Initially, monkeys were given a control test with each of the training conditions (Δ⁹-THC and VEH). Further testing was initiated once a successful control test with each training condition was completed, *i.e.*, monkeys were required to complete the first FR on the correct lever and maintain greater than 80% correct-lever responding. After the monkeys had met these criteria, stimulus generalization tests with Δ⁹-THC and then CP 55,940 were begun. Control tests with each of the training conditions both preceded and followed each dose-effect curve. Doses of CP 55,940 were administered i.m., in a volume of 0.1 ml/kg, 30 min presession.

Because different training doses of Δ⁹-THC were used, the results of control and substitution tests in monkeys are presented for individual subjects. Reported are both the %DLR and overall rates of responding. MEDs for each monkey were obtained for the production of Δ⁹-THC-like discriminative stimulus effects defined as the lowest dose resulting in > 80 %DLR.

Results

Dose-effect curves for Δ⁹-THC and CP 55,940 in the rat. Control tests at both 30 (fig. 1) and 90 (fig. 2) min after administration of the training dose of Δ⁹-THC (3 mg/kg) produced averages of greater than 80 %DLR, whereas tests with VEH produced less than 30 %DLR (*i.e.*, greater than 70% responding on the VEH lever). For control tests conducted after 30 min, Δ⁹-THC and VEH resulted in similar response rates (fig. 1); however, by 90 min after administration, response rates after 3.0 mg/kg of Δ⁹-THC were slightly, but significantly ($P < .05$), lower than VEH when the averages of the control points preceding both cannabinoid dose-effect evaluations were compared (fig. 2).

The results of stimulus generalization tests with different doses of Δ⁹-THC and CP 55,940 at the 30-min postinjection interval are shown in figure 1. Δ⁹-THC produced a dose-dependent generalization from the training dose of 3.0 mg/kg. The graded nature of the dose-effect curve results from averaging subjects whose lever selection tended to be exclusively on one lever or the other. Response rates were decreased with the 10 mg/kg dose of Δ⁹-THC, with only 4 of 10 rats responding at greater than 0.05 responses/sec. CP 55,940 was also dose-dependently generalized from Δ⁹-THC, but was considerably more potent. A dose of 0.1 mg/kg produced greater than 80 %DLR in 8 of 10 rats and was accompanied by only modest

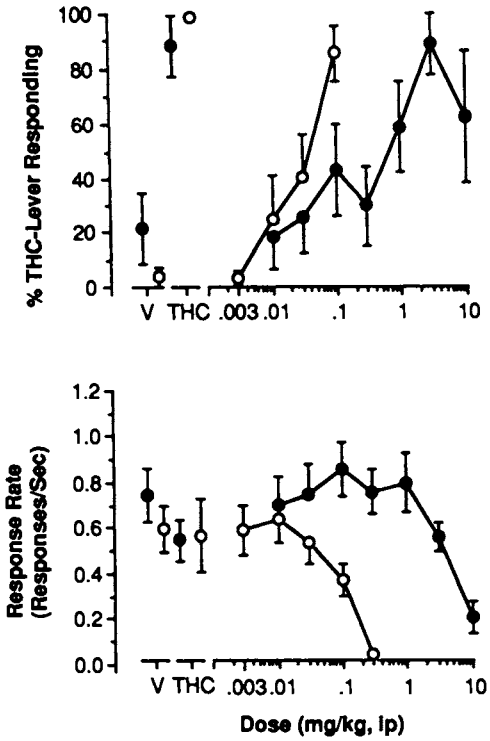

Fig. 1. Percentage of Δ⁹-THC-lever responding (upper panel) and response rates (lower panel) 30 min after Δ⁹-THC (●) and CP 55,940 (○) administered to rats trained to discriminate Δ⁹-THC (3.0 mg/kg i.p.) from VEH. V and THC, corresponding VEH and Δ⁹-THC control tests conducted before each dose-effect curve determination. Values represent the mean (±S.E.) for 8 to 10 rats, except the highest dose of Δ⁹-THC for which only 4 rats are included in the mean for percentage of Δ⁹-THC-lever responding.

response-rate suppression. With the next higher dose tested, 0.3 mg/kg, none of the rats (total of 10) responded greater than 0.05 responses/sec. The results of stimulus generalization tests with different doses of Δ⁹-THC and CP 55,940 at the 90-min postinjection interval are presented in figure 2. There was very little difference in the dose-effect curves for %DLR and response-rate effects of both Δ⁹-THC and CP 55,940 at 30 and 90 min postinjection.

Potency estimates in both milligrams per kilogram and micromoles per kilogram for the discriminative and response-rate effects of Δ⁹-THC and CP 55,940 at 30 min postinjection are shown in table 1. Comparing ED_{50} values, CP 55,940 was 28-fold more potent than Δ⁹-THC for engendering Δ⁹-THC-lever responding and 88-fold more potent for decreasing rates of responding. MEDs in both milligrams per kilogram and micromoles per kilogram for substitution are also presented in table 1 for comparison with similar data in monkeys.

Doses of Δ⁹-THC between 0.1 and 3.0 mg/kg produced less than 20% immobility (*i.e.*, less than 60 sec of immobility in a 300-sec session) when measured after both the 30- and 90-min operant tests (fig. 3). VEH injection was associated with approximately 30 sec of immobility, or 10% time immobile. Only the 10-mg/kg dose of Δ⁹-THC, which was associated with marked response-rate suppression in the operant task, was associated with catalepsy (approximately 40% of the 5-min session). CP 55,940 produced similar degrees of catalepsy (less than 20% time immobile) over a dose range of 0.003 to 0.1 mg/kg. The highest dose of CP 55,940, 0.3 mg/kg, which abolished responding in the operant procedure, produced immobility for

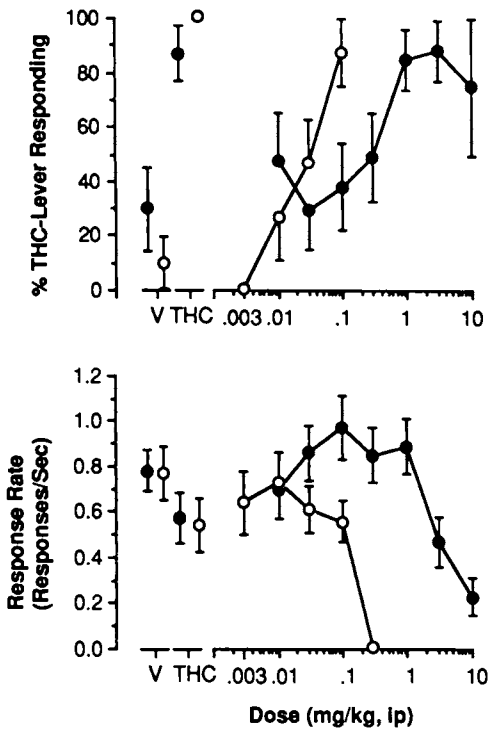

Fig. 2. Percentage of Δ^9-THC-lever responding (upper panel) and response rates (lower panel) 90 min after Δ^9-THC (●) and CP 55,940 (○) administered to rats trained to discriminate Δ^9-THC (3.0 mg/kg i.p.) from VEH. V and THC, corresponding VEH and Δ^9-THC (3.0 mg/kg) control tests conducted before each dose-effect curve determination. Values represent the mean (±S.E.) for 8 to 10 rats, except the highest dose of Δ^9-THC for which only 4 rats are included in the mean for percentage of Δ^9-THC-lever responding.

30% of the time at the 30-min postinjection interval and 53% at the 90-min postinjection interval.

Time course for Δ^9-THC and CP 55,940 in rats. Stimulus generalization was measured at various times after injection with either Δ^9-THC or CP 55,940 (fig. 4). Doses of 3.0 mg/kg of Δ^9-THC and 0.1 mg/kg of CP 55,940 were chosen because they were roughly equipotent in producing greater than 80 %DLR with minimal effects on rates of responding in the acute dose-effect determination. Drug-lever selection was maximal (100%) after Δ^9-THC when measured at the 90- and 180-min postinjection intervals. After 360 min, the %DLR dropped to intermediate levels. This time point was tested twice; the first time 57 ± 20 %DLR was obtained, whereas 12 ± 12 %DLR was obtained upon redetermination. By 600 min postinjection, drug-lever selection had diminished to near zero. CP 55,940 produced discriminative stimulus effects over a similar time course as Δ^9-THC (fig. 4). CP 55,940 fully substituted at 90 min postinjection and maintained 71 ± 18 %DLR at 180 min postinjection. Responding on the drug-lever fell to 12 ± 10% at 360 min and further to 1% by 600 min postinjection, although only four of the rats were tested at this time point.

Dose-effect curves for Δ^9-THC and CP 55,940 in the monkey. All four monkeys acquired the Δ^9-THC/VEH discrimination. The drug discrimination training phase for the monkeys took considerably longer than the rats. In the present study, monkeys were trained in their home cages in the vivarium. Extraneous disturbances in the animal room were found to disrupt the behavior of the monkeys during the early phases of training. However, once the monkeys had become experienced with the experimental situation, their behavior was not often perturbed. At the outset, all subjects were given the same training dose of Δ^9-THC (0.2 mg/kg). It became apparent that the monkeys demonstrated differing sensitivities to the sedative effects of Δ^9-THC. If a monkey was observed to respond consistently on VEH days but was unable to respond on THC days, the training dose was lowered. Ultimately, the dose of Δ^9-THC which did not severely disrupt responding on drug days was the dose which was used to train the discrimination. This approach resulted in a 4-fold difference in training dose from the most sensitive monkey trained with 0.04 mg/kg to the least sensitive monkey trained with 0.16 mg/kg (table 2). Also, some tolerance appeared to develop to the sedative properties of Δ^9-THC during the acquisition period. Doses of Δ^9-THC that had severely disrupted responding when tried initially as a training dose were not associated with significant response-rate suppression when tested as part of the acute dose-effect curve. For example, monkey 306, who required a reduction of training dose from 0.2 to 0.04 mg/kg, was able to respond between 1 and 1.5 responses/sec with doses of 0.08 to 0.2 mg/kg of Δ^9-THC after many months of training with 0.04 mg/kg. Similarly, monkey 831 maintained responding at a rate of 1.6 responses/sec with a dose of 0.4 mg/kg, whereas doses greater than 0.1 mg/kg were associated with severe response-rate disruption during earlier training. In contrast, no adjustments of training dose were necessary once the monkeys acquired the discrimination, indicating that tolerance did not develop to the discriminative stimulus properties of Δ^9-THC.

After acquisition of the discrimination had been established, control tests with the training dose of Δ^9-THC consistently produced greater than 80 %DLR, whereas tests with VEH produced less than 20 %DLR (fig. 5), indicating that all mon-

TABLE 1

Potency of Δ^9-THC and CP 55,940 for substitution for Δ^9-THC and for decreasing rates of responding in rats trained to discriminate Δ^9-THC from VEH

	Substitution				Response Rate	
	MED[a]		ED_{50}[b] (95% CL)		ED_{50}[c] (95% CL)	
	mg/kg	μmol/kg	mg/kg	μmol/kg	mg/kg	μmol/kg
Δ^9-THC	3.0	9.5	0.7 (0.3–1.7)	2.2 (1.0–5.4)	7.5 (3.2–17.4)	23.8 (10.2–55.3)
CP 55,940	0.1	0.27	0.03 (0.02–0.06)	0.08 (0.05–0.16)	0.10 (0.05–0.2)	0.27 (0.13–0.5)
Ratio (μmol/kg) THC/CP 55,940		35		28		88

[a] MED for producing an average of greater than 80% Δ^9-THC-lever responding.
[b] ED_{50} for substitution is that dose expected to produce 50% Δ^9-THC-lever responding.
[c] ED_{50} for response rates is that dose expected to reduce rates by 50% relative to the corresponding VEH control rates.

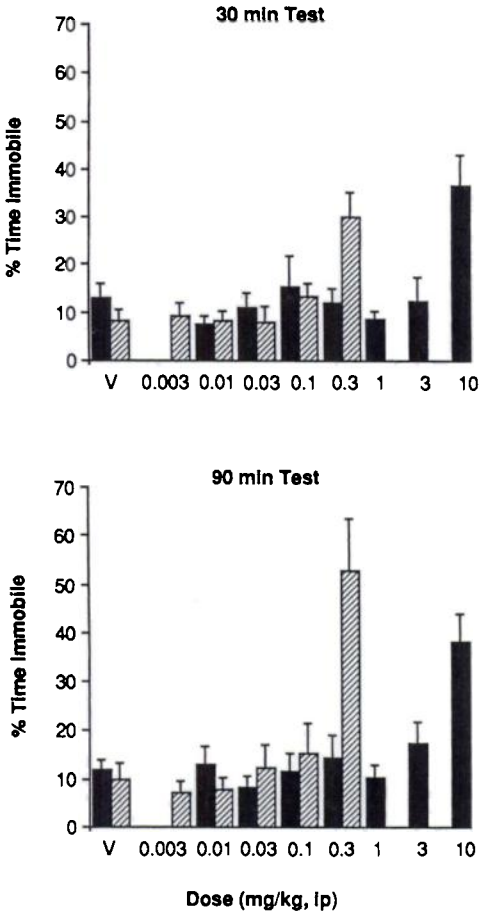

Fig. 3. Percentage of time immobile after Δ⁹-THC (■) and CP 55,940 (▨) administration in rats trained to discriminate Δ⁹-THC (3.0 mg/kg i.p.) from VEH. Catalepsy was measured immediately after the 30- (upper panel) and 90-min (lower panel) drug discrimination tests. V, results of VEH control tests conducted before each dose-effect curve determination. Values represent the mean (±S.E.) for 10 rats.

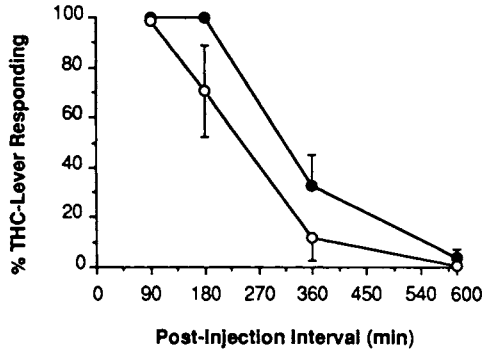

Fig. 4. Effects of Δ⁹-THC (3.0 mg/kg, ●) and CP 55,940 (0.1 mg/kg, ○) on Δ⁹-THC-lever selection measured at various times after injection. Values represent the mean (±S.E.) for 7 to 10 rats; however, only 4 rats were tested at the final time point for CP 55,940.

keys were under good stimulus control. Response rates, typically between 2 and 3 responses/sec, were comparable for the training dose and VEH for individual monkeys.

The results of the stimulus generalization tests for various doses (0.005–0.4 mg/kg) of Δ⁹-THC presented for individual subjects are shown in figure 5. In all subjects, Δ⁹-THC generalized from the training dose over several doses. The MED for producing greater than 80 %DLR covaried with the training dose used in each subject (table 2), with lower training doses

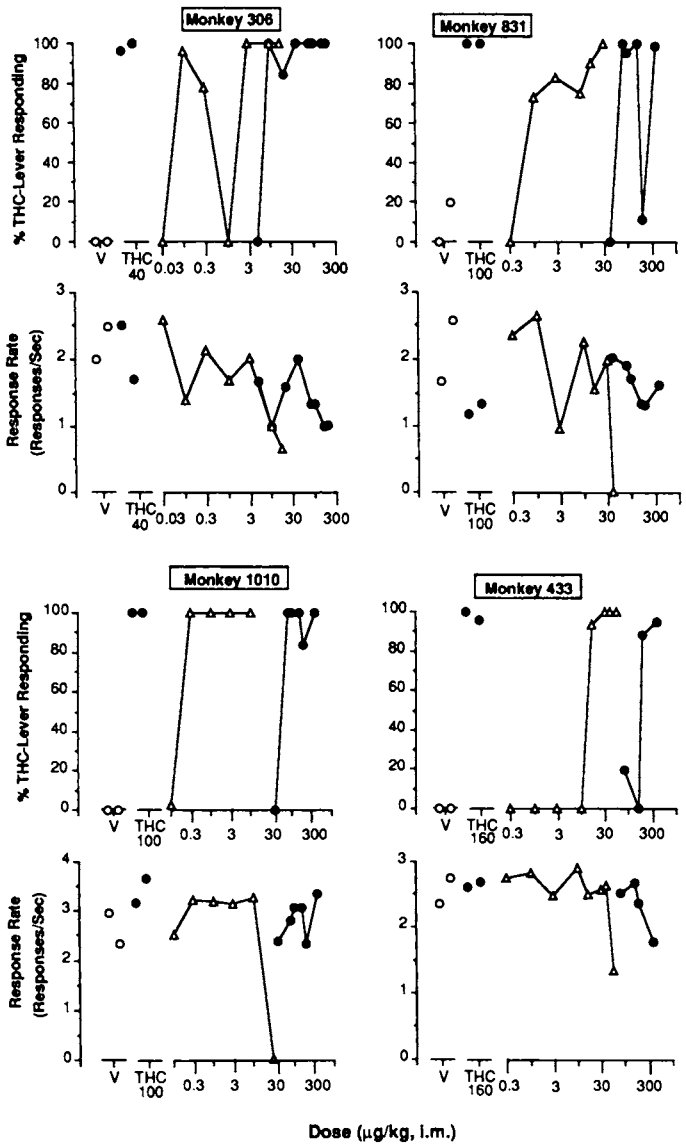

Fig. 5. Percentage of Δ⁹-THC-lever responding and response rates in monkeys trained to discriminate Δ⁹-THC from VEH. V and THC, corresponding VEH and Δ9-THC control tests conducted before each dose-effect curve determination. Data are shown for four individual monkeys. The training dose for each monkey is indicated underneath the Δ⁹-THC control point.

associated with lower MEDs. Response rates were fairly stable across the range of doses tested in each subject. Even doses which produced observable ataxia, sedation and ptosis were not found to reduce rates of responding during generalization tests.

CP 55,940 fully substituted for Δ⁹-THC in the monkeys (fig. 5). The MED for greater than 80 %DLR ranged from 0.1 μg/kg in the most sensitive monkey to 17 μg/kg in the least sensitive monkey (table 2). The highest doses tested in each subject produced marked ataxia, sedation and ptosis and were associated with response-rate suppression. Monkey 433, whose rates were suppressed by 51% with 56 μg/kg, was still able to respond 400 times, exclusively on the drug lever. Responding was almost completely abolished in monkey 1010 with 30 μg/kg; however, five responses were emitted on the drug lever. Comparing MEDs, CP 55,940 was considerably more potent than Δ⁹-THC in all four monkeys (table 2), with potency differences ranging from 14- to 310-fold. The unusually high

TABLE 2

MEDs for production of greater than 80% drug-lever responding in rhesus monkeys trained to discriminate Δ^9-THC from VEH

Monkey	Training Dose	Δ^9-THC MED		CP 55,940 MED		Ratio THC/CP 55,940
	mg/kg	mg/kg	μmol/kg	mg/kg	μmol/kg	μmol/kg
306	0.04	0.01	0.03	0.0001	0.0003	100
831	0.10	0.08	0.25	0.0030	0.0080	31
1010	0.10	0.08	0.25	0.0003	0.0008	310
433	0.16	0.20	0.64	0.0170	0.0451	14

MED for CP 55,940 in monkey 433 suggests that the lower potency difference between Δ^9-THC and CP 55,940 in this subject may underestimate the actual potency differences which would be predicted to occur in this species.

Discussion

The proposed cannabinoid receptor recently labeled with [^{3}H]-CP 55,940 is a saturable, high-affinity binding site which exhibits characteristics of a receptor associated with a G-protein (Devane *et al.*, 1988; Howlett *et al.* 1990a). The potencies of many cannabinoids, both natural and synthetic, as competitors of binding at this site (Devane *et al.*, 1988) are well correlated with their relative potencies in several biological assays (Razdan, 1986). In addition, the localization of the binding site in brain has been shown to be distributed in regions which are considered to be likely anatomical substrates for the various pharmacological and behavioral effects of Δ^9-THC (Herkenham *et al.*, 1990). In total, this evidence suggests that the receptor defined by [^{3}H]CP 55,940 may mediate many of the actions of Δ^9-THC *in vivo*.

The results of this study provide additional evidence in support of an important role for the recognition site labeled with [^{3}H]CP 55,940 as a neural substrate for the *in vivo* actions of cannabinoids. CP 55,940 produced Δ^9-THC-like discriminative stimulus effects in both rats and rhesus monkeys, and in both species it was as least 20-fold more potent than Δ^9-THC. This potency corresponds to its 30-fold greater affinity than Δ^9-THC typically obtained in binding studies (Herkenham *et al.*, 1990). The fact that both the efficacy and potency of CP 55,940 for discriminative stimulus effects was predicted by binding affinity supports the hypothesis that this site plays a role in cannabinoid discrimination. To the extent that the discriminative stimulus properties of drugs are predictive of their subjective effects in humans (Balster, 1991; Schuster and Johanson, 1988), these data further suggest that this site may be important in the abuse of cannabis. The evidence provided here that CP 55,940 produces discriminative stimulus effects isomorphic with those of Δ^9-THC also supports the further use of this compound as a tool for probing the neurobehavioral basis for the effects of cannabinoids. The evidence also suggests that CP 55,940 may produce Δ^9-THC-like subjective effects were it to be developed for human use.

There is also a good correlation between binding affinity for the site labeled with [^{3}H]CP 55,940 and potency for discriminative stimulus effects in rats (Balster and Prescott, 1992; present results). For example, 11-hydroxy-Δ^8-THC-dimethylheptyl displaces [^{3}H]-CP 55,940 binding in rat brain membranes with an affinity similar to that of CP 55,940 (Howlett *et al.*, 1990b) and its ED_{50} for Δ^9-THC-like discriminative stimulus effects is approximately 0.04 μmol/kg (Järbe *et al.*, 1989), comparable to the ED_{50} of 0.08 μmol/kg for CP 55,940 in the present study. Similarly, 9-nor-9β-hydroxy-hexahydrocannabinol, which has an affinity for the cannabinoid recognition site over 20-fold higher than its 9-nor-9α-enantiomer (Herkenham *et al.*, 1990), also shows similar enantiomeric selectivity for Δ^9-THC-like discriminative stimulus effects (Ford *et al.*, 1984). Finally, cannabidiol, which has little affinity for the [^{3}H]CP 55,940 site (Devane *et al.*, 1988; Herkenham *et al.*, 1990) also does not substitute for Δ^9-THC in drug discrimination studies (Ford *et al.*, 1984). Similar correlations have been observed between binding and other behavioral and pharmacological effects of cannabinoids (Herkenham *et al.*, 1990), suggesting that actions at this site may be the initial event leading to the diverse pharmacological effects of this class of chemicals. The fact that the potencies for potentially useful effects of cannabinoids, such as their analgesic effects, and their discriminative stimulus effects are closely correlated suggests a common neural basis for these actions and points to a potential difficulty in developing therapeutically useful cannabinoids without Δ^9-THC-like abuse potential. The prototype bicyclic cannabinoid, CP 47,497, was found to generalize from Δ^9-THC with an ED_{50} of 0.1 mg/kg (Weissman *et al.*, 1982). CP 55,940 was 3.4 times more potent than CP 47,497 in the phenylbenzoquinone antinociceptive test (Howlett *et al.*, 1988) and was 3.3 times more potent when the ED_{50} values for generalization from Δ^9-THC in rats were compared (Weissman *et al.*, 1982; present results).

In the present experiment, the potency for the discriminative stimulus of Δ^9-THC in rats was comparable to those reported previously in the literature as reviewed by Balster and Prescott (1992). A comparison of the ED_{50} values for decreasing response rates and %DLR reveals a ratio of around 10 for Δ^9-THC and only 3.3 for CP 55,940, suggesting a greater separation between the discriminative stimulus effects and response-rate suppression in the natural *vs.* the synthetic compound. Both Δ^9-THC and CP 55,940 fully substituted at a dose just below that which disrupted operant responding and produced significant catalepsy. It would appear that the discriminative stimulus effects and the sedative effects of these compounds can be separated in the dose range which produces significant drug-lever selection. These results are comparable with those of Prescott *et al.* (1992), collected under very similar conditions, who found greater than 80 %DLR with Δ^9-THC at a dose of 3 mg/kg and response-rate suppression and greater than 50% immobility with a dose of 10 mg/kg.

Rhesus monkeys have been used frequently to evaluate the discriminative stimulus properties of a number of drug classes with the notable exception of the cannabinoids. Discrimination training typically takes longer in monkeys. In some cases this may occur because the monkeys can hit both levers simultaneously, which confounds learning (Kamien and Woolverton, 1989). In the present study, discrimination training took considerably longer compared to other drug discriminations trained in our laboratory. Relatively slow acquisition of Δ^9-THC discrimination in monkeys administered p.o. was also observed in a previous report (Ferraro *et al.*, 1974). Subjects in that study had been trained initially on a pentobarbital discrimination in the same complex task consisting of a concurrent chain schedule. The current investigation differed from this earlier one in route of administration and the consequence of incorrect responses. Incorrect responses are not reinforced in our procedure; whereas incorrect responses were reinforced but with lesser frequency in the complex schedule task. The biggest

difference between the presently developed procedure and previous monkey drug discrimination research is that it was conducted in the home cages of the animals. This protocol eliminated the need to transfer the subjects to the laboratory but exposed them to external distractions and environmental disturbances in the vivarium.

Evidence for discriminative stimulus effects of Δ⁹-THC in the range of 0.01 to 0.4 mg/kg without significant effects on rates of responding is consistent with previous studies of the behavioral effects of Δ⁹-THC in monkeys. Stark and Dews (1980) found that a dose of 3.0 mg/kg i.m. of Δ⁹-THC abolished responding under a multiple FR 50 fixed-interval 1000 sec schedule. Beardsley *et al.* (1987) reported that cumulative i.v. doses ≥ 0.1 mg/kg totally suppressed FR responding, consistent with observations of Edery *et al.* (1971) of stupor, ataxia and suppression of motor activity after an i.v. dose of 0.1 mg/kg. Even a lower i.v. dose of 0.03 mg/kg disrupted behavior in a temporal differentiation task (Schulze *et al.*, 1988). Effective doses of CP 55,940 in monkeys ranged from 0.1 to 17 μg/kg. Potencies in the micrograms per kilogram range in monkeys are quite rare for psychoactive drugs and suggests very specific action at neuronal receptors.

In the monkey, CP 55,940 fully substituted for Δ⁹-THC. All four monkeys identified several doses of both Δ⁹-THC and CP 55,940 as Δ⁹-THC-like, without exhibiting disruption in responding. The MEDs represent the lowest doses which were determined to be Δ⁹-THC-ike, although these values are restricted to the particular doses chosen for testing. In monkeys, doses of both Δ⁹-THC and CP 55,940 several-fold higher than the MED for drug-lever responding had minimal effects on response rates. Thus, there was a greater separation between the discriminative stimulus and response-rate effects of both cannabinoids in the monkey, compared to the rat. These results emphasize the importance of measuring the same behaviors in several species, particularly inasmuch as species differences have been reported previously for other cannabinoids (Beardsley *et al.*, 1987; Martin *et al.*, 1981; Semjonow and Binder, 1985; Sullivan *et al.*, 1987). On the other hand, similarities in binding characteristics (Howlett *et al.*, 1990a) and receptor densities determined by autoradiographic localization of the binding site (Herkenham *et al.*, 1990) have been demonstrated across species, suggesting that the cannabinoid receptor is highly conserved.

Δ⁹-THC and CP 55,940 produced discriminative stimulus effects over similar time courses in rats. Drug-lever selection was sustained for at least 3 hr and declined by 6 hr postinjection. These results are concordant with those reported previously for Δ⁹-THC (Järbe *et al.*, 1981; Prescott *et al.*, 1992). To our knowledge, complete time course analyses have not been reported for other behavioral effects of CP 55,940; however, CP 55,940 is extremely potent in tests of analgesia, hypothermia and catalepsy when evaluated at the time when Δ⁹-THC elicits a peak effect (Johnson and Melvin, 1986; Little *et al.*, 1988). Taken together with the results from the present investigation, these data suggest that many of the Δ⁹-THC-like effects produced by CP 55,940 occur within a similar time period as Δ⁹-THC. This time course is in contrast with other potent synthetically derived cannabinoids, such as those in the dimethylheptyl series, which are known to possess considerably longer durations of action (Edery *et al.*, 1971; Järbe *et al.*, 1989; B. R. Martin, unpublished observations). This similarity in the time course of effects of Δ⁹-THC and CP 55,940 provides some practical advantages for the use of CP 55,940 *in vivo* to study Δ⁹-THC-like effects.

In summary, a drug discrimination procedure was developed to train rhesus monkeys to discriminate Δ⁹-THC from VEH. Tests with the novel cannabinoid CP 55,940 conducted in these subjects and in rats trained similarly revealed that it has potent Δ⁹-THC-like discriminative stimulus effects in both species. In monkeys, CP 55,940 was effective in producing discriminative stimulus effects at doses between 0.1 and 17 μg/kg, making it one of the most potent behaviorally active compounds known. Because radiolabeled CP 55,940 has been used to identify a cannabinoid recognition site in brain tissue for which it has a very high affinity, the present demonstration of its efficacy and potency for discriminative stimulus effects provides support for the continued use of this potent probe to investigate the neural basis for the behavioral effects of cannabinoids.

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Send reprint requests to: Billy R. Martin, Ph.D., Department of Pharmacology and Toxicology, Medical College of Virginia, PO. Box 613, Richmond, VA 23298–0613.