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Effects of the cannabinoid CB_1 receptor antagonist, SR141716A, after Δ^9-tetrahydrocannabinol withdrawal ☆

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Abstract

Rats were trained to lever press according to variable interval 10 s schedules during daily experimental sessions composed of six 3 min food reinforcement periods and were treated twice daily for 6 days with either vehicle or escalating regimens of Δ^9-tetrahydrocannabinol. On days 7 and 8, the rats were challenged with vehicle and cumulative doses of SR141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4,-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxyamide hydrochloride), a cannabinoid CB_1 receptor antagonist, up to 3 and 9 mg/kg, respectively. Response rates increased during Δ^9-tetrahydrocannabinol withdrawal and towards those of the vehicle treatment group suggesting a waning of the direct effects of Δ^9-tetrahydrocannabinol. SR141716A reduced response rates but only in rats pre-treated with Δ^9-tetrahydrocannabinol. These data suggest that dependence upon Δ^9-tetrahydrocannabinol was induced and SR141716A precipitated withdrawal.

Keywords: THC (tetrahydrocannabinol); Dependence; Withdrawal; (Rat); Operant behavior; Behavioral dependence; SR141716A

1. Introduction

Marijuana is amongst the most widely used recreational substances, and it has been promoted by some for a variety of medical applications as well. Repetitive use of behaviorally active substances can sometimes induce dependence, as evidence by physiological or behavioral disruptions upon abstinence. There have been reports of withdrawal effects in humans upon abstinence from marijuana and other cannabis products, and these effects have included hyperirritability, nervousness, tremor, dysphoria, and sleep disturbance (Jones and Benowitz, 1976; Jones et al., 1981; Mendelson et al., 1984; Wiesbeck et al., 1996). When reports of these withdrawal effects were critically reviewed, however, reviewers have concluded that even under the most intense exposure regimens the effects are typically mild in most subjects and are not of major medical consequence (Compton et al., 1990). Attempts to experimentally induce dependence in laboratory animals upon Δ^9-tetrahydrocannabinol, the main behaviorally active constituent of marijuana, have led to conflicting results. When relying upon spontaneous withdrawal effects to infer dependence, several attempts have been unable to demonstrate dependence (McMillan et al., 1970; Dewey et al., 1972; Harris et al., 1974; Leite and Carlini, 1974), while other attempts were reported as successful (Deneau and Kaymakcalan, 1971; Fredericks and Benowitz, 1980; Beardsley et al., 1986). The failure to consistently observe withdrawal effects following Δ^9-tetrahydrocannabinol administration may, in part, be due to the mild nature of the dependence syndrome. Maximizing the sensitivity of dependent measures to potential withdrawal-induced perturbations maximizes the likelihood of detecting mild states of dependence. Two general methods have been used to maximize the likelihood of detecting states of dependence. These methods have included using antagonists to precipitate withdrawal and by using baselines of operant (i.e., schedule-maintained) behavior for revealing withdrawal-induced perturbations.

Antagonist challenge to animals treated with dependence-producing drugs typically elicits more intense withdrawal reactions than does non-precipitated withdrawal

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(Aceto, 1990). SR141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)- 1-(2,4,-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxyamide hydrochloride) has been identified as a central cannabinoid CB_1 receptor antagonist (Rinaldi-Carmona et al., 1994) and is effective in antagonizing cannabinoid agonist effects in vivo (Rinaldi-Carmona et al., 1994; Compton et al., 1996; Mansbach et al., 1996). SR141716A has also been reported to precipitate withdrawal effects in Δ^9-tetrahydrocannabinol-treated rats indicative of physical dependence (Aceto et al., 1995,1996; Tsou et al., 1995). Precipitated withdrawal effects during these studies included disturbed locomotor activity including retropulsion, wet-dog shakes, forepaw fluttering and ptosis. The precipitated withdrawal effects reported in these studies are amongst the most intense ever reported during withdrawal from Δ^9-tetrahydrocannabinol in rats, and suggest that challenging Δ^9-tetrahydrocannabinol-treated animals with a cannabinoid antagonist may effectively reveal the dependence state.

Disruptions of operant baselines during withdrawal, that is, deviations from vehicle control rates, have also been used as effective measures for detecting dependence upon a variety of drugs (e.g., Thompson and Schuster, 1964; Holtzman and Villarreal, 1973; Slifer et al., 1984; Beardsley and Balster, 1987; Beardsley et al., 1986; Carroll et al., 1989). At times, these withdrawal effects are observed in the absence, or in minimal presence of the effects on physiological (autonomically-mediated) responses, and they have been used to infer a type of dependence referred to as ''behavioral dependence'' (Holtzman and Villarreal, 1973; Slifer et al., 1984; Beardsley and Balster, 1987; Beardsley et al., 1986). We had previously observed marked disruptions of operant performance during withdrawal from chronic i.v. infusions of Δ^9-tetrahydrocannabinol in rhesus monkeys, from which we inferred the ability of Δ^9-tetrahydrocannabinol to induce behavioral dependence (Beardsley et al., 1986). Combining antagonist challenges with using operant baselines for monitoring withdrawal effects, and thereby maximizing the likelihood of detecting a mild dependence state, has not been previously reported in Δ^9-tetrahydrocannabinol-treated animals.

The purpose of the present study was to evaluate whether behavioral dependence could be induced in rats following several days of twice daily (b.i.d.) dosings with Δ^9-tetrahydrocannabinol. We hypothesized that a change in response rates away from vehicle control levels during Δ^9-tetrahydrocannabinol withdrawal would be indicative of behavioral dependence. Three different Δ^9-tetrahydrocannabinol regimens were used in an attempt to maximize the Δ^9-tetrahydrocannabinol doses administered while avoiding the total suppression of response rates by Δ^9-tetrahydrocannabinol. These regimens included either acute or cumulative dosages exceeding those reported to induce profound physical dependence upon Δ^9-tetrahydrocannabinol in rats (Tsou et al., 1995). In this study, SR141716A did not affect response rates in rats given chronic vehicle regimens. SR141716A, but not Δ^9-tetrahydrocannabinol discontinuation (i.e., non-precipitated withdrawal), produced changes in response rates away from vehicle control levels in rats with recent Δ^9-tetrahydrocannabinol exposure consistent with a precipitated withdrawal effect.

2. Materials and methods

2.1. Subjects

Twenty-four adult male Long-Evans Hooded rats (Harlan, Dublin, VA) were individually housed in an animal room maintained at 20°C illuminated under a 12 h light/dark cycle with continuous access to water. The rats were maintained at 85% of their ad libitum body weight by supplements with rodent chow following each experimental session, and during the day on those days in which experimental sessions were not scheduled. All animals received care according to the ''Guide for the Care and Use of Laboratory Animals'', DHHS Publication, Revised, 1985. The facilities are certified by the American Association for the Accreditation of Laboratory Care. These studies were approved by the Institutional Animal Care and Use Committee at the Virginia Commonwealth University.

2.2. Apparatus

Eight identical two-lever operant chambers (Lafayette Instrument, Lafayette, IN) were used. Each chamber was enclosed in a sound- and light-attenuating chamber and ventilated by an exhaust fan that produced a constant masking noise. The two response levers were located on the front panel of these chambers and were separated by a food hopper into which 45 mg food pellets (Bio-Serve, Frenchtown, NJ) could be delivered by activation of an automated dispenser. A 4-W stimulus lamp was located above each lever. The recording of lever presses, activation of lights, and dispensing of food pellets were automatically controlled by a microcomputer operating MED-PC software (MED Associates, St. Albans, VT).

2.3. Procedure

Subjects were initially trained to press the right lever under a fixed-ratio 1 (FR 1) schedule of reinforcement in which each right side lever press resulted in a pellet delivery. Subsequently, the rats were trained daily for 78-min experimental sessions (usually Monday–Friday, however, if the testing protocol required it for longer than 5 consecutive days) under a multiple timeout of 10 min and variable interval of 10 s (for 3 min) schedule of reinforcement reinforced with food pellet delivery. Each experimental session consisted of six 10-min timeout periods alternating with six 3-min food availability periods. An experimental session would always begin with a 10-min timeout period during which the test chamber was darkened and food pellets were unavailable although lever

presses were recorded. During the 3-min food availability periods, the right stimulus lamp was illuminated and food pellets were delivered according to the variable interval of 10 s schedules. During food pellet deliveries, the right stimulus lamp was extinguished for 0.2 s and then re-illuminated. According to the variable interval of 10 s schedules, the first press of the right lever, following an average of 10 s since the last pellet delivery or the beginning of the food period resulted in a pellet delivery. Thirty-nine intervals ranging from 0.5 to 19.5 s in 0.5 s increments were used for the random selection of variable interval values. The variable interval schedule was chosen because its contingencies permitted reinforcement to continue even under low rate conditions expected during Δ^9-tetrahydrocannabinol administration.

Each rat was trained until five consecutive experimental sessions occurred in which its overall response rates (right lever presses/s) during the first and fifth sessions did not exclusively represent the highest and lowest rates for those five sessions, and the rate during each individual session was $\pm 20\%$ of the average rate across these sessions. After a rat's performance satisfied these training criteria, it was habituated to receiving within- and post-session vehicle injections until five consecutive sessions occurred during which overall response rates during sessions 4 and 5 were $\geq 75\%$ of the average response rates across sessions 1–3. Once a rat meet these habituation criteria, subsequent testing begin.

Six rats were randomly distributed into four groups for the dependence tests. All rats received 6 consecutive days of b.i.d. Δ^9-tetrahydrocannabinol or vehicle injection followed by tests with vehicle and SR141716A on days 7 and 8. The conditions for these groups differed only with respect to the Δ^9-tetrahydrocannabinol doses administered on days 1–6 as conditions were identical for all groups on days 7 and 8. During the first 6 days of treatment, all rats received an injection at approximately 0800 h, and another immediately after the experimental sessions at approximately 1600 h. VEH group (i.e., vehicle group) rats received injections of vehicle during these 6 days. The Δ^9-tetrahydrocannabinol treatment groups received Δ^9-tetrahydrocannabinol injections on these 6 days and differed by how rapidly the Δ^9-tetrahydrocannabinol dose was increased and on the highest dose of Δ^9-tetrahydrocannabinol administered (30, 40, or 50 mg/kg for groups 30 THC, 40 THC, and 50 THC, respectively). These Δ^9-tetrahydrocannabinol regimens were chosen because: (1) pilot studies had found administering 10 mg/kg Δ^9-tetrahydrocannabinol for 3 days neither suppressed operant responding nor induced dependence; (2) given these pilot study results, we hoped that gradually increasing the Δ^9-tetrahydrocannabinol regimen would induce tolerance and would permit, at least at the lowest dosage regimen, continued expression of operant responding; (3) we wanted to exceed in acute and in the cumulative b.i.d. dosage of i.p. regimens reported to induce profound physical dependence in rats (Tsou et al., 1995) in order to maximize the likelihood of establishing dependence. It was not an objective to parametrically manipulate the Δ^9-tetrahydrocannabinol dosage regimen in these groups but to bracket a 6-day Δ^9-tetrahydrocannabinol dosing condition which would permit tolerance to develop, permitting the expression of operant behavior while maximizing the likelihood of dependence. The 30 THC group received 10, 20, and 30 mg/kg b.i.d. Δ^9-tetrahydrocannabinol on days 1–2, 3–4, and 5–6, respectively; the 40 THC group received 10, 20, 30, and 40 mg/kg b.i.d. Δ^9-tetrahydrocannabinol on days 1–2, 3, 4–5, and 6, respectively; and the 50 THC group received 10, 20, 30, 40, and 50 mg/kg b.i.d. Δ^9-tetrahydrocannabinol on days 1, 2, 3, 4–5, and 6, respectively. All rats received the same test regimens during the experimental sessions on days 7 and 8 which consisted of injections given at the beginning of the first, third, and fifth timeout periods. On day 7, these injections consisted of vehicle, 1 and 2 mg/kg SR141716A; on day 8, these injections were vehicle, 3 and 6 mg/kg SR141716A. Injections on day 7 and 8 thus began approximately 23 and 47 h following the last injection of Δ^9-tetrahydrocannabinol, respectively.

2.4. Drugs

Δ^9-Tetrahydrocannabinol was obtained from the National Institute on Drug Abuse and SR141716A was provided by Pfizer Central Research, Groton, CT. Both drugs were dissolved in a solvent consisting of 1:1:18 of absolute ethanol/emulphor (EL-620, GAF, Linden, NJ)/sterile 0.9% saline. All injections were given s.c. in a volume equivalent to 3 ml/kg. When tests with vehicle are indicated, the 1:1:18 mixture of ethanol/emulphor/saline was used.

2.5. Data analysis

Lever pressing counts during food components 1 and 2, 3 and 4, and 5 and 6 were summated to form a cumulative response to the first, second, and third injections, respectively, occurring during the precipitated withdrawal tests of days 7 and 8. To statistically assess whether lever pressing activity was different for rats treated with Δ^9-tetrahydrocannabinol, lever pressing counts were modeled with the SAS v. 6.12 GENMOD procedure (SAS, Cary, NC). A generalized linear model (McCullagh and Nelder, 1989; Dobson, 1990) with Poisson distribution and loglink was fit to the data, with a mean structure analogous to that used in analysis of variance. The repeated measures in nature of the data were exploited with generalized estimating equations (Liang and Zeger, 1986; Diggle et al., 1994). The between subject correlation pattern was modeled as first-order autoregressive. Overdispersion observed in the fitted model was adjusted for with quasi-likelihood methodology (McCullagh, 1983) Using the model described above, comparisons of interest were tested with contrasts analo-

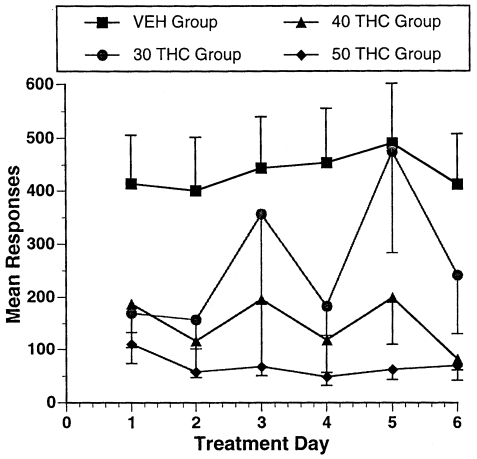

Fig. 1. Mean lever presses during the 6 days of b.i.d. vehicle or Δ^9-tetrahydrocannabinol treatment. Numbers of presses of the right lever across the six, 3-min food-reinforced components were summated for each of the six rats within each experimental group and then averaged within each group. Bars through the symbols indicate the S.E.M.

gous to those utilized in analysis of variance. These comparisons were considered significantly different when they yielded *P*-values of $P \leq 0.05$.

3. Results

3.1. Baseline performance

Within-session vehicle administration was without effect indicating the rats had habituated to the multiple injection procedure. The average number of lever presses emitted during the food components of the three control sessions immediately preceding the two vehicle baseline testdays was 504.1 and slightly increased on each of the testdays to 550.8 and 562.5 responses, respectively. Average (mean ± S.E.M.) response rates, when individual rates were expressed as a percentage of each rat's immediately preceding 3-day control rate, were 113.7% (±5.3) and 107.7% (±4.1) during vehicle testday 1 and 2, respectively.

3.2. Effects of Δ^9-tetrahydrocannabinol regimens

The Δ^9-tetrahydrocannabinol regimens dose-dependently reduced rates of responding in the Δ^9-tetrahydrocannabinol-treatment groups relative to the VEH group. Fig. 1 shows the average number of daily lever presses

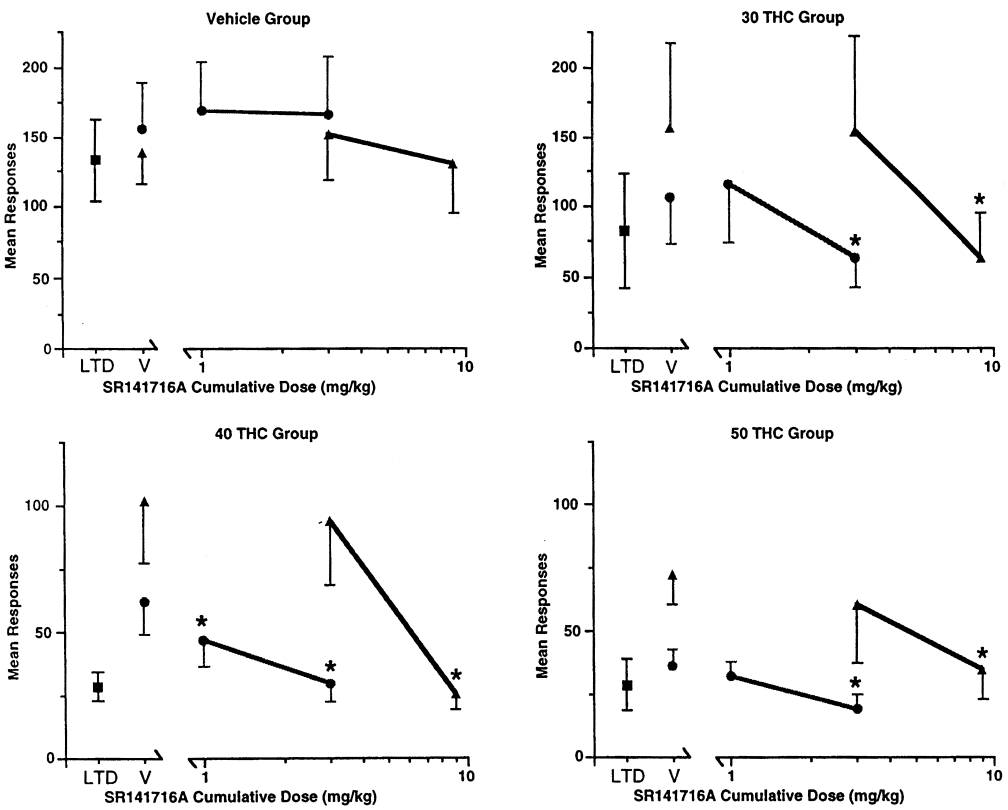

Fig. 2. Mean lever presses during food components 1–2 on the last treatment day (■, ''LTD''), and following a vehicle challenge (''V'') during testday 1 (●) and testday 2 (▲) and during food components 3–4 and 5–6 on testday 1 (●) and testday 2 (▲) which followed cumulative doses of SR141716A of 1, 3 and 9 mg/kg, respectively. Numbers of presses of the right lever during food components 1 and 2 were first summated for each of the six rats within each experimental group during treatment day 6, and during food components 1 and 2, 3 and 4, and 5 and 6 during the two testdays, and then averaged within each group. Bars through the symbols indicate the S.E.M. ''*'' indicates $P \leq 0.05$. Note that the scale of the ordinates of the upper frames is different from the lower frames.

emitted during the food components for each treatment group during the 6-day regimens. The number of responses emitted by the VEH group remained relatively constant across the 6 days of treatment and was greater each day than those of the Δ^9-tetrahydrocannabinol-treatment groups. Variability in response rates characterized the 30 Δ^9-tetrahydrocannabinol group between days 2 and 5 and by day 6, this group's rate was approximately half of that of the VEH group. Less variable but lower response rates were indicative of the 40 THC and 50 THC groups.

3.3. Effects of SR141716A challenges

Fig. 2 shows mean response rates for each treatment group following injections of vehicle, and cumulative doses of 1 and 3 mg/kg SR141716A on testday 1, and of vehicle, 3 and 9 mg/kg SR141716A on testday 2. Also shown are the mean response rates during the combined first two food components on the last (i.e., sixth) day of treatment immediately preceding the first testday.

Response rates of the VEH group were similar within each test session, and were unaffected by injections of either vehicle or SR141716A (Fig. 2). None of the doses of SR141716A produced statistically significant ($P \leq 0.05$) changes in response rates relative to their respective within-session vehicle control rates. These data indicate that SR141716A did not affect the lever pressing rates of the VEH group at any of the doses tested. In addition, along with the results from the habituation sessions described above they indicate that the multiple, within-session injection procedure was not disruptive to lever pressing in itself. Average response rates were similar or greater, following vehicle injections for each of the Δ^9-tetrahydrocannabinol treatment groups on each testday relative to the rates during corresponding food components on the final Δ^9-tetrahydrocannabinol treatment day (see data points labeled ''V'' vs. those labeled ''LTD'' in Fig. 2).

Although SR141716A did not affect response rates of the VEH group, SR141716A dose-dependently reduced response rates in each of the Δ^9-tetrahydrocannabinol treatment groups (Fig. 2). These reductions were statistically significant ($P \leq 0.05$) relative to the effects of within-session vehicle control injections for all Δ^9-tetrahydrocannabinol treatment groups at 3 (testday 1) and 9 mg/kg of SR141716A, and also at 1 mg/kg SR141716A for the 40 THC group, and approached significance ($P \leq 0.087$) at 1 mg/kg SR141716A in the 50 THC group. Response rates were at least twice as great on testday 2 relative to testday 1 at 3 mg/kg SR141716A for all Δ^9-tetrahydrocannabinol treatment groups.

Although not systematically nor blindly scored, overt signs of spontaneous withdrawal effects were not observed before the start, nor immediately following either test session in any of the Δ^9-tetrahydrocannabinol treatment groups.

4. Discussion

Previously, we had reported (Beardsley et al., 1986) that withdrawal from Δ^9-tetrahydrocannabinol infusion in chronically treated rhesus monkeys resulted in abstinence effects marked by suppression of food-reinforced response rates. Similar to conventional dependence effects induced by other drugs, Δ^9-tetrahydrocannabinol-withdrawal suppressed responding observed in this earlier study was reversed with the readministration of Δ^9-tetrahydrocannabinol (Beardsley et al., 1986). We inferred that the monkeys had become behaviorally dependent upon Δ^9-tetrahydrocannabinol in keeping with the prior use of this term (Schuster and Thompson, 1969) denoting that changes in response rates away from control levels had occurred upon drug withdrawal. Withdrawal effects of dependence-producing drugs can also be precipitated with the administration of an antagonist. In the present study, administration of the cannabinoid antagonist, SR141716A, precipitated reductions of food-maintained response rates (and away from VEH group rates) only in rats previously treated with Δ^9-tetrahydrocannabinol.

Precipitation of withdrawal effects by SR141716A in presumably Δ^9-tetrahydrocannabinol-dependent rats have been reported by other labs. SR141716A (10 mg/kg) precipitated a withdrawal syndrome characterized by scratching, face rubbing, licking, wet-dog shakes, back arching, and ptosis in rats treated with escalating regimens of continuously i.p.-infused Δ^9-tetrahydrocannabinol for 4 days and terminating with either 20 or 100 mg/kg per day (Aceto et al., 1995, 1996). At times, instances of the most intense precipitated withdrawal effects included biting, tongue rolling, retropulsion, head shakes, limb extension, ataxia, myoclonic spasms, and front paw treading (Aceto et al., 1996). SR141716A (5 mg/kg) also precipitated a ''dramatic abstinence syndrome'' in rats treated with 6.5 days of b.i.d. 15 mg/kg Δ^9-tetrahydrocannabinol treatment which was characterized by retropulsion, wet-dog shakes, forepaw fluttering, and increases in vertical and horizontal activity (Tsou et al., 1995). The results of the present study systematically extend these earlier reports to now include SR141716A-precipitated disruptions of learned behavior in previously Δ^9-tetrahydrocannabinol-treated rats. A noteworthy difference between the present results and these earlier reports, however, was our inability to observe signs indicative of physical dependence despite our use of Δ^9-tetrahydrocannabinol regimens and SR141716A challenge doses overlapping or exceeding those reported effective. An important procedural difference which qualifies comparisons between these other studies and our own, however, was that we did not systematically observe and rate our subjects for these other signs. Nevertheless, the rats of the present study were handled and observed daily during weighings, injections, and transport to and from the operant chambers. It would seem unlikely that unusual motor behaviors or other intense

physiological effects would escape our detection following SR141716A challenge. Another important procedural difference was that Δ^9-tetrahydrocannabinol was administered b.i.d. and subcutaneously in the present study while it was either infused continuously (Aceto et al., 1995, 1996) or given b.i.d. by the i.p route (Tsou et al., 1995). Although the route of administration could be an important difference between these studies all the Δ^9-tetrahydrocannabinol-treated rats in the present study received higher doses of both Δ^9-tetrahydrocannabinol and SR141716A than that administered i.p. during the Tsou et al. study (Tsou et al., 1995) which would likely compensate for any differences in potency of Δ^9-tetrahydrocannabinol between the i.p. and the s.c. routes. Other procedural differences between these studies would more likely account for the differences in results.

Within the dose range tested, SR141716A did not affect the response rates of the vehicle-treated group but did dose-dependently reduce rates of lever pressing in each of the Δ^9-tetrahydrocannabinol-treated groups. These response-rate reductions occurred in the absence of obvious autonomic and other signs typically reported to infer physical dependence. In this sense, antagonist-precipitated disruptions of operant behavior were the more sensitive measure of Δ^9-tetrahydrocannabinol dependence. Others have reported that changes in operant response rates can be a more sensitive measure of dependence upon drugs than relying upon more conventional signs entailing unlearned behavior. For example, marked disruptions of operant responding in rhesus monkeys dependent upon phencyclidine (Slifer et al., 1984) or morphine (Holtzman and Villarreal, 1973), and in rats dependent upon phencyclidine (Beardsley and Balster, 1987) or nicotine (Carroll et al., 1989) have occurred in the absence of observable changes in gross behavior. The present study further extends the utility of this approach.

Each of the Δ^9-tetrahydrocannabinol groups had greater response rates following vehicle injections during the first and second test sessions than during the corresponding first two food components on the last day of Δ^9-tetrahydrocannabinol treatment. If there had been non-precipitated withdrawal effects, they would have likely manifested themselves in reductions of response rates during these components that occur at 23 and 47 h post-withdrawal. The elevations of response rates during these periods, relative to the last day of Δ^9-tetrahydrocannabinol treatment, were most likely attributable to a diminution of the rate-suppressant effects of Δ^9-tetrahydrocannabinol. Signs of non-precipitated withdrawal including wet-dog shakes and facial rubbing have been observed in rats at 48 h of withdrawal from 4-day, continuously infused Δ^9-tetrahydrocannabinol regimens which had terminated with 100 mg/kg per day of i.p. Δ^9-tetrahydrocannabinol (Aceto et al., 1996). If the rats in the present study, for example, had been engaging in bouts of wet-dog shakes or intensive facial rubbing during the beginning of the test sessions prior to SR141716A challenge, lever pressing would likely have been compromised and rates reduced to low levels, but were not. Other researchers have reported a lack of non-precipitated withdrawal effects in rats from Δ^9-tetrahydrocannabinol or cannabis extract regimens as well (Leite and Carlini, 1974).

Although the observation that SR141716A only reduced response rates in previously Δ^9-tetrahydrocannabinol-treated rats in the present study is consistent with an inference of precipitated withdrawal and the presence of dependence, another explanation is possible. SR141716A can reduce operant response rates if high enough doses are administered (Mansbach et al., 1996). It was an objective of the present study to administer doses of SR141716A which, by themselves, would not alter the responding of control animals otherwise it would have been impossible to discriminate a precipitated withdrawal effect from a non-specific rate reducing effect. Although this objective was achieved, the possibility remains that the effects of submaximal doses of SR141716A summated with the residual response rate reducing effects of Δ^9-tetrahydrocannabinol to result in reductions in rates relative to either the levels during Δ^9-tetrahydrocannabinol administration or to VEH group levels. The likelihood of this explanation is strengthened by the absence of non-precipitated withdrawal effects, as well as by the observation that response rates following vehicle injections on testday 2 were greater than on testday 1 for the Δ^9-tetrahydrocannabinol groups suggesting that the rate-suppressing effects of Δ^9-tetrahydrocannabinol were diminishing over these 2 days. On the other hand, there are no other reports of SR141716A and Δ^9-tetrahydrocannabinol having synergistic effects in any other procedure. For example, SR141716A blocks the sedative, hypothermic, and antinociceptive effects of Δ^9-tetrahydrocannabinol in mice rather than potentiating them (Compton et al., 1996). Given that SR141716A has the potential to non-specifically reduce operant response rates (Mansbach et al., 1996), and that a precipitated withdrawal effect would have also manifested itself as further reductions in response rates away from VEH group levels, it will have to be left to future research to definitively discriminate between these two possible explanations.

The present results demonstrating SR141716A-precipitated disruptions of operant behavior in previously Δ^9-tetrahydrocannabinol-treated rats, along with reports of SR141716A-precipitated withdrawal effects entailing non-learned behaviors in rats (Tsou et al., 1995; Aceto et al., 1996), when considered amidst reports of spontaneous withdrawal effects from discontinuing Δ^9-tetrahydrocannabinol or cannabis administration in laboratory animal and human subjects (Deneau and Kaymakcalan, 1971; Stadnicki et al., 1974; Jones and Benowitz, 1976; Fredericks and Benowitz, 1980; Mendelson et al., 1984; Beardsley et al., 1986), strengthens the hypothesis that Δ^9-tetrahydrocannabinol can induce a type of dependence. Given that the selective cannabinoid CB_1 receptor antagonist,

SR141716A, appears to have the ability to precipitate withdrawal-like effects under some Δ^9-tetrahydrocannabinol dosing conditions it appears that this receptor plays a role in mediating these Δ^9-tetrahydrocannabinol dependence effects. It is too early to attempt to position the importance of these dependence effects within the natural history of the chronic marijuana user although, if present, they would likely affect the maintenance, and perhaps the relapse to, marijuana usage.

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