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Precipitated cannabinoid withdrawal is reversed by Δ^9-tetrahydrocannabinol or clonidine

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Abstract

The availability of the cannabinoid antagonist, SR 141716A, to precipitate withdrawal following repeated cannabinoid administration provides a model to investigate the mechanisms underlying cannabinoid dependence as well as potential treatments to alleviate withdrawal symptoms. The goal of the present study was to determine whether SR 141716A-precipitated withdrawal symptoms in Δ^9-tetrahydrocannabinol (Δ^9-THC)-tolerant mice could be alleviated by either readministration of Δ^9-THC or clonidine, an α_2-receptor agonist. SR 141716A elicited paw tremors in Δ^9-THC-tolerant mice, but produced a significant increase in head shakes independently of repeated Δ^9-THC treatment. Readministration of Δ^9-THC, following SR 141716A-precipitated withdrawal, reversed paw tremors ($ED_{50} = 9.9$ mg/kg), but failed to reduce head shaking behavior. Clonidine reversed SR 141716A-precipitated paw tremors ($ED_{50} = 0.18$ mg/kg) and blocked head shakes at all doses tested. The reversal effects did not appear to be the result of motor impairment because neither decreases in spontaneous locomotor activity nor motor incoordination, as assessed in the inverted screen test, could account for the effects. These findings suggest that SR 141716A precipitates paw tremors in mice by competing with Δ^9-THC at the CB_1 receptor, though it also produced head shaking in nondependent animals. Finally, the observation that clonidine alleviated SR 141716A-precipitated paw tremors suggests its potential as a treatment for cannabinoid dependence.

Keywords: Cannabinoid; Δ^9-THC; SR 141716A; Dependence; Clonidine; Withdrawal; Tolerance

1. Introduction

Marijuana continues to be the most commonly used illicit drug in the United States (Johnston et al., 1998). Further increasing its potential for use is that public sentiment appears to favor allowing marijuana consumption for medicinal uses, as reflected by its decriminalization for this purpose in several states. The high prevalence of marijuana use, with the positive relationship between marijuana use and marijuana dependency (Chen et al., 1997), suggests that the occurrence of physical withdrawal effects could become an issue when a recreational user or patient discontinues the drug. In fact, an abrupt cannabinoid withdrawal syndrome has been described in humans following discontinuation from chronic Δ^9-tetrahydrocannabinol (Δ^9-THC) (Jones and Benowitz, 1976; Jones et al., 1976). Recent studies have also demonstrated abstinence symptoms that included subjective effects of anxiety, irritability, and stomach pain, as well as decreases in food intake, following abrupt withdrawal from continued administration of either oral Δ^9-THC (Haney et al., 1999a) or marijuana smoke inhalation (Haney et al., 1999b). These findings taken together suggest that daily marijuana use may be continued in part to alleviate abstinence symptoms.

Initial research investigating cannabinoid dependence in laboratory animals yielded conflicting findings. Evidence of withdrawal was reported upon abrupt cessation of drug treatment following repeated dosing in rats (Karler and Turkanis, 1980) and monkeys (Beardsley et al., 1986; Kaymakcalan, 1979). On the other hand, other studies failed to observe abrupt withdrawal in pigeons (McMillan et al., 1970), mice (Chesher and Jackson, 1974), rats (Leite and Carlini, 1974), dogs (Dewey et al., 1972), and monkeys (Harris et al., 1974). With the availability of SR 141716A, however, reliable withdrawal effects have been precipitated in mice (Cook et al., 1998; Hutcheson et al., 1998), rats

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(Aceto et al., 1995; Beardsley and Martin, 2000; Tsou et al., 1995), and dogs (Lichtman et al., 1998) following repeated administration of Δ^9-THC. SR 141716A also precipitated withdrawal in rats following repeated treatment of either HU-210 (Rodriguez de Fonseca et al., 1997) or CP 55,940 (Rubino et al., 1998), two potent synthetic cannabinoids. These models can now be used to investigate possible neurochemical mechanisms underlying cannabinoid dependence as well as to develop potential treatments that would alleviate withdrawal symptoms.

The primary goal of the present study was to determine whether SR 141716A-precipitated withdrawal effects in Δ^9-THC-dependent mice could be pharmacologically alleviated. If SR 141716A was eliciting withdrawal effects by displacing Δ^9-THC from the receptors, then readministration of Δ^9-THC is predicted to reverse the precipitated cannabinoid withdrawal effects by competing for the CB_1 receptor. It has been well established that clonidine, as well as other α_2-agonists, abrogates many of the withdrawal effects in morphine-dependent animals (Fielding et al., 1978; Sparber and Meyer, 1978; van der Laan, 1985). Therefore, we sought to determine whether clonidine would also ameliorate SR 141716A-precipitated withdrawal in mice treated repeatedly with Δ^9-THC. Because any apparent reduction in withdrawal-related behavior might result from nonspecific effects such as sedation or motor impairment, additional groups of mice were evaluated for gross locomotor activity and assessed for incoordination in the inverted screen test.

According to the DSM-IV criteria for substance dependence, physiological dependence is specified if either tolerance or withdrawal is found (American Psychiatric Association, 1994). Therefore, we evaluated whether our Δ^9-THC dosing regimen would also lead to tolerance in the spontaneous locomotor activity, tail-flick test, and rectal temperature assays.

2. Materials and methods

2.1. Subjects

ICR male mice (Harlan Laboratories, Indianapolis, IN) weighing between 22 and 29 g served as subjects. The subjects were housed in the animal care quarters maintained at 22 ± 2°C on a 12-h light/dark cycle, and food and water were available ad lib. The mice were brought to the test environment (22°C to 24°C, 12-h light–dark cycle) and allowed 24 h to recover from movement and handling. Naïve subjects ($n = 6$ per group) were used each experiment.

2.2. Drugs

Δ^9-THC and SR 141716A were provided by the National Institute on Drug Abuse (Rockville, MD). Δ^9-THC and SR 141716A were dissolved in a 1:1 mixture of absolute ethanol and Emulphor-620 (Rhone-Poulenc, Princeton, NJ) and diluted with saline to form a final vehicle mixture of ethanol/Emulphor/saline (1:1:18). Clonidine was mixed in physiological saline.

2.3. Evaluation for tolerance

Mice were administered two daily subcutaneous injections of either Δ^9-THC (10 mg/kg) or vehicle once between 0900 and 1000 h and again between 1600 and 1700 h on two consecutive days. On the third day, each subject was given a baseline tail-flick test, assessed for rectal temperature, and given an intravenous injection of either vehicle or Δ^9-THC (1, 3, 10, or 30 mg/kg). Subjects were assessed for spontaneous activity from 5 to 15 min, nociception at 20 min, and for body temperature at 60 min. Body temperature was determined by inserting a thermocouple probe 2.5 cm into the rectum and temperature was obtained from a telethermometer (Yellow Springs Instrument, Yellow Springs, OH). Nociception was assessed using a standard tail-flick apparatus with a 10-s cut-off (D'Amour and Smith, 1941; Dewey et al., 1970). The heat emitted from the apparatus was adjusted to maintain a noxious stimulus sufficient to elicit tail-flick latencies of approximately 2–2.5 s.

2.4. Evaluation for precipitated withdrawal

Using a similar regimen as the Δ^9-THC tolerance protocol described above, mice were given two daily subcutaneous injections of either Δ^9-THC (10 mg/kg) or vehicle (i.e., ethanol/Emulphor/saline, 1:1:18) once between 0900 and 1000 h and again between 1600 and 1700 h for 2 days. On the third day, each subject was given its respective subcutaneous injection between 0900 and 1000 h, and 4 h later challenged with an intraperitoneal injection of SR 141716A (10 mg/kg) or vehicle. The mice were then placed in clear cages and the number of paw tremor (i.e., a lateral clapping behavior of the front paws) and head shake (i.e., turning or twisting of the head from side to side) incidents were scored (Cook et al., 1998). The mice were also observed for other signs including writhing, ptosis, piloerection, scratching, and mastication. The observation period began 15 min following the intraperitoneal injection and continued for 30 min.

In the reversal experiments, Δ^9-THC-tolerant mice received either an intravenous injection of ethanol/Emulphor/saline vehicle or Δ^9-THC (1, 3, 10, 30 mg/kg) or an intraperitoneal injection of saline or clonidine (0.125, 0.25, 0.5, 1 mg/kg) 5 min after the challenge with SR 141716A. Observations of withdrawal signs began 35 min after the initial challenge and continued for 30 min. The experimenter was blind to the drug conditions in all experiments.

2.5. Evaluation of motor impairment

Spontaneous locomotor activity was assessed by placing mice in individual photocell activity cages (6.5×11

in.) consisting of 16 photocell beams per chamber. Individual mice were placed into one of six chambers activity chambers, and interruptions of the photocell beams were recorded for 30 min using a Digiscan Animal Activity Monitor (Omnitech Electronics, Columbus, OH). Activity in the chamber was expressed as the total number of beam interruptions.

Motor incoordination was evaluated in the inverted screen test (Coughenour et al., 1977). The mice were placed on a wire screen that was immediately inverted and the percentage of animals that climbed onto the top within 60 s was recorded. Subjects were given three baseline trials prior to SR 141716A challenge, which all mice successfully completed. Subjects were then given seven tests at 5-min intervals beginning 15 min after receiving SR 141716A. The means of these latencies were then calculated.

2.6. Statistical analyses

Rectal temperature was expressed as the difference between pre- and postinjection values obtained from each mouse. Antinociception was calculated by transforming the tail-flick data to percent maximum possible effect (%MPE), where %MPE = 100 × (postinjection latency − preinjection latency)/(cut-off time − preinjection latency). ANOVA was conducted on the data from each experiment and Dunnett's test was used for post hoc comparison when appropriate. In addition, the Bonferroni t test was used for planned comparisons. Differences were considered significant at the $P < .05$ level. A temperature of $-4.3°C$ was selected as the E_{max} value for hypothermia. ED_{50} values were determined by least squares linear regression analysis followed by calculation of 95% confidence limits (CL) (Bliss, 1967). Potency ratios were determined as previously described (Colquhoun, 1971).

3. Results

As shown in Fig. 1, tolerance occurred to the antinociceptive and locomotor depressant effects of Δ^9-THC following two daily drug injections for 2 days. Significant main effects of repeated drug administration occurred for spontaneous activity, $F(1,4) = 11$, $P < .05$, and antinociception, $F(1,4) = 10.7$, $P < .05$. The main effect of repeated drug administration for change in body temperature did not achieve statistical significance, $F(1,4) = 3.6$, $P = .06$. The main effect of acute dose was significant for each of the

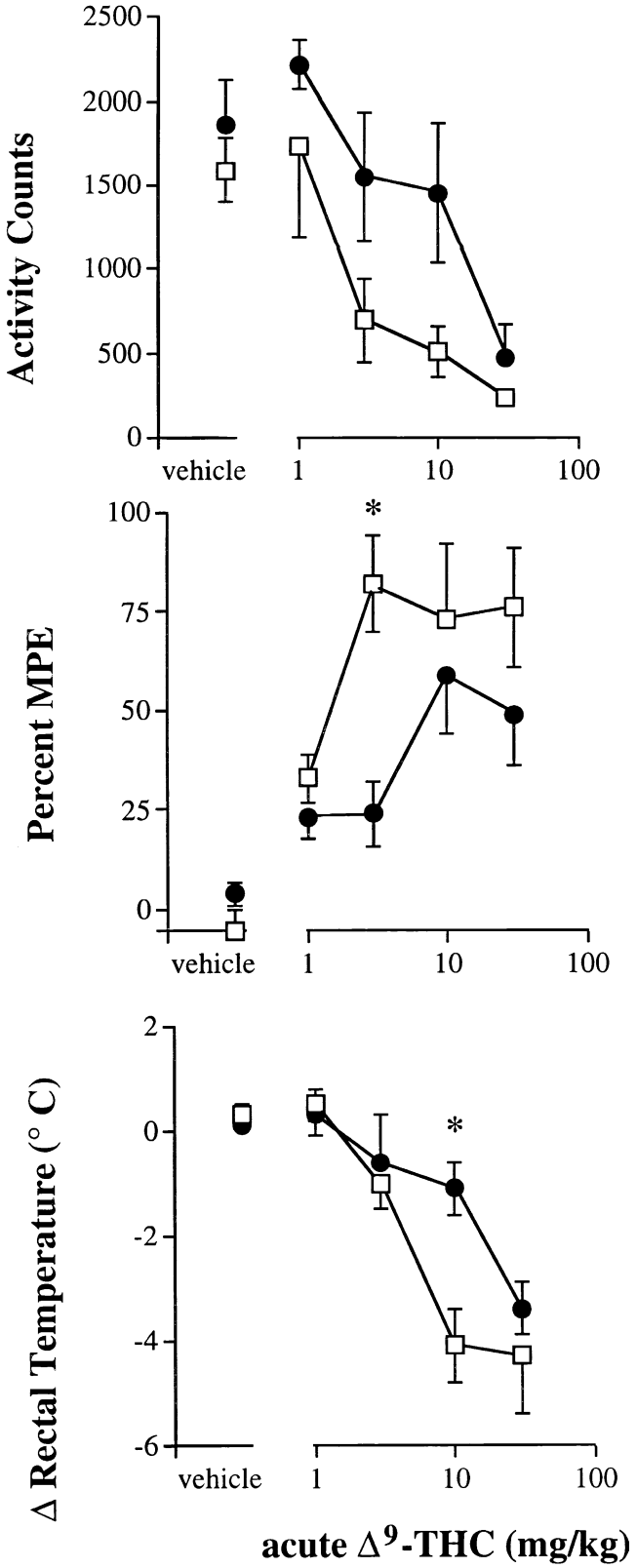

Fig. 1. Mice were given two daily injections for 2 days of either vehicle (□) or 10 mg/kg Δ^9-THC (●). On the morning of the third day, each mouse was injected with either vehicle or Δ^9-THC (1, 3, 10, or 30 mg/kg) and evaluated for spontaneous activity (top panel), antinociception (middle panel), and hypothermia (bottom panel). * Significant difference between the groups that received repeated administration of Δ^9-THC and vehicle (Bonferroni t test, $P < .01$).

Table 1
Two daily injections of Δ^9-THC for two consecutive days are sufficient to produce pharmacological tolerance

Dependent measure	Repeated vehicle ED_{50} (95% CL) (mg/kg)	Repeated Δ^9-THC ED_{50} (95% CL) (mg/kg)	Acute potency ratio of Δ^9-THC (95% CL) (vehicle: Δ^9-THC)
Locomotor activity	3.3 (1.5–6.8)	12 (5.9–23)	3.5 (1.7–13)
Antinociception	1.2 (0.2–6.8)	59% ± 15 MPE at 10 mg/kg 49% ± 13 MPE at 30 mg/kg	>14
Hypothermia	7.3 (4.0–13)	18 (6.7–47)	not different

three indices ($P<.05$), however, none of the interactions between repeated drug administration and acute dose reached significance. Following repeated Δ^9-THC treatment, the drug's potency for inhibiting locomotor activity and producing antinociception was decreased approximately 3-fold and greater than 14-fold, respectively (Table 1). In addition, Δ^9-THC was considerably less efficacious in producing antinociception in the tolerant mice than in the nontolerant mice.

Shown in Fig. 2 are the effects of SR 141716A in eliciting paw tremors and head shakes in mice that were treated repeatedly with either vehicle or Δ^9-THC (10 mg/kg). SR 141716A dose-dependently increased paw tremors in the Δ^9-THC-treated mice, but was completely without effect in the control group as reflected by a significant interaction between Δ^9-THC and SR 141716A, $F(3,40)=12.2$, $P<.05$. In the mice that received repeated Δ^9-THC administration, both 3 and 10 mg/kg SR 141716A

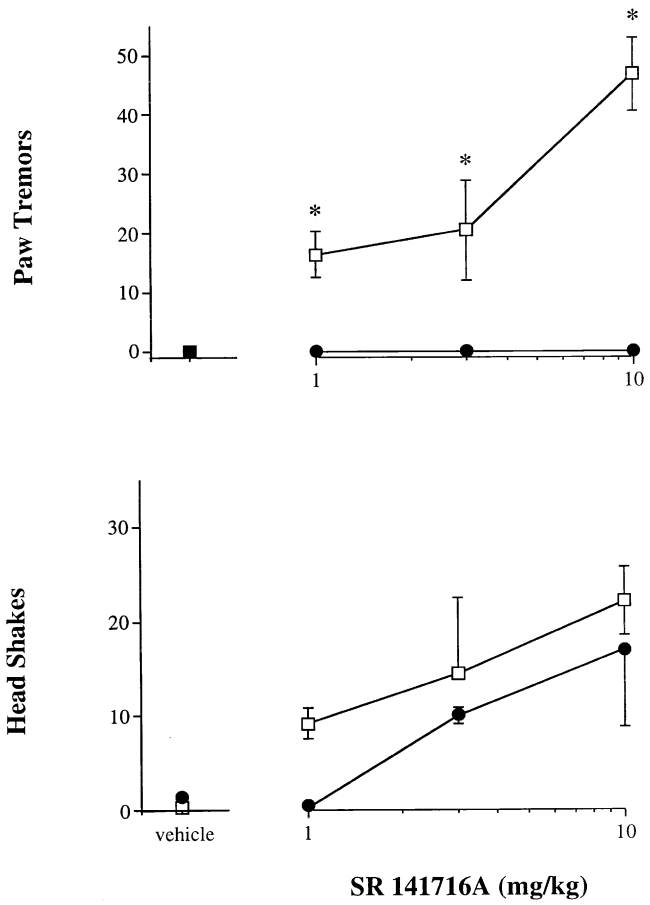

Fig. 2. Effects of SR 141716A in mice treated repeatedly with either (□) 10 mg/kg of Δ^9-THC or vehicle (●). Top panel: SR 141716A precipitated paw tremors in Δ^9-THC-treated, but not vehicle-treated, mice. Bottom panel: SR 141716A elicited head shakes regardless of Δ^9-THC treatment. * Significant difference between the groups that received repeated administration of Δ^9-THC and vehicle (Bonferroni t test, $P<.01$).

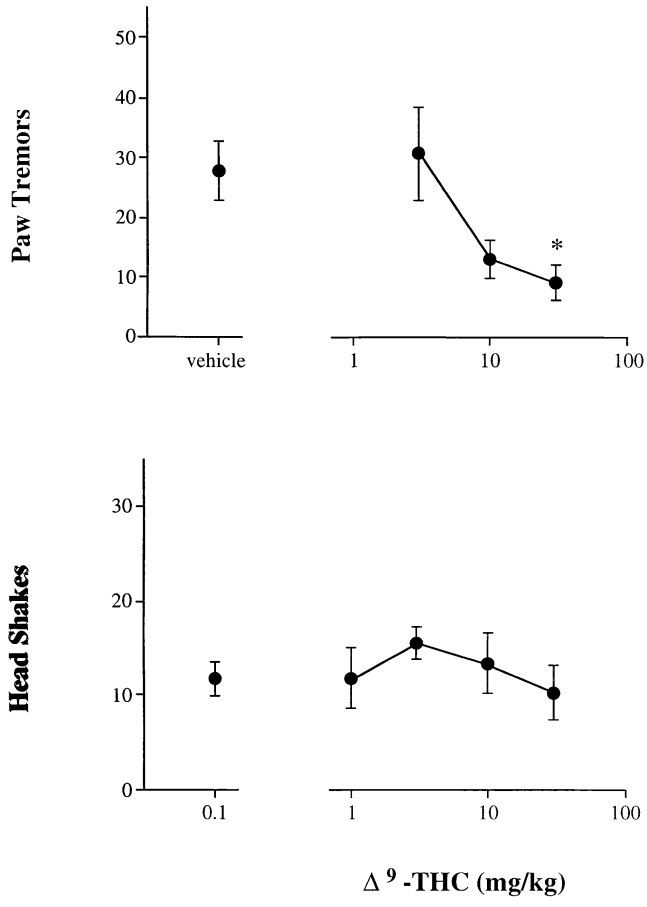

Fig. 3. Δ^9-THC reversal of SR 141716A precipitated withdrawal. Δ^9-THC-dependent mice were challenged with SR 141716A, and given an intravenous injection of either vehicle or Δ^9-THC 5 min later. Δ^9-THC reversed SR-141716A-induced increases in forepaw tremors (top panel), but failed to alter significantly the number of head shakes (bottom panel). * Significantly different from the vehicle treatment (Dunnett's test, $P<.05$).

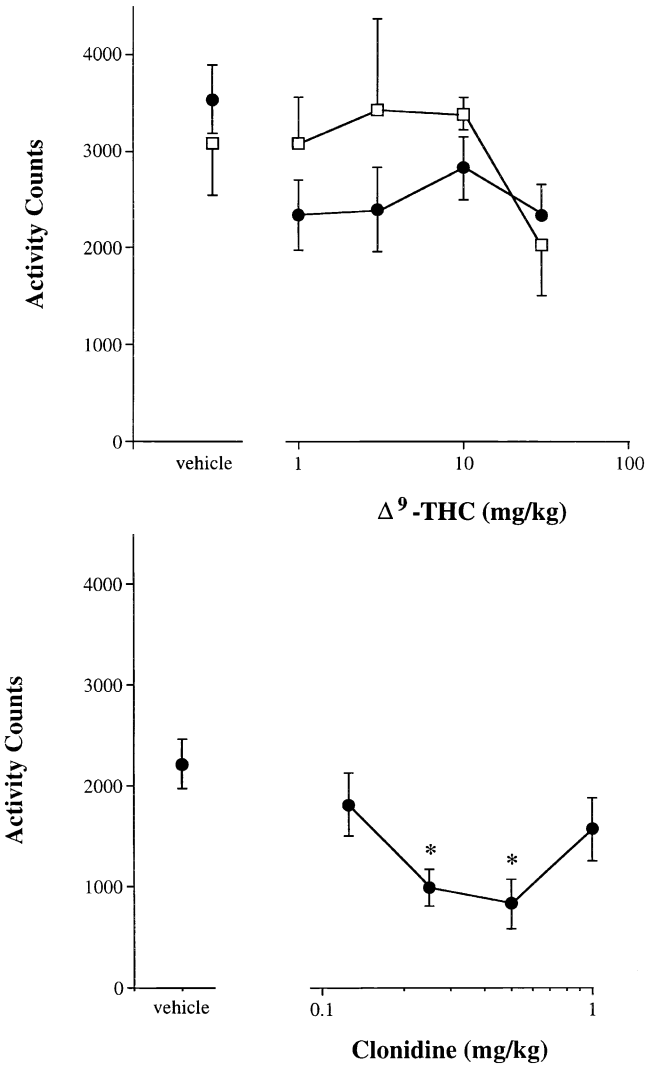

Fig. 4. Top panel: Δ^9-THC failed to produce any locomotor activity depression in Δ^9-THC-dependent mice challenged with SR 141716A. Subjects were given repeated injections of vehicle (□) or 10 mg/kg of Δ^9-THC (●), challenged with SR 141716A (10 mg/kg), 5 min later given a reversal dose of Δ^9-THC, and then assessed for locomotor activity for 30 min. Bottom panel: Clonidine produced locomotor activity depression only at intermediate doses. Δ^9-THC-tolerant mice were challenged with SR 141716A (10 mg/kg), given a reversal dose of clonidine 5 min later, and then assessed for locomotor activity for 30 min. * Significantly different from the vehicle treatment (Dunnett's test, $P<.05$).

significantly differed from the vehicle control group. In the case of head shakes, however, SR 141716A elicited head shakes regardless of the Δ^9-THC treatment, as only the main effect of SR 11716A achieved statistical significance, $F(3,40)=7.6$, $P<.05$. There were no significant differences of each respective dose of SR 141716A between the vehicle- and Δ^9-THC-treated groups. No other behavioral signs indicative of precipitated cannabinoid withdrawal such as ptosis, writhing, or piloerection were observed, though SR 141716A increased scratching behavior regardless of Δ^9-THC treatment.

Fig. 3 depicts the effects of an intravenous injection of vehicle or 3, 10, or 30 mg/kg Δ^9-THC in reversing SR 141716A-induced paw tremors and head shakes. Readministration of Δ^9-THC significantly blocked SR 141716A-induced paw tremors, $F(3,26)=4.7$, $P<.05$, with an ED_{50} (95% CL) of 9.9 (4.8 to 20) mg/kg. However, readministration of Δ^9-THC failed to affect SR 141716A-induced head shakes. Readministration of Δ^9-THC also failed to affect either locomotor activity (Fig. 4, top panel) or motor incoordination as assessed in the inverted screen test (data not shown) in mice treated repeatedly with Δ^9-THC. In fact, no significant effects were found for either measure.

The effects of saline or clonidine in reversing SR 141716A-precipitated effects in Δ^9-THC-tolerant mice are illustrated in Fig. 5. Clonidine dose-dependently reversed SR 141716A-induced paw tremors, $F(4,37)=15.1$, $P<.05$,

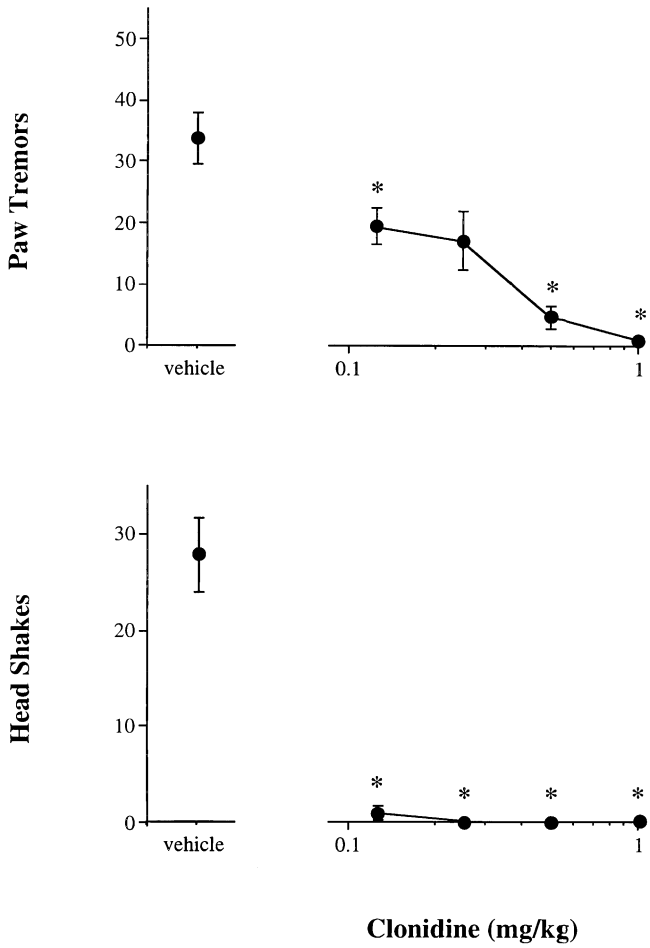

Fig. 5. Clonidine reversal of SR 1431716A precipitated withdrawal. Δ^9-THC-dependent mice were challenged with SR 141716A, and given either vehicle or clonidine 5 min later. Clonidine reversed SR-141716A-induced increases in forepaw tremors in a dose-dependent fashion (top panel) and completely blocked SR 141716A-induced head shakes at all doses tested (bottom panel). * Significantly different from the vehicle treatment (Dunnett's test, $P<.05$).

with an ED_{50} (95% CL) of 0.18 (0.14 to 0.23) mg/kg, and completely blocked SR 141716A-induced head shakes at each dose tested, $F(4,37)=24.4$, $P<.05$. Although a significant effect was found for spontaneous activity, $F(4,37)=4.4$, $P<.05$, the effect was not dose-related as only the two intermediate doses significantly differed from the vehicle treatment (Fig. 4, bottom panel). Once again, no effect was found for motor incoordination as assessed in the inverted screen test (data not shown, $P<.50$).

4. Discussion

The results of the present study indicate that both cannabinoid tolerance and cannabinoid dependence can be rapidly acquired. The development of tolerance found here was even more rapid than a previous study from our laboratory in which mice were treated with drug for 6{1/2} days (Fan et al., 1994). Similarly, Cook et al. (1998) also reported a rapid acquisition of cannabinoid dependence and a comparable magnitude of paw tremors using a similar dosing regimen as that used here (Cook et al., 1998; Hutcheson et al., 1998). In the present study, precipitated paw tremors in Δ^9-THC-tolerant mice administration. Importantly, the Δ^9-THC reversal treatment failed to affect locomotor activity or performance in the inverted screen test suggesting that the decrease in paw tremors was not due to sedation or incoordination. Clonidine did not produce any incoordination and though it did produce some locomotor suppression, this effect only occurred at the intermediate doses and was not dose-related. These results indicate that SR 141716A-precipitated withdrawal paw tremors can be reversed.

In contrast to its effects on paw tremors, SR 141716A increased head shaking regardless of Δ^9-THC treatment. Moreover, intravenously administered Δ^9-THC failed to reverse SR 141716A-induced head shakes in either Δ^9-THC-dependent or nondependent mice, though clonidine did reverse this effect. Thus, head shakes was not an SR 141716A-precipitated withdrawal sign in ICR mice using our Δ^9-THC dosing regimen. Cook et al. (1998) also found that SR 141716A increased head shakes in ICR mice injected repeatedly with vehicle as well as in naïve mice, however, that effect was augmented in Δ^9-THC-dependent mice. A similar pattern of results was described in CD-1 mice (Hutcheson et al., 1998). Interestingly, it has been recently demonstrated that SR 141716A-induced head twitches were completely blocked by a serotonergic 2A/C receptor antagonist and were partially blocked by an AMPA/kainate receptor antagonist as well as by a tachykinin NK1 antagonist (Darmani and Pandya, 2000). SR 141716A has also been found to produce mild withdrawal-like effects in naïve (Rodriguez de Fonseca et al., 1997) or vehicle-treated rats (Aceto et al., 1996, 1998). These results taken together suggest that SR 141716A can elicit head shakes by activating monoaminergic and other neurochemical pathways through its own intrinsic activity either at the CB_1 receptor or through a noncannabinoid site of action. SR 141716A has also been found to increase spontaneous locomotor activity in naïve ICR mice (Compton et al., 1996). Indeed, several studies suggest that SR 141716A can act as an inverse agonist at the CB_1 receptor. In contrast to cannabinoid agonists that stimulate G-protein activity (Burkey et al., 1997), SR 141716A inhibits G-protein activity (Bourboula et al., 1997; Landsman et al., 1997), and has also been found to alter the mRNA levels of $G\alpha s$ and $G\alpha i$ subunits in nontolerant rats (Rubino et al., 1998). The results of the present study indicate that head shakes are due, at least in part, to SR 141716A itself in mice. However, it should be noted that the Δ^9-THC dosing regimen employed in the present study (i.e., 10 mg/kg of Δ^9-THC per injection for 2.5 days) was considerably more mild than the regimens used in the other studies.

A considerable amount of research has demonstrated that clonidine alleviates many opioid withdrawal symptoms. In rats, this α-adrenergic agonist blocks naloxone precipitated morphine withdrawal signs, including wet-dog shakes, prevents weight loss, reduces escape attempts, and reduces pressor effects (Buccafusco, 1983; Fielding et al., 1978; Sparber and Meyer, 1978; Tseng et al., 1975; van der Laan, 1985). While clonidine alleviated precipitated withdrawal jumping in mice (Valeri et al., 1989), it failed to block this effect in rats (Fielding et al., 1978). In addition to reducing opioid withdrawal in laboratory animals, clonidine has long been known to alleviate withdrawal signs in human opioid addicts (Gold et al., 1978). Although clonidine has been demonstrated to produce a marked reduction of withdrawal symptoms, it does not completely eliminate all the effects, and there is some concern about hypotensive side effects (Gossop, 1998). For example, it significantly reduced the number of opioid withdrawal signs, however, about 7% of the patients developed severe hypotension in a single blind study conducted in India (Gupta and Jha, 1988). Therefore, the hypotensive action of clonidine must be considered before developing it for alleviating cannabinoid withdrawal.

In summary, SR 141716A precipitated an increase in forepaw tremors in Δ^9-THC-tolerant mice. In contrast to the results of previous studies (Cook et al., 1998; Hutcheson et al., 1998), SR 141716A failed to elicit a greater increase in head shakes in Δ^9-THC-tolerant mice than in the nontolerant mice. The fact that we gave Δ^9-THC injections for only 2.5 days, while the other studies used a dosing regimen of 5.5 (Hutcheson et al., 1998) or 6.5 days (Cook et al., 1998) may account for this difference. In addition, readministration of Δ^9-THC in the dependent mice reversed SR 141716A-precipitated paw tremors, but failed to affect SR 141716A-induced head shakes. This pattern of results is consistent with the explanation that the head shakes observed in the present study was an effect of SR 141716A alone and not a precipitated withdrawal

effect. Finally, clonidine reversed SR 141716A-precipitated forepaw tremors as well as SR 141716A-induced head shakes. These observations suggest that clonidine, or other α_2-agonists, may hold some promise as a potential treatment for cannabinoid dependence.

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References

Aceto M, Scates S, Lowe J, Martin B. Cannabinoid precipitated withdrawal by the selective cannabinoid receptor antagonist SR 141716A. Eur J Pharmacol 1995;282:R1–2.

Aceto M, Scates S, Lowe J, Martin B. Dependence on Δ^9-tetrahydrocannabinol: studies on precipitated and abrupt withdrawal. J Pharmacol Exp Ther 1996;278:1290–5.

Aceto MD, Scates SM, Razdan RK, BR M. Anandamide, an endogenous cannabinoid, has a very low physical dependence potential. J Pharmacol Exp Ther 1998;287:598–605.

American Psychiatric Association. DSM-IV: diagnostic and statistical manual of mental disorders (DSM-IV). Washington (DC): American Psychiatric Association, 1994. p. 181.

Beardsley PM, Martin BR. Effects of the cannabinoid CB(1) receptor antagonist SR141716A, after Delta(9)-tetrahydrocannabinol withdrawal. Eur J Pharmacol 2000;387:47–53.

Beardsley PM, Balster RL, Harris LS. Dependence on tetrahydrocannabinol in rhesus monkeys. J Pharmacol Exp Ther 1986;239:311–9.

Bliss CI. Statistics in biology. New York: McGraw-Hill, 1967. p. 439.

Bouaboula M, Perrachon S, Milligan L, Canat X, Rinaldi-Carmona M, Portier M, Barth F, Calandra B, Pecceu F, Lupker J, Maffrand JP, Le Fur G, Casellas P. A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1. Evidence for a new model of receptor/ligand interactions. J Biol Chem 1997;272:22330–9.

Buccafusco JJ. Cardiovascular changes during morphine withdrawal in the rat: effects of clonidine. Pharmacol, Biochem Behav 1983;18:209–15.

Burkey TH, Quock RM, Consroe P, Ehlert FJ, Hosohata Y, Roeske VR, Yamamura HI. Relative efficacies of cannabinoid CB1 receptor agonists in the mouse brain. Eur J Pharmacol 1997;336:295–8.

Chen K, Kandel DB, Davies M. Relationships between frequency and quantity of marijuana use and last year proxy dependence among adolescents and adults in the United States. Drug Alcohol Depend 1997;46:53–67.

Chesher GB, Jackson DM. The effect of withdrawal from cannabis on pentylenetrazol convulsive threshold in mice. Psychopharmacology 1974;40:120–35.

Colquhoun D. Lectures on biostatistics: an introduction to statistics with applications in biology and medicine. Oxford: Clarendon Press, 1971. pp. 327–32.

Compton D, Aceto M, Lowe J, Martin B. In vivo characterization of a specific cannabinoid receptor antagonist (SR141716A): inhibition of Δ^9-tetrahydrocannabinol-induced responses and apparent agonist activity. J Pharmacol Exp Ther 1996;277:586–94.

Cook SA, Lowe JA, Martin BR. CB1 receptor antagonist precipitates withdrawal in mice chronically exposed to Δ9-THC. J Pharmacol Ther Exp 1998;285:1150–6.

Coughenour LL, McLean JR, Parker RB. A new device for the rapid measurement of impaired motor function in mice. Pharmacol, Biochem Behav 1977;6:351–3.

D'Amour FE, Smith DL. A method for determining loss of pain sensation. J Pharmacol Exp Ther 1941;72:74–9.

Darmani NA, Pandya DK. Involvement of other neurotransmitters in behaviors induced by the cannabinoid CB1 receptor antagonist SR 141716A in naive mice (in process citation)J Neural Transm 2000;107: 931–45.

Dewey WL, Harris LS, Howes JF, Nuite JA. The effect of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone tests. J Pharmacol Exp Ther 1970;175:435–42.

Dewey WL, Jenkins J, Rourke T, Harris LS. The effects of chronic administration of *trans*-Δ^9-tetrahydrocannabinol on behavior and the cardiovascular system. Arch Int Pharmacodyn Ther 1972;198:118–31.

Fan F, Compton DR, Ward S, Melvin L, Martin BR. Development of cross-tolerance between Δ^9-THC, CP 55,940 and WIN 55,212. J Pharmacol Exp Ther 1994;271:1383–90.

Fielding S, Wilker J, Hynes M, Szewczak M, Novick WJ, Lal H. A comparison of clonidine with morphine for antinociceptive and antiwithdrawal actions. J Pharmacol Exp Ther 1978;207:899–905.

Gold MS, Redmond DEJ, Kleber HD. Clonidine blocks acute opiate-withdrawal symptoms. Lancet 1978;2:599–602.

Gossop M. Clonidine and the treatment of the opiate withdrawal syndrome. Drug Alcohol Depend 1998;21:253–9.

Gupta AK, Jha BK. Clonidine in heroin withdrawal syndrome: a controlled study in India. Br J Addict 1988;83:1079–84.

Haney M, Ward AS, Comer SD, Foltin RW, Fischman MW. Abstinence symptoms following oral THC administration to humans. Psychopharmacology (Berlin) 1999a;141:385–94.

Haney M, Ward AS, Comer SD, Foltin RW, Fischman MW. Abstinence symptoms following smoked marijuana in humans. Psychopharmacology (Berlin) 1999b;141:395–404.

Harris RT, Waters W, McLendon D. Evaluation of reinforcing capability of Δ^9-tetrahydrocannabinol in rhesus monkeys. Psychopharmacologia 1974;37:23–9.

Hutcheson DM, Tzavara ET, Smadja C, Valjent E, Roques BP, Hanoune J, Maldonado R. Behavioural and biochemical evidence for signs of abstinence in mice chronically treated with delta-9-tetrahydrocannabinol. Br J Pharmacol 1998;125:1567–77.

Johnston LD, O'Malley PM, Bachman JG. National survey results on drug use from the Monitoring the Future Study, 1975–1997: Vol. I. Secondary school students (NIH Publication No. 98-4345) and Vol. II: College students and young adults (NIH Publication No. 98-4346). Rockville, MD: National Institute on Drug Abuse, 1998.

Jones RT, Benowitz N. The 30-day trip — clinical studies of cannabis tolerance and dependence. In: Braude MC, Szara S, editors. Pharmacology of marihuana. New York: Raven Press, 1976. pp. 627–42.

Jones RT, Benowitz N, Bachman J. Clinical studies of cannabis tolerance and dependence. Ann NY Acad Sci 1976;282:221–39.

Karler R, Turkanis SA. Subacute cannabinoid treatment: anticonvulsant activity and withdrawal excitability in mice. Br J Pharmacol 1980;68: 479–84.

Kaymakcalan S. Physiological and psychological dependence on the in rhesus monkeys. In: Nahas GG, Paton WDM, editors. Marihuana: biological effects. Adv Biosci. Oxford: Pergamon Press, 1979. pp. 142–9.

Landsman RS, Burkey TH, Consroe P, Roeske WR, Yamamura HI. SR141716A is an inverse agonist at the human cannabinoid CB1 receptor. Eur J Pharmacol 1997;331:R1–2.

Leite JR, Carlini EA. Failure to obtain "cannabis-directed behavior" and abstinence syndrome in rats chronically treated with cannabis sativa extracts. Psychopharmacologia 1974;36:133–45.

Lichtman AH, Wiley JL, LaVecchia KL, Neviaser ST, Arthur DB, Wilson BR, Martin BR. Effects of SR 141716A after acute or chronic cannabinoid administration in dogs. Eur J Pharmacol 1998;357:139–48.

McMillan DE, Harris LS, Frankenheim JM, Kennedy JS. L-Δ^9-*trans*-Tet-

rahydrocannabinol in pigeons: tolerance to the behavioral effects. Science 1970;169:501–3.

Rodriguez de Fonseca F, Carrera M, Navarro M, Koob K, Weiss F. Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. Science 1997;276:2050–4.

Rubino T, Patrini G, Massi P, Fuzio D, Vigano D, Giagnoni G, Parolaro D. Cannabinoid-precipitated withdrawal: a time-course study of the behavioral aspect and its correlation with cannabinoid receptors and G protein expression. J Pharmacol Exp Ther 1998;285:813–9.

Sparber SB, Meyer DR. Clonidine antagonizes naloxone-induced suppression of conditioned behavior and body weight loss in morphine-dependent rats. Pharmacol, Biochem Behav 1978;9:319–25.

Tseng LF, Loh HH, Wei ET. Effects of clonidine on morphine withdrawal signs in the rat. Eur J Pharmacol 1975;30:93–9.

Tsou K, Patrick S, Walker JM. Physical withdrawal in rats tolerant to Δ^9-tetrahydrocannabinol precipitated by a cannabinoid receptor antagonist. Eur J Pharmacol 1995;280:R13–5.

Valeri P, Martinelli B, Pimpinella G, Severini C. Effects of dapiprazole, clonidine and yohimbine on the development of dependence and withdrawal behaviour in mice. Drug Alcohol Depend 1989;23:73–7.

van der Laan JW. Effects of alpha 2-agonists on morphine withdrawal behaviour: potentiation of jumping mediated by alpha 2-receptors. Naunyn-Schmiedeberg's Arch Pharmacol 1985;329:293–8.