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The pharmacological activity of inhalation exposure to marijuana smoke in mice

Aron H. Lichtman [a,*], Justin L. Poklis [b], Alphonse Poklis [a,b], David M. Wilson [a], Billy R. Martin [a]

[a] *Department of Pharmacology and Toxicology, MCV Campus, Medical College of Virginia, Virginia Commonwealth University, P.O. Box 980613, Richmond, VA 23298-0613, USA*

[b] *Department of Pathology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613, USA*

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Abstract

Although the majority of cannabinoid users smoke marijuana, the preponderance of laboratory animal research is based on administration of Δ^9-tetrahydrocannabinol (Δ^9-THC) or other cannabinoid agents via injection. The aim of the present study was to evaluate the impact of inhaling marijuana, or ethanol-extracted placebo smoke in the mouse model of cannabinoid activity by assessing inhibition of spontaneous activity, antinociception, catalepsy, and body temperature. In order to determine dosimetry, blood levels of Δ^9-THC were obtained following either marijuana exposure or intravenous injection of Δ^9-THC. Inhalation exposure to marijuana produced dose-related increases in antinociception and catalepsy, with estimated ED_{50} doses of Δ^9-THC of 2.4 and 3.8 mg/kg, respectively. However, hypothermia and locomotor depression occurred in both the placebo- and marijuana-exposed mice. The CB_1 receptor antagonist, SR 141716A antagonized the antinociceptive effects of marijuana ($AD_{50} = 0.6$ mg/kg), but only slightly decreased marijuana-induced catalepsy, and failed to alter either the hypothermic or locomotor depressive effects. In contrast, SR 141716A antagonized the antinociceptive, cataleptic, and hypothermic effects of intravenously administered Δ^9-THC in mice that were exposed to air alone, though all subjects exhibited locomotor depression, possibly related to the restraint. In accordance with reports of others, these data suggest that exposure to smoke alone has pharmacological consequences. Our findings also indicate that marijuana-induced antinociception is mediated through a CB_1-receptor mechanism of action and are consistent with the notion that Δ^9-THC is mainly responsible for this effect.

Keywords: Marijuana smoking; Cannabinoid; Δ^9-THC; SR 141716A; Antinociception; Analgesia; Catalepsy; Hypothermia

1. Introduction

In addition to marijuana being the most commonly used illicit drug in the United States (Johnston et al., 1998), there is a growing public sentiment favoring its consumption for medicinal purposes. Considerable research has been dedicated to investigating this drug and great progress has been made in understanding its mechanisms of action using Δ^9-tetrahydrocannabinol (Δ^9-THC), its major psychoactive constituent, as well as many other naturally occurring and synthetic analogs. Through the use of structure-activity relationship (Razdan, 1986) and binding (Devane et al., 1988) studies, it was firmly established that cannabinoids bind to a specific receptor. Consequently, two cannabinoid receptors have been cloned and named CB_1 and CB_2. The CB_1 receptor is located primarily in the central nervous system (Matsuda, 1992) and the CB_2 receptor, predominantly associated with the immune system, has only been found in the periphery (Munro et al., 1993). Both CB_1 and CB_2 receptors are coupled to G-proteins and inhibit adenylyl cyclase activity (Howlett, 1995). Finally, the discovery of anandamide and other endogenous cannabinoids (Devane et al., 1992; Facci et al., 1995; Mechoulam et al., 1995) suggest the existence of a cannabinoid neurochemical system.

* Corresponding author. Tel.: +1-804-8288480; fax: +1-804-8282117.

E-mail address: alichtma@hsc.vcu.edu (A.H. Lichtman).

Cannabinoid agonists produce a distinct spectrum of behavioral effects in mice, including decreases in spontaneous activity, antinociception, catalepsy, and hypothermia, that is highly predictive of binding affinity to the CB_1 receptor (Little et al., 1988; Compton et al., 1993). Also, supporting a receptor mechanism of action is that the CB_1 receptor antagonist, SR 141716A blocks these four behavioral effects (Rinaldi-Carmona et al., 1994; Compton et al., 1996) as well as other actions of cannabinoids (Varga et al., 1995; Wiley et al., 1995; Lichtman and Martin, 1996). Of importance, SR 141716A is highly selective for CB_1 receptors and does not bind to CB_2, histamine, dopamine, opioid, 5-HT, adenosine, and several other receptors and ion channels (Rinaldi-Carmona et al., 1994). Although considerable research has evaluated the pharmacological effects of marijuana-plant material in humans (Linton et al., 1975; Cone et al., 1986; Chait, 1989; Foltin et al., 1990; Heishman et al., 1997), relatively few published reports have examined its behavioral effects in laboratory animals. Rats exposed to marijuana smoke exhibited modest alterations in cortical and hippocampal electrical activity as well as an apparent suppression in spontaneous locomotor activity (Fried and Nieman, 1973). Alterations in these measures also occurred following exposure to placebo smoke. Similarly, mice exposed to either marijuana or placebo smoke showed a substantial decrease in locomotor activity compared with the air-exposed control group (Weinberg et al., 1977b).

Presently, there are no published reports that have examined the pharmacological profile of marijuana inhalation in mice using the full tetrad of responses. Therefore, the primary goal of this study was to evaluate mice for spontaneous activity, antinociception, catalepsy, and hypothermia following inhalation exposure to placebo or marijuana smoke. The influence of restraint to which the subjects were exposed during inhalation exposure was assessed by comparing the pharmacological effects of intravenously-administered Δ^9-THC in mice that were either restrained and exposed to air alone or allowed to remain freely moving following the injection. Previous studies examining marijuana inhalation in rodents did not determine the actual Δ^9-THC blood levels, but estimated the doses based on the amount of drug to which the animals were exposed (Fried and Nieman, 1973; Rosenkrantz and Braude, 1974; Fleischman et al., 1975). In order to determine the relationship between the pharmacological effects following inhalation of marijuana smoke and Δ^9-THC, we calculated dosimetry based on the Δ^9-THC blood levels. The final goal of this study was to determine whether the pharmacological effects produced by inhalation exposure to marijuana were mediated via CB_1 receptors by assessing whether the effects were blocked by SR 141716A. Once again, to control for restraint, the ability of SR 141716A to antagonize the effects of intravenously-administered Δ^9-THC was assessed in another group of mice that was restrained in the holding tubes and exposed to air alone prior to testing.

2. Methods

2.1. Subjects

ICR male mice (Harlan Laboratories, Indianapolis, IN) weighing between 20–25 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 libitum. The mice were brought to the test environment (22–24°C, 12-h light/dark cycle) and allowed 24 h to recover from movement and handling.

2.2. Drugs

Marijuana (containing 3.46% Δ^9-THC, 0.18% cannabinol, 0.17% cannabidiol, 0.14% cannabigerol, and 0.05% tetrahydrocannabivarin), ethanol-extracted marijuana (placebo), Δ^9-THC, and SR 141716A were obtained from the National Institute for 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).

2.3. Exposure system

The exposure system was similar to that described elsewhere (Lichtman et al., 1996) with a few modifications. A 15 cm long corncob pipe was used to burn the plant material. The smoke from the pipe was drawn through a 27.5 cm length of tygon tubing to the manifold at a flow rate of 400 ml/mm using a vacuum pump and flow regulator. A solenoid puffing device was used to alternate the flow of smoke and fresh air to the animals every 8 s. The subjects were placed into holding tubes that fit snugly into a manifold, consisting of six ports for a nose-only exposure. Tygon tubing, containing 0.5 g of glass-wool fiber to sequester the smoke, was connected to the exhaust of the manifold. Mice were exposed to the smoke until the plant material was consumed, typically occurring within a 2–4 min time period. If the material ceased to burn at any time, it was lit again until completely consumed.

2.4. Determination of Δ^9-THC blood levels

Twenty minutes following either i.v. injection of Δ^9-THC (1, 3, or 10 mg/kg) or inhalation exposure to smoke from either 50, 100, or 200 mg of marijuana, the

animals were decapitated and the blood collected in heparinized (Elkins-Sinn, Inc., Cherry Hill, NJ) tubes. The blood was then prepared so that the Δ^9-THC could be extracted and quantified via gas chromatography/mass spectrometry (GC/MS) Calibrators were prepared from blank whole blood. Fifty μl of deuterated Δ^9-THC (1 ng/μl, Radian Corporation, Austin TX) were added to 1.0 ml of calibrator blood and samples were allowed to stand overnight. The following day, 2.5 ml of cold acetonitrile (Fisher Scientific, Raleigh, NC) was added drop-wise, the mixture was vortexed, centrifuged (Marathon 6 K Centrifuge, Fisher Scientific) at 4000 rpm for 10 min, and then stored in the freezer (-20°C) overnight, allowing three layers to form. The acetonitrile layer was removed, 2 ml of 0.2 N NaOH was added, and the mixture was vortexed. Next, 4 ml of 9:1 hexane:ethyl acetate (Fisher Scientific, Raleigh, NC) was added and the vials were then vortexed and spun at 30 rpm for 30 min. After mixing, the vials were centrifuged (4000 rpm) for 10 min. Once again, the organic layer was removed and evaporated to dryness while heated to 40°C. Upon drying, 50 μl of derivatizing agent (Regisil plus 10% TMCS, Regis Technologies, Morton Grove, Il) was added and vortexed. The vials were heated at 40°C for 1 h.

Each sample was injected into a GC/MS (Hewlett Packard 6890, Palo Alto, CA) with a split/splitless injection port and a Hewlett Packard 7683 autosampler for quantitative analysis. The mass selective detector (MSD) was a Hewlett Packard model 5973. The initial oven temperature was 190°C and the final temperature was 230°C. The injection-port temperature was 230°C and the transfer temperature was 280°C. An HP-1 column, 12 m × 0.2 mm, 0.33 μm film thickness was used. The MSD was operated in the selected ion monitoring (SIM) mode using the following ions. 315, 343, and 386 for Δ^9-THC; 318 and 346 for deuterated Δ^9-THC. Dwell times were set at 40 ms. The calibration curve for the assay was constructed with eight calibrators with concentrations of 0, 2, 5, 10, 20, 50, 100, and 200 ng Δ^9-THC per ml. The calibrator was based on a linear regression using peak-area ratios of the first ion listed for each analyte with a limit of detection of 2 ng/ml.

2.5. Behavioral evaluation

2.5.1. Locomotor activity testing protocol

Locomotor activity was assessed by placing mice in individual-photocell-activity cages (6.5 × 11 inches) consisting of eight photocell beams per chamber. Individual mice were placed into one of six chambers for 5 min following smoke exposure for a total duration of 10 min, and total beam interruptions were recorded using a Digiscan Animal Activity Monitor (Med Associates, Inc., St Albans, VT). Activity in the chamber was expressed as the total number of beam interruptions.

2.5.2. Antinociception

The tail-flick response to radiant heat (D'Amour and Smith, 1941) was used to assess antinociception. The intensity of the heat stimulus was fixed to yield control latencies of 2–4 s, and an automatic 10-s cut-off was used.

2.5.3. Rectal temperature

Core temperature to the nearest 0.1°C was recorded by inserting a rectal probe, connected to a telethermometer (Yellow Spring Industries Inc., Yellow Springs, Ohio) to a depth of 2.5 cm.

2.5.4. Catalepsy

A ring-test procedure was used to evaluate catalepsy in mice (Pertwee, 1972) in which each subject was placed on a ring (5.5 cm diameter) that was elevated 16 cm from a table top for a 5-min observation period. The amount of time each subject remained motionless, except for respiratory movements, was recorded to the nearest second.

2.5.5. Procedure

Each subject was given a baseline tail-flick test and assessed for rectal temperature prior to smoke exposure from 10, 50, 100, 200, or 300 mg of marijuana or placebo material. Following exposure, each subject was assessed from 5–15 min for spontaneous activity, at 20 min in the tail-flick test, at 40–45 min for ring immobility, and at 60 min for rectal temperature. Two additional groups of mice were given i.v. injections of Δ^9-THC (0, 0.5, 1, 2, 4, or 8 mg/kg) and assessed in each of the four tests, using the same time points employed in the inhalation experiments. The first group served as an air control group, in which each mouse was individually placed in a holding tube after the injection and exposed to air alone for not more than 4 min. Subjects in the second group served as unrestrained controls and were returned to their cages following the i.v. injection. In the first antagonism experiment, 10 mg/kg of SR 141716A or vehicle was given intravenously 10 min prior to inhalation exposure to either 200 mg of placebo plant material or marijuana. In the second antagonism experiment, 10 mg/kg of SR 141716A or vehicle was given intravenously 10 min prior to an i.v. injection of vehicle or 5.6 mg/kg of Δ^9-THC and immediately placed in the holding tubes and exposed to air alone for approximately 4 min. These quantities of marijuana and Δ^9-THC yielded equivalent blood levels of Δ^9-THC at 20 min. Throughout this study, separate groups of naïve mice were employed for each condition.

2.6. Statistical analysis

Rectal temperature was expressed as the difference between pre- and post-injection values obtained from each mouse. Antinociception was calculated by transforming the tail-fl ick data to per cent maximum possible effect (%MPE, where %MPE = 100 × (post-injection latency − pre-injection latency) ÷ (cut-off time − pre-injection latency)). Statistical analyses were conducted using ANOVA with differences considered significant at $P < 0.05$. Post hoc analyses included Dunnett's test, the Bonferroni t-test, or Tukey's test, and are indicated throughout the text. ED_{50} values of marijuana quantity of Δ^9-THC dose in producing pharmacological effects were determined by least squares linear-regression analysis followed by calculation of 95% confidence limits (Bliss, 1967). The doses of Δ^9-THC that the mice received from marijuana smoke were determined by obtaining the concentration of Δ^9-THC in blood and then calculating the dosage level based on the Δ^9-THC blood levels following i.v. administration of Δ^9-THC.

3. Results

The blood levels of Δ^9-THC obtained 20 min after either inhalation exposure to marijuana or i.v. Δ^9-THC administration are presented in Table 1. ANOVA revealed significant differences in drug blood levels for inhalation, $F(2,26) = 5.7$, $P < 0.05$, and intravenous, $F(2,12) = 424$, $P < 0.05$, routes of administration. A linear relationship between the intravenous dose and Δ^9-THC blood levels was found ($r = 1.00$), with a slope of 71.8 and an origin of 3.1. Using these parameters determined from the i.v. Δ^9-THC blood levels, the 50, 100, and 200 mg marijuana inhalation-exposure groups were calculated to have received Δ^9-THC doses of 2.0, 3.5, and 5.6 mg/kg, respectively. The temperature of the smoke upon entry into the manifold was 19.6°C, equal to ambient temperature under the hood.

The pharmacological effects of placebo and marijuana smoke are shown in Fig. 1. Significant interactions between the amount of material burned and marijuana vs. placebo were found for antinociception, $F(1,4) = 12.6$, $P < 0.05$, and catalepsy, $F(1,4) = 10.5$, $P < 0.05$. The interactions resulted from dose-related increases in the marijuana-exposed, but not the placebo-exposed, mice. The ED_{50} values, with 95% confidence limits (C.L.), for the total amount of marijuana burned for antinociception and catalepsy were 60 (43–85) and 103 (63–170) mg, respectively. Based on the blood levels of Δ^9-THC, these amounts of marijuana corresponded to 2.4 and 3.8 mg/kg, respectively. Increasing the amount of plant material, irrespective of the type significantly depressed locomotor activity and lowered body temperature, $F(1,4) = 11.4$, $P < 0.05$ and $F(1,4) = 6.9$, $P < 0.05$, respectively. There were no differences between marijuana and placebo for these measures as the significant main effect of marijuana and interaction between the amount of material burned and type of material (i.e. marijuana vs. placebo) failed to achieve significance.

The effects of Δ^9-THC given intravenously in unrestrained mice and mice restrained in the holding tubes and exposed to air are shown in Fig. 2. Δ^9-THC elicited significant dose-related increases for antinociception, $F(5,108) = 17.1$, $P < 0.05$, and hypothermia, $F(5,108) = 17.5$, $P < 0.05$. There was no significant main effect of restraint or interaction between restraint and drug for either of these measures. The ED_{50} values (95% C.L. of Δ^9-THC for antinociception was 2.8 (2.0–4.0) mg/kg and for hypothermia was 3.4 (2.6–4.3) mg/kg. Δ^9-THC also inhibited spontaneous activity, $F(5,107) = 2.6$, $P < 0.05$, and caused a dose-related increase in catalepsy, $F(5,108) = 14.4$, $P < 0.05$. However, restraint also significantly increased catalepsy, $F(1,108) = 16.9$, $P < 0.05$, and decreased overall spontaneous activity, $F(1,107) = 9.5$, $P < 0.05$. The interaction between restraint and drug for each of these measures failed to achieve statistical significance. The ED_{50} values, with 95% C.L. of Δ^9-THC in producing

Table 1
Comparison of mouse Δ^9-THC blood levels 20 min following either inhalation exposure to marijuana smoke or an i.v. injection of Δ^9-THC

Drug	Administration route	Dose* (mg/kg)	Δ^9-THC blood levels (ng/ml)
Marijuana	Inhalation of smoke from:		
	50 mg marijuana	2.0	149 ± 43
	100 mg marijuana	3.5	254 ± 34
	200 mg marijuana	5.6	402 ± 77**
Δ^9-THC	i.v. injection	1	$69 \pm 7^{\dagger}$
		3	$226 \pm 21^{\dagger}$
		10	$720 \pm 20^{\dagger}$

*, Inhalation doses were extrapolated from the Δ^9-THC blood levels;
**, significantly different from 50 mg dose (Tukey's test);
†, significantly different from all other groups (Tukey's test).

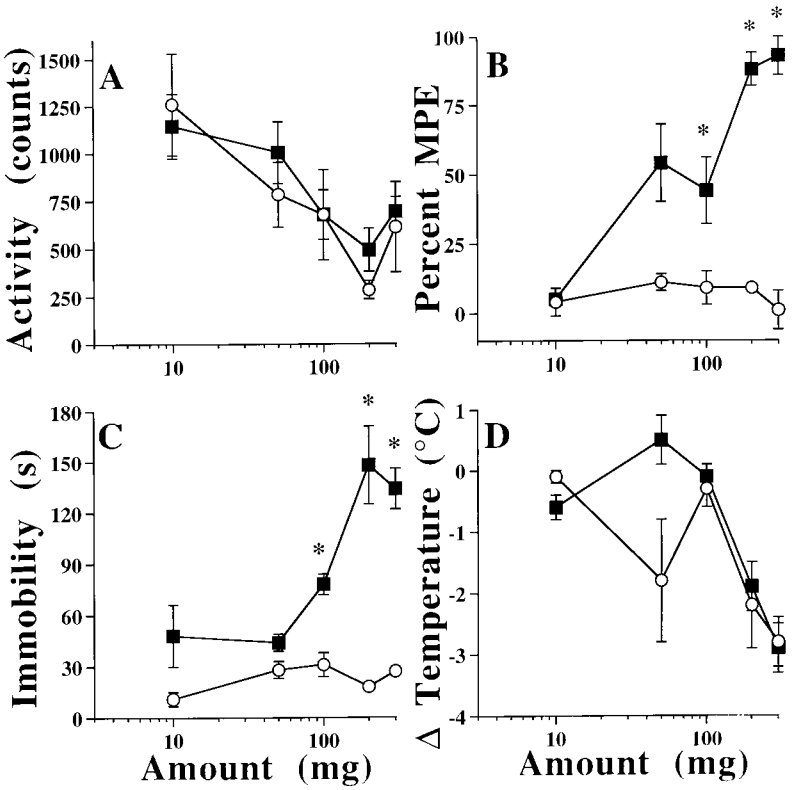

Fig. 1. Mice were exposed to varying quantities of smoke from either placebo or (–○–) marijuana (–■–) and assessed from 5 to 15 min for spontaneous activity (panel A), at 20 min for antinociception (panel B), at 40 min for catalepsy (panel C), and at 60 min for hypothermia (panel D). The results are presented as mean ± S.E., $n = 6$ mice per group. *, Significantly different from the respective placebo control group (Bonferonni t-test, $P < 0.01$).

catalepsy were virtually identical between both conditions, 1.9 (1.4–2.8) mg/kg in the non-restrained group, and 1.7 (1.0–2.9) mg/kg in the air-exposed group. The ED_{50} values were not determined for spontaneous activity because even at the highest dose tested, the drug failed to produce more than a 50% effect in the unrestrained group.

The time course of the antinociceptive effects following inhalation exposure to smoke from 200 mg of marijuana is depicted in Fig. 3. Subjects exhibited significant increases in antinociception, $F(4,25) = 5.2$, $P < 0.05$, that occurred immediately following exposure and lasted for approximately 1 h.

The ability of SR 141716A to antagonize the tetrad of behavioral effects elicited from inhalation exposure to smoke generated from 200 mg of either placebo or marijuana is presented in Fig. 4. A significant interaction was found between plant material (i.e. placebo vs. marijuana) and injection (i.e. vehicle vs. SR 141716A) for antinociception, $F(1,1) = 32$, $P < 0.05$. A Bonferrom t-test, with an alpha level of 0.025 to protect from making a Type-1 error for the two comparisons, indicted that SR 141716A significantly attenuated antinociception in the marijuana-exposed mice, $t(34) = 7.2$, $P < 0.025$, but was without effect in the placebo-exposed subjects ($P > 0.50$). A significant interaction was also found for the cataleptic effects between plant material and SR 141716A, $F(1,1) = 4.6$, $P < 0.05$, though the antagonism was small in magnitude such that a considerable degree of catalepsy was still evident. According to planned comparisons, SR 141716A failed to produce a significant antagonism of catalepsy in the marijuana-exposed mice, $t(34) = 2.3$, $P = 0.027$, and failed to elicit even a marginal attenuation in the placebo smoke-exposed mice, $P > 0.50$. Finally, there were no significant effects of plant material, SR 141716A, or the interaction between the two factors for either spontaneous activity or rectal temperature ($P > 0.25$ for each effect).

The dose–response relationship of SR 141716A in antagonizing the antinociceptive effects following inhalation exposure to the smoke from 200 mg of marijuana is shown in Fig. 5. A significant effect of SR 141716A was found, $F(5,42) = 5.9$, $P < 0.05$. The AD_{50} (95% C.L.) of SR 141716A in antacronizing the antinociceptive effects following inhalation exposure to smoke from 200 mg of marijuana was 0.6 (0.3–1.2) mg/kg.

Shown in Fig. 6 are the pharmacological effects of vehicle or SR 141716A pretreatment in mice given an i.v. injection of vehicle or Δ^9-THC (5.6 mg/kg), placed in the holding tubes, and exposed to air alone. SR

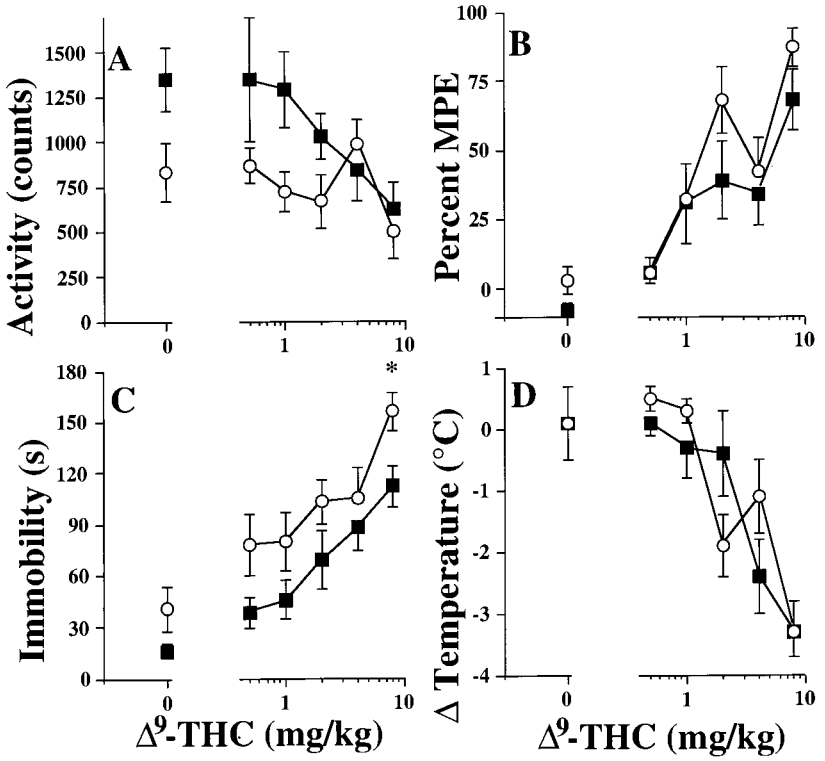

Fig. 2. Mice were given an i.v. injection of vehicle or Δ^9-THC, either left alone (–■–) or placed in a holding tube and exposed to air alone (–○–) and assessed from 5 to 15 min for spontaneous activity (panel A), at 20 min for antinociception (panel B), at 40 min for catalepsy (panel C), and at 60 min for hypothermia (panel D). The results are presented as mean ± S.E., $n = 10$ mice per group. *, Significantly different from the respective placebo control group (Bonferonni t-test, $P < 0.0083$).

141716A antagonized the antinociceptive ($F(1,20) = 58$, $P < 0.05$), cataleptic ($F(1,20) = 24$, $P < 0.05$), and hypothermic ($F(1,20) = 16$, $P < 0.05$) effects produced by Δ^9-THC as indicated by significant interactions between the two drugs. According to planned comparisons, SR 141716A-significantly blocked each of these pharmacological effects in the Δ^9-THC-injected mice, $P < 0.025$ for each comparison. In addition, significantly more immobility in the ring test was found in the vehicle-treated mice that were pretreated with SR 141716A than in the vehicle-treated mice that were also pretreated with vehicle, $P < 0.025$. Also, SR 141716A led to a slight but significant drop in body temperature in the vehicle mice, $P < 0.025$. Finally, no significant effects were found for spontaneous activity, $P > 0.10$ for each effect.

4. Discussion

Mice were used to investigate the inhalation route of marijuana administration. Inhalation of marijuana elicited some pharmacological effects that were similar to those obtained from i.v. administration Δ^9-THC. In order to study the pharmacological effects elicited by inhalation exposure to marijuana smoke, approaches

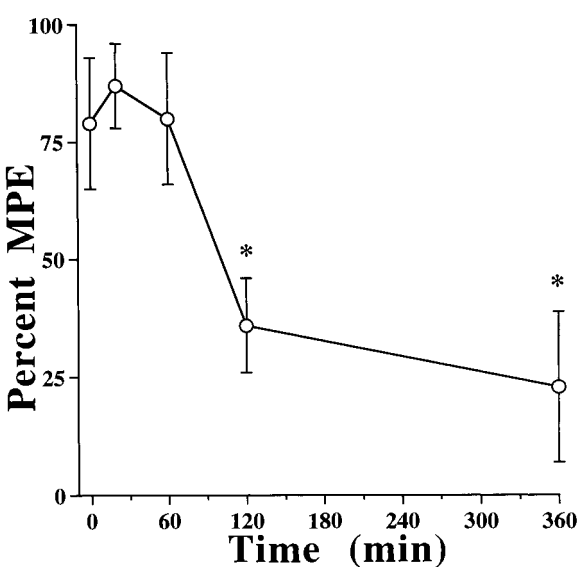

Fig. 3. Time course of the antinociceptive effects following inhalation exposure to 200 mg of marijuana smoke. The results are presented as mean ± S.E. of % MPE, $n = 6$ mice per group. A separate group of mice was used for each time point. *, Significantly different from the groups tested 0, 20, and 60 min following exposure (Tukey's test, $P < 0.05$).

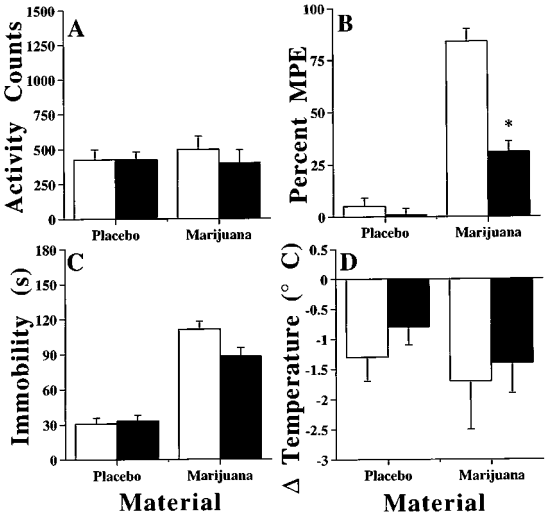

Fig. 4. The impact of vehicle (□) or 10 mg/kg of SR 141716A (■) given before inhalation exposure to the smoke generated from burning 200 mg of placebo or marijuana was evaluated on spontaneous activity (panel A), antinociception (panel B), catalepsy (panel C), and hypothermia (panel D). The results are presented as mean ± S.E., $n = 6$–18 mice per group. *, Significantly different from the respective vehicle control group (Bonferonni t-test, $P < 0.025$).

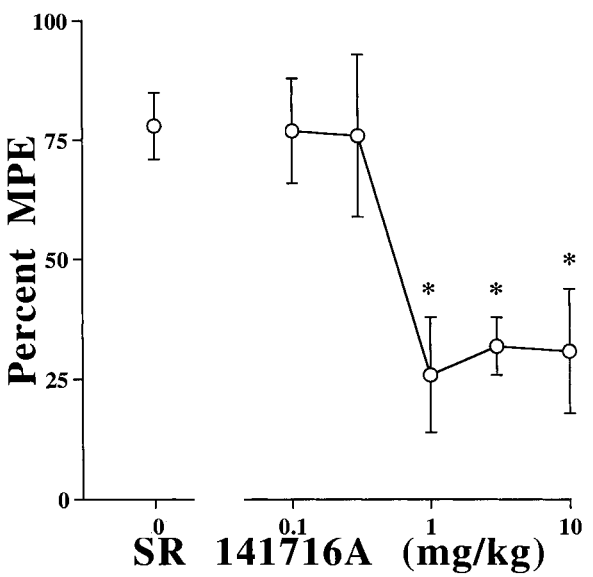

Fig. 5. SR 141716A antagonized the antinociceptive effects following inhalation exposure to smoke generated from 200 mg of marijuana with an AD_{50} dose (95% C.L.) of 0.6 (0.3–1.2) mg/kg. The results are presented as mean ± S.E., $n = 6$ mice per group. *, Significantly different from the respective placebo control group (Dunnett's test, $P < 0.05$).

were taken to examine the relationship between its pharmacological potency upon inhalation and Δ^9-THC blood levels. Determining Δ^9-THC blood levels enabled us to evaluate the relationship between smoking and Δ^9-THC. The most striking pharmacological effect that inhalation exposure to marijuana smoke elicited was a dose-dependent increase in antinociception that occurred immediately following exposure and lasted approximately 60 min. The calculated antinociceptive ED_{50} dose of Δ^9-THC following marijuana exposure of 2.4 mg/kg is remarkably similar to the i.v. antinociceptive ED_{50} dose of 2.8 mg/kg. These findings are consistent with the notion that Δ^9-THC is chiefly responsible for the antinociceptive effects of marijuana. Moreover, the ability of SR 141716A to block the antinociceptive effects following injected Δ^9-THC and inhalation of marijuana smoke indicates a CB_1-receptor mechanism of action. Finally, the AD_{50} (95% C.L.) value of SR 141716A found here to be 0.6 (0.3–1.2) mg/kg is in good concordance with its 1.6 (0.2–11) and 0.12 (0.02–0.66) mg/kg AD_{50} values in antagonizing the antinociceptive effects of WIN 55 212–2 (Rinaldi-Carmona et al., 1994) and Δ^9-THC (Compton et al., 1996), respectively.

Marijuana also produced a dose-dependent increase in catalepsy with an estimated ED_{50} Δ^9-THC dose of 3.8 mg/kg. Conversely, the failure of SR 141716A to produce more than a slight reversal of marijuana-induced catalepsy suggests a predominant noncannabinoid component. In addition, an equivalent degree of hypothermia and locomotor suppression occurred between the marijuana-exposed and placebo-exposed mice at each quantity of material tested. Inhalation exposure to marijuana or placebo smoke has also been previously reported to lead to similar degrees of locomotor depression in mice (Weinberg et al., 1977a,b) and rats (Fried and Nieman, 1973; Fried, 1976). Moreover, the failure of SR 141716A to block either the

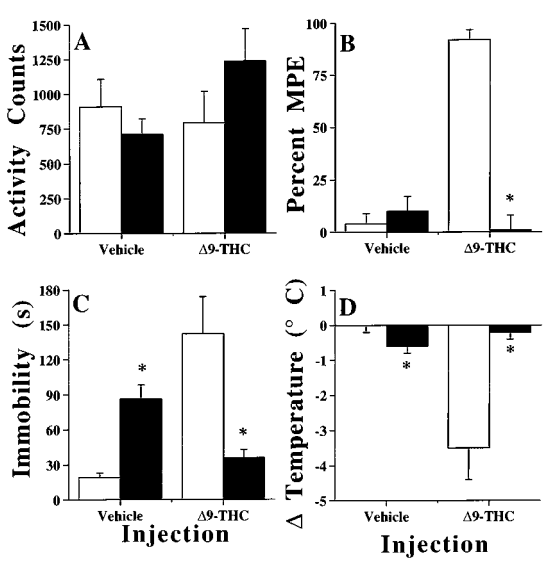

Fig. 6. Evaluation of vehicle (□) or 10 mg/kg of SR 141716A (■) given 10 before an i.v. injection of vehicle or Δ^9-THC (5.6 mg/kg) on spontaneous activity (panel A), antinociception (panel B), catalepsy (panel C), and hypothermia (panel D). The results are presented as mean ± S.E., $n = 6$ mice per group. *, Significantly different from the respective vehicle-control group (Bonferonni t-test, $P < 0.025$).

hypothermic or the locomotor-depressive effects suggests that a CB_1-receptor mechanism of action does not mediate the pharmacological effects resulting from smoke inhalation. The degree of locomotor-activity depression elicited by the smoke and restraint is likely to have obscured any cannabinoid-mediated effects on this measure. Finally, the hypothermic effects were quite modest, also reducing the likelihood of observing a significant reduction by SR 141716A. Because of the issue of toxicity, higher quantities of marijuana were not tested.

The pharmacological effects of marijuana exposure are in stark contrast to the effects of i.v. administered cannabinoids in which all four pharmacological effects occur (Little et al., 1988; Compton et al., 1993). The impact of restraining the mice in the holding tubes may have confounded some of the pharmacological measures of interest. Whereas freely moving mice exhibited the full tetrad of responses following i.v. Δ^9-THC, placement in the restraining tubes alone led to a reduction in the level of spontaneous activity and increased catalepsy as assessed in the ring immobility test. In fact, the restrained mice failed to exhibit a further reduction in locomotor activity following Δ^9-THC administration. Nonetheless, SR 141716A antagonized the antinociceptive, hypothermic and cataleptic effects of i.v. administered Δ^9-THC in mice that were restrained in the holding tubes and exposed to air alone for 4 min. Interestingly, SR 141716A significantly increased catalepsy in the mice given a vehicle injection and exposed to air alone. Although SR 141716A has been previously found to stimulate locomotor activity (Compton et al., 1996), it failed to affect locomotor behavior in this same group of mice.

The results of the present study also demonstrate that smoke inhalation of ethanol-extracted marijuana, containing no detectable levels of cannabinoids, led to locomotor inhibitory, hypothermic, and cataleptic effects. It is unlikely that these effects resulted from heat because the smoke was at ambient temperature by the time it reached the mice, though the smoke may have still been irritating to the respiratory tract. It remains to be determined whether these effects resulted specifically from the noncannabinoid constituents alone or as consequence of indirect effects, such as hypoxia. Exposure to marijuana smoke has been found to increase carboxyhemoglobin levels in mice (Watson et al., 1987), suggesting that hypoxia may have played a contributing role to the pharmacological effects observed in the present study. Indeed, the mice in both the placebo and the marijuana groups, but not the air-exposed mice, exhibited prostration upon removal from the restraining tubes in the present study, suggesting behavioral toxicity. Conversely, noncannabinoid chemicals may have pharmacological consequences, some of which may impact upon the effects of Δ^9-THC. Truitt et al. (1976) previously examined the pharmacological effects of different fractions of marijuana-smoke condensate given intravenously to rats. They found that some of the noncannabinoid compounds present in the smoke produced effects on their own and also altered some of the pharmacological effects of Δ^9-THC (Truitt et al., 1976). Although these effects may limit the direct comparison of smoked marijuana to injected Δ^9-THC, an inhalation model may better approximate cannabinoid use in humans than injecting rodents with Δ^9-THC or other cannabinoid agonists.

Exposure to chronic marijuana smoke has not been causally linked to pulmonary diseases or deleterious cardiovascular consequences; however, polycyclic aromatic hydrocarbons known to have carcinogenic properties, such as benz[a]pyrene, have been isolated from marijuana-smoke condensate (Lee et al., 1976). Conversely, smoke from ethanol-extracted marijuana is not innocuous, levels of aromatic carcinogens- in monkey lungs did not differ between subjects that were exposed to marijuana smoke and animals exposed to placebo smoke (Talaska et al., 1992). Similarly, exposure to marijuana or placebo smoke has been found to impair the pulmonary antibacterial defense system (Huber et al., 1980). These results indicate that exposure to noncannabinoid constituents of marijuana smoke may have long-term consequences in addition to the acute pharmacological effects.

As only a small fraction of the total amount of available drug is inhaled during the exposure period, the issue of dosimetry becomes an important concern in inhalation studies. Although there are many cannabinoids present in marijuana, Δ^9-THC is its major psychoactive ingredient and is generally accepted to be responsible for most of marijuana's psychoactive effects. The present study calculated the drug dose by extrapolating the Δ^9-THC blood levels following marijuana exposure to the drug blood levels following i.v. Δ^9-THC administration. Because the tests were conducted at different times following smoke exposure or injection and blood levels were determined at only one time point, it is possible that the time differences may account for the differences in pharmacological effects found. The accuracy of the extrapolated doses based on i.v. Δ^9-THC injection is dependent on similar absorption and pharmacokinetics between the two routes of administration. However, this assumption is supported by previous work that demonstrated that the subjective effects and blood levels of Δ^9-THC exhibited similar time courses following inhalation of marijuana smoke or i.v. injection of Δ^9-THC in humans (Ohlsson et al., 1980; Hollister et al., 1981).

Previous research used an indirect approach to determine Δ^9-THC doses following marijuana inhalation. The total amount of drug available for inhalation was adjusted by accounting for pyrolysis and side-stream

smoke, and this value was then divided by the body weight of the subject to calculate dose (Rosenkrantz and Braude, 1974). One such calculation employed for determining dose was that approximately 50% of the total Δ^9-THC present in marijuana is lost due to pyrolysis (Truitt, 1971) and that the 30–45% of the remaining drug was inhaled (Rosenkrantz and Braude, 1974). Thus, using these parameters for the present study in which the 60 mg ED_{50} amount of marijuana containing 3.46% Δ^9-THC is divided by six, to account for the six nose-only-exposure portals in our system, each mouse would have received between 0.052 and 0.078 mg of Δ^9-THC. These amounts translate to an estimated dose of 2.1–3.1 mg/kg in a 25-g mouse, a value virtually identical to the 2.4-mg/kg dose we determined by extracting Δ^9-THC from blood and extrapolating the blood levels to those following i.v. administration.

In conclusion, our findings indicate that inhalation exposure to marijuana smoke elicits some effects that are similar to those produced by systemic administration of Δ^9-THC. Although the Δ^9-THC blood levels achieved should have been sufficient to produce the full tetrad of responses, the fact that exposure to placebo smoke alone affected these indices is likely to have overshadowed some of the cannabinoid-mediated events. Thus, exposure to smoke alone also has an important pharmacological impact and should not be neglected when a rodent model is used to study marijuana abuse. Nonetheless, the Δ^9-THC blood levels are consistent with the interpretation that Δ^9-THC is mainly responsible for the antinociceptive effects. Finally, the antagonism of marijuana-induced antinociception by SR 141716A implicates a CB_1-receptor mechanism of action.

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