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Pharmacological evaluation of aerosolized cannabinoids in mice

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Abstract

The reemergence on the debate of the use of marijuana for medicinal purposes has been the impetus for developing an acceptable delivery form of aerosolized cannabinoids. The goals of the present study were to: (1) develop and characterize the physical properties of an aerosolized form of Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive constituent present in marijuana; and (2) assess the pharmacological effects of cannabinoid inhalation in mice. A Small Particle Aerosol Generator (SPAG) nebulizer, used to generate the aerosol, had an output of approximately 0.154 mg/l of aerosolized Δ^9-THC with a 2.0 μm mass median aerodynamic diameter and a 2.2 geometric standard deviation (GSD). Virtually all the particles were less than 5.0 μm in diameter suggesting that they were sufficiently small to penetrate deeply into the lungs. Inhalation exposure to aerosolized Δ^9-THC in mice elicited antinociceptive effects that were dependent on concentration and exposure time with an estimated Δ^9-THC dose of 1.8 mg/kg. On the other hand, inhalation exposure to Δ^9-THC failed to produce two other indices indicative of cannabinoid activity, hypothermia and decreases in spontaneous locomotor activity. The antinociceptive effects occurred within 5 min of exposure and lasted approximately 40 min in duration. The cannabinoid receptor antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide HCl (SR 141716A), but not naloxone, blocked these antinociceptive effects (AD_{50} = 0.09 mg/kg) indicating a cannabinoid receptor mechanism of action. Similarly, inhalation exposure to a water soluble cannabinoid analog, 3-(5′-cyano-1′,1′dimethylheptyl)-1-(4-*N*-morpholino-butyrloxy)-Δ^8-tetrahydrocannabinol (O-1057), produced antinociception that was blocked by SR 141716A. These results demonstrate that the development of an aerosolized form of cannabinoids for human medicinal use is feasible. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Cannabis was first introduced into Western cultures more than 150 years ago for the relief of a wide range of disorders (O'Shaughnessy, 1842). In addition to its potential therapeutic value, marijuana also possesses psychoactive properties such as a psychological high that has resulted in its popularity as a recreational drug of abuse. In an attempt to curtail its use, the Marijuana Tax Act was passed in 1937, and the drug was eventually made illegal. However, marijuana remains the most abused illicit drug in the United States today (Johnston et al., 1998a,b). Recently, the debate on medical marijuana has reemerged, despite the availability of an oral dosage form of Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive constituent present in marijuana. Synthetic Δ^9-THC (Marinol®), has been available in capsule form in the United States since 1985 and has been demonstrated to relieve nausea and vomiting related to cancer chemotherapy (Frytak et al., 1979; Sallan et al., 1980, 1975), and

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to stimulate appetite in patients suffering from acquired immunodeficiency syndrome (AIDS) wasting syndrome (Beal et al., 1997, 1995). When taken orally, however, Δ^9-THC undergoes considerable first pass metabolism and is slowly and erratically absorbed, with a delayed onset of action between 30 min and 2 h (Lemberger, 1973; Wall and Perez-Reyes, 1981). Conversely, marijuana smoke contains aerosolized Δ^9-THC and its inhalation is characterized by fast absorption through the lungs resulting in an immediate elevation of arterial blood drug concentration, and a higher bioavailability due to avoiding drug metabolism by the liver (Lemberger, 1973; Wall and Perez-Reyes, 1981). Consequently, these properties of marijuana smoke should presumably allow patients to titrate their doses better than the oral route of administration.

During the last few years, advocates have successfully petitioned for legislation in several states that sanction the use of marijuana for medical purposes. Although some have advocated that marijuana be made available for certain patients (Stiefel, 1996; Taylor, 1998), the general medical community has not accepted marijuana as a therapeutic agent (Schwartz and Voth, 1995; Voth and Schwartz, 1997). This continuing controversy led the Office of the National Drug Control Policy to petition the Institute of Medicine (IOM) to assess the scientific literature and make recommendations for a reasonable course of action. Although the IOM report acknowledged that marijuana may indeed be a beneficial treatment relieving discomfort in some seriously ill patients, it concluded that smoked marijuana represented an unacceptable delivery form of Δ^9-THC (Joy et al., 1999). Marijuana smoke contains hundreds of other chemicals that have not been fully characterized, some of which are irritating to lung including polycyclic aromatic hydrocarbons known to have carcinogenic properties, such as benz{*a*}pyrene (Lee et al., 1976). Exposure to marijuana or placebo smoke has also been found to impair the pulmonary antibacterial defense system (Huber et al., 1980) and is known to expose humans to greater amounts of carbon monoxide and insoluble particulates than smoking a similar quantity of tobacco (Wu et al., 1988).

Because marijuana smoke poses health problems for patients who are severely health compromised, the IOM report recommended that an alternative delivery system be developed (Joy et al., 1999). A Δ^9-THC aerosol device would provide a safe and controlled method to administer the drug, without the harmful effects of smoke constituents. In fact, there was some interest in the 1970s in developing aerosolized Δ^9-THC generated from either a metered-dose inhaler (Tashkin et al., 1976; Williams et al., 1976) or a nebulizer (Vachon et al., 1976) for its bronchodilatory properties in humans. Although inhalation of aerosolized Δ^9-THC produced a bronchodilatory effect (Tashkin et al., 1976; Vachon et al., 1976; Williams et al., 1976), it also caused some chest discomfort and coughing in some normal subjects and bronchoconstriction in some asthmatics (Tashkin et al., 1977). Consequently, this area of research has been left relatively untouched for more than 20 years.

The first goal of the present study was to develop and characterize a Δ^9-THC aerosol. A Small Particle Aerosol Generator (SPAG) nebulizer was employed to generate aerosolized Δ^9-THC. The output of Δ^9-THC was determined by capturing the aerosol in a filter and the particle size analysis of the aerosol was determined using the Andersen Cascade impactor.

The second goal of this study was to deliver a systemic dose of cannabinoids via inhalation and determine whether pharmacological effects could then be elicited in mice. A serious limitation of Δ^9-THC and most other cannabinoids is that they are virtually insoluble in aqueous solution, requiring the addition of solvents and/or surfactants to make a solution or suspension (Garrett and Hunt, 1974). However, a water soluble cannabinoid analog, 3-(5′-cyano-1′,1′dimethylheptyl)-1-(4-*N*-morpholinobutyrloxy)-Δ^8-tetrahydrocannabinol (O-1057), is available and has been found to bind to the cannabinoid receptor with relatively high affinity and produce the full pharmacological spectrum of cannabinoid effects with great potency (Martin and Razdan, 1998; Pertwee et al., 2000). Therefore, we evaluated the antinociceptive effects of aerosolized Δ^9-THC or O-1057 in the tail-flick test to radiant heat. In order to assess whether the antinociceptive effects were mediated through cannabinoid receptors, some subjects were pretreated with the CB_1 receptor antagonist, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide HCl (SR 141716A) (Rinaldi-Carmona et al., 1994), prior to aerosol exposure. Similarly, some subjects were pretreated with naloxone prior to inhalation exposure to aerosolized Δ^9-THC to infer the involvement of opioid receptors. Finally, the blood levels of Δ^9-THC were determined following inhalation exposure in order to provide direct evidence that the drug was actually absorbed following aerosol exposure. The blood was taken 20 min following exposure, a time point at which reliable antinociceptive effects were observed. Obtaining the blood levels of Δ^9-THC enabled us to compare the antinociceptive effects between intravenous and inhalation routes of administration at similar drug blood levels.

2. Materials and methods

2.1. Subjects

ICR male mice (Harlan Laboratories, Indianapolis, IN) weighing between 20 and 24 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 subjects 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

Δ^9-THC and SR 141716A were obtained from the National Institute for Drug Abuse (NIDA). Naloxone–HCl was purchased from Sigma (St. Louis, MO) and O-1057 was supplied by Organix (Woburn, MA). Δ^9-THC and SR 141716A were dissolved in a 1:1 mixture of absolute ethanol (Aaper Alcohol and Chemical, Shelbyville, KY) and Emulphor-620 (Rhone-Poulenc, Princeton, NJ) and diluted with saline to form a final vehicle mixture of ethanol/emulphor/saline (1:1:18). Saline was used as the vehicle for both naloxone and O-1057. The concentrations of Δ^9-THC and O-1057 put into the reservoir of the nebulizer, unless otherwise noted, were 10 and 1.0 mg/ml, respectively. SR141716A was injected into a tail vein in a volume of 10 ml/kg, 10 min before exposure to nebulized drug or vehicle, and naloxone was administered subcutaneously 5 min before the exposure.

2.3. Exposure system

The exposure system was similar to that described elsewhere (Lichtman et al., 1996) with a few modifications. The subjects were placed into the holding tubes that fit snugly into the manifold for a nose only exposure. Tygon tubing, containing 0.5 g of glass wool fiber to sequester the aerosol, was connected to the exhaust of the manifold. The air flow was produced by a vacuum pump. The aerosol generator was a SPAG-2, 6000 series (ICN Pharmaceutical, Costa Mesa, CA) nebulizer with an air flow of 7.5 l/min and a drying-air flow of 9 l/min. Subjects were placed in animal holders that were connected to a manifold where they were given a nose only exposure to aerosolized drug or vehicle for 10 min, unless otherwise noted.

2.4. Physiochemical characterization of aerosolized Δ^9-THC

In order to determine the Δ^9-THC output of the nebulizer, the aerosol was collected on filter paper for the first 2 and last 2 min from a 10-min nebulization. The filter papers containing Δ^9-THC were added to 5 ml of ethanol and the amounts of Δ^9-THC in the ethanol were determined using a Hewlett-Packard (Avondale, PA) GC/MS equipped with a HP Ultra I column coated with cross-linked methyl silicone gum (0.2 mm i.d., 20 m length, 0.33 μm film thickness). A total ion scan with a range 50–500 mw was used to detect the drug. Standard curves generated from the experimental stock solution (0.5–6.0 μg of Δ^9-THC) yielded correlation coefficients of $r = 0.998$ for each experiment.

Particle size analysis of Δ^9-THC was determined using the Andersen Cascade Impactor (Andersen Sanplers, Atlanta, GA). The Andersen consists of a preseparator, eight stages, and an absolute filter. Each stage has a number of orifices of different diameters arranged in a radial pattern on each stage. The aerodynamic sizes, which are collected with 50% efficiency at each stage of the sampler, are 9.0, 5.8, 4.7, 3.3, 2.1, 1.1, 0.7 and 0.4 at stages 0 through 7, respectively. The samples were drawn through the impactor at a volumetric flow rate of 28.3 l/min. The Δ^9-THC deposition was determined by high-performance liquid chromatography. Three replicates were preformed both for assessing the output of the nebulizer and the particle size analysis.

2.5. Behavioral testing protocol

Subjects were assessed for nociception on a standard tail-flick apparatus (D'Amour and Smith, 1941; Dewey et al., 1970). The heat emitted from the apparatus was maintained at an intensity sufficient to elicit tail-flick latencies of 2–4 s, with a 10-s cut-off time. Each subject was tested once for a baseline value prior to exposure and again 20 min after inhalation exposure, unless otherwise noted.

Locomotor activity was assessed by placing mice in individual photocell activity cages (6.5×11 in.) containing 16 photocell beams per chamber. The subjects were first placed in the exposure system and allowed to breath either aerosolized vehicle or Δ^9-THC for 10 min. Immediately after the exposure, the mice were put in the activity chambers, and interruptions of the photocell beams were recorded for 40 min using a Digiscan Animal Activity Monitor (Omnitech Electronics, Columbus, OH). Activity in the chamber was expressed as the total number of beam interruptions.

Rectal 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). Subjects were assessed for rectal temperature 5, 20, 40, and 60 min following the exposure.

2.6. Determination of Δ^9-THC blood levels

A total of 12 mice in two separate experiments were given a 10-min inhalation exposure to aerosolized Δ^9-THC from a concentration of 10 mg/ml. Twenty minutes following the exposure, each animal was decapitated and using methods previously described (Lichtman et al., in press) the Δ^9-THC was extracted from blood using a modified method from Peel and Perrigo (1981). The blood was collected in heparinized (Elkins-Sinn, Cherry Hill, NJ) tubes and the calibrators were prepared from blank whole blood. The standard consisted of 1 mg/ml Δ^9-THC in methanol (Radian, Austin, TX) and diluted to 2, 5, 10, 20, 50, 100, and 200 ng/ml. The standard curve was linear and had a correlation coefficient of $r = 0.999$. A total of 50 μl of deuterated Δ^9-THC (1 ng/μl, Radian, Austin, TX) were added to 1.0 ml of calibrator blood and samples were allowed to stand overnight. Δ^9-THC was converted to

its trimethyl silyl derivative for gas chromatography/mass spectrophotometry (GC/MS) using a modification of a published procedure (Foltz et al., 1980) in which 50 μl of N_2O–Bis(Trimethylsilyl)Trifluoroacetamide (BSTFA) with 10% trimethylchlorosilane (Regis Technologies, Morton Grove, IL) was added. Each sample was injected into a GC/MS (Hewlett Packard 6890, Palo Alto, CA) with a split/splitless injection port and a 7683 autosampler for quantitative analysis. The mass selective detector (MDS) was a Hewlett Packard model 5973. The lower limit of detection for Δ^9-THC was 2.0 ng/ml.

2.7. Statistical analysis

Antinociception was calculated by transforming the tail-flick data to percent maximum possible effect (%MPE), where %MPE = 100 × ((post-injection latency − pre-injection latency)/(cut-off time − pre-injection latency)). Rectal temperature was expressed as the difference between pre- and post-injection values obtained from each mouse. Statistical analyses were conducted using *t*-tests, analysis of variance (ANOVA) or two-way ANOVA with differences considered significant at $p < 0.05$. Tukey's test was used for all post hoc analyses, unless otherwise noted. The AD_{50} value of SR 141716A in antagonizing the antinociceptive effects of inhalation exposure to aerosolized Δ^9-THC was determined by least squares linear regression analysis followed by calculation of 95% confidence limits (Bliss, 1967).

3. Results

Using cascade impaction, the mass median aerodynamic diameter of the Δ^9-THC particles emitted from the nebulizer was determined to be 2 μm with a geometric standard deviation (GSD) of 2.2. All the particles were less than 4.7 μm in diameter. The mean (±S.E.M.) aerosol Δ^9-THC output by the nebulizer for the first and final 2 min of the 10-min exposure period were 0.155 ± 0.01 and 0.154 ± 0.005 mg/l, respectively. There was no statistical difference in the amount of aerosol generated between these two time blocks ($p > 0.9$).

A 10-min exposure to aerosolized Δ^9-THC (10 mg/ml), led to a mean (±S.E.M.) drug concentration in blood of 133 ± 36 ng/ml 20 min after inhalation exposure. The dose of Δ^9-THC achieved by inhalation exposure was determined by first obtaining the concentration of Δ^9-THC in blood and then calculating the dose based on the blood levels following i.v. administration of the drug. We have previously found a linear relationship between the intravenous dose and Δ^9-THC blood levels ($r = 1.00$) 20 min following drug administration, with a slope of 71.8 and an origin of 3.1 (Lichtman et al., in press). The inhalation dose was calculated from the regression of the i.v. injected dose by plugging the Δ^9-THC blood levels following inhalation exposure into the following equation: 133 = 71.8 × inhalation dose + 3.1. Solving the equation for a line, the inhalation dose was determined to be 1.8 mg/kg of Δ^9-THC.

As shown in Fig. 1, the antinociceptive effects of a 10 mg/ml Δ^9-THC solution were dependent on exposure time, $F(2,15) = 7.8$, $p < 0.05$, with the 10-min exposure leading to significantly longer tail-flick latencies than either the 1- or 3-min exposures. There was also a significant effect of Δ^9-THC concentration following the 10-min exposure period, $F(4,25) = 3.5$, $p < 0.05$ (Fig. 2). The 10 mg/ml concentration of Δ^9-THC produced a significant antinociceptive effect compared to the vehicle alone and the 0.625 mg/ml concentration of drug, but no other statistical differences were found. On the other hand, inhalation exposure to aerosolized Δ^9-THC failed to produce either hypothermia at 5, 20, 40, and 60 min or decreases in spontaneous activity in mice from 0 to 40 min (data not shown).

The time course for the antinociceptive effects following a 10-min exposure to 10 mg/ml Δ^9-THC or vehicle is presented in Fig. 3. A significant interaction between time following exposure and drug was found, $F(4,15) = 2.8$, $p < 0.05$. Significant antinociceptive effects between the Δ^9-THC group and each corresponding vehicle group were found within 5 min following exposure and lasted for 40 min.

The antinociceptive effects produced by aerosolized Δ^9-THC were blocked by SR 141716A in a dose-dependent fashion, $F(5,30) = 4.6$, $p < 0.05$ with an AD_{50} (95% C.L.) of 0.09 (0.02–0.26) mg/kg (Fig. 4). In contrast,

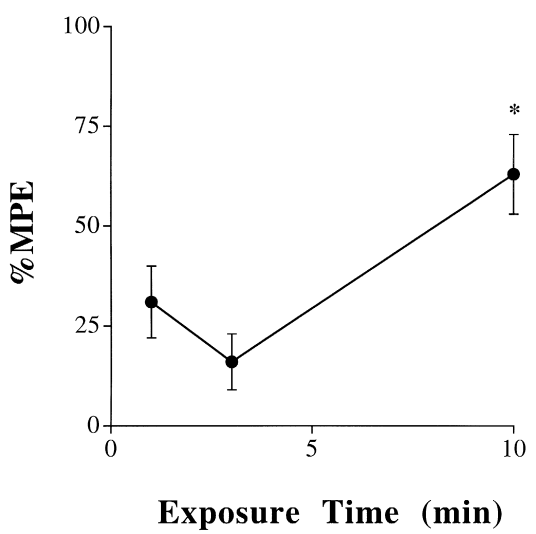

Fig. 1. The antinociceptive effects following inhalation exposure to aerosolized Δ^9-THC are dependent on the duration of exposure time. Subjects were exposed to aerosolized Δ^9-THC (10 mg/ml) for 1, 3, or 10 min. Twenty minutes following the exposure the subjects were assessed in the tail-flick test. The results are presented as means ± S.E.M. of %MPE, $n = 6$ mice per group. A separate group of mice was used for each group. * The subjects exposed to Δ^9-THC for 10 min exhibited significantly more antinociception than the other groups.

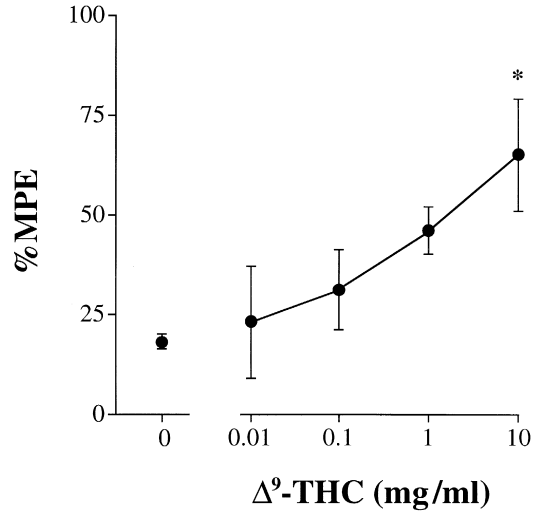

Fig. 2. The antinociceptive effects following inhalation exposure to aerosolized Δ^9-THC are dependent on concentration. Subjects were exposed to aerosolized vehicle or Δ^9-THC for 10 min and tested in the tail-flick test 20 min following the exposure. The results are presented as means ± S.E.M. of %MPE, $n = 6$ mice per group. A separate group of mice was used for each group. * The subjects exposed to the 10 mg/ml concentration of Δ^9-THC exhibited significantly more antinociception than the vehicle-exposed and lowest dosage of Δ^9-THC groups.

naloxone failed to block the antinociceptive effects following inhalation exposure to nebulized Δ^9-THC (Fig. 5). Both the main effect of naloxone and the interaction between aerosolized Δ^9-THC and naloxone failed to approach statistical significance ($p > 0.25$), though there was a significant main effect of Δ^9-THC, $F(1,20) = 16.6$, $p < 0.05$.

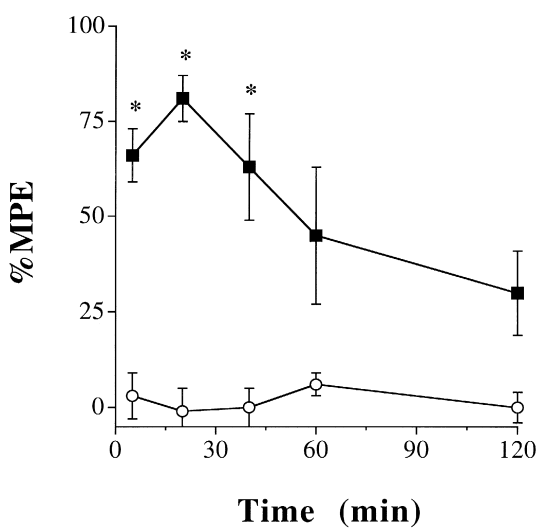

Fig. 3. Time course of the antinociceptive effects of aerosolized Δ^9-THC. Subjects were given a 10-min exposure to either nebulized vehicle (—○—) or nebulized Δ^9-THC (—■—) and assessed in the tail-flick test at 5, 20, 40, 60, or 120 min later. The results are presented as means ± S.E.M. of %MPE, $n = 6$–12 mice per group. A separate group of mice was used for each time point. * Significantly different from the respective placebo control group (Bonferonni t test, $p < 0.05$).

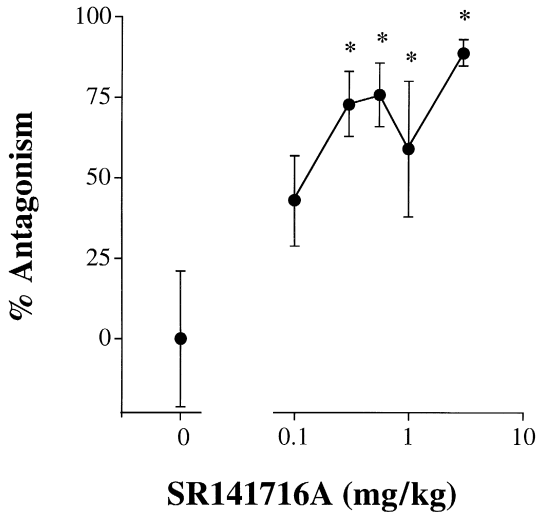

Fig. 4. SR 141716A antagonized the antinociception produced by inhalation exposure to aerosolized Δ^9-THC. Subjects were given an i.v. injection of vehicle or SR141716A, and 10 min later given a 10-min exposure to either aerosolized vehicle or aerosolized Δ^9-THC (10 mg/ml). Twenty minutes following the exposure the subjects were assessed in the tail-flick test. Inhalation exposure to aerosolized Δ^9-THC in the mice pretreated with vehicle produced an antinociceptive effect of 71 ± 15%MPE. The ordinate reflects the percentage of antagonism of that effect at each dose of SR141716A pretreatment. The AD_{50} of SR141716A was determined to be 0.09 mg/kg. The results are presented as means ± S.E.M. of %MPE, $n = 6$ mice per group. Significantly different from the 0 mg/kg SR 141716A group (Dunnett's test, $p < 0.05$).

Inhalation exposure to the water-soluble cannabinoid analog O-1057 produced a significant increase in antinociception compared to the saline exposure group, $t(10) = 3.3$, $p < 0.05$ (Fig. 6, top panel). This antinociceptive effect of

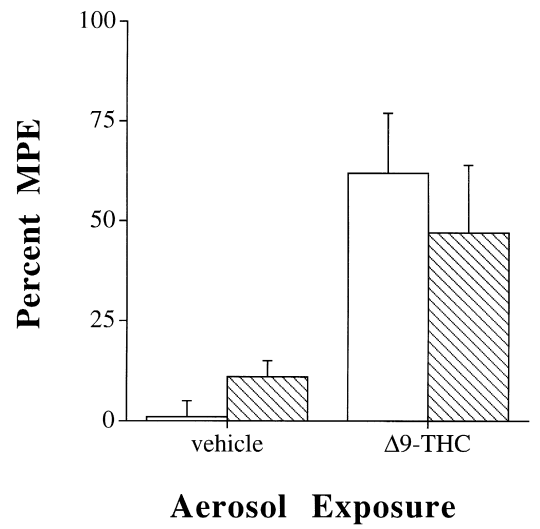

Fig. 5. Naloxone (1 mg/kg) failed to antagonize the antinociception produced by inhalation exposure to aerosolized Δ^9-THC. Subjects were given an i.v. injection of vehicle (□) or naloxone (▨) and 5 min later given a 10-min exposure to either aerosolized vehicle or aerosolized Δ^9-THC (10 mg/ml). Twenty minutes following the exposure the subjects were assessed in the tail-flick test. The results are presented as means ± S.E.M. of %MPE, $n = 6$ mice per group.

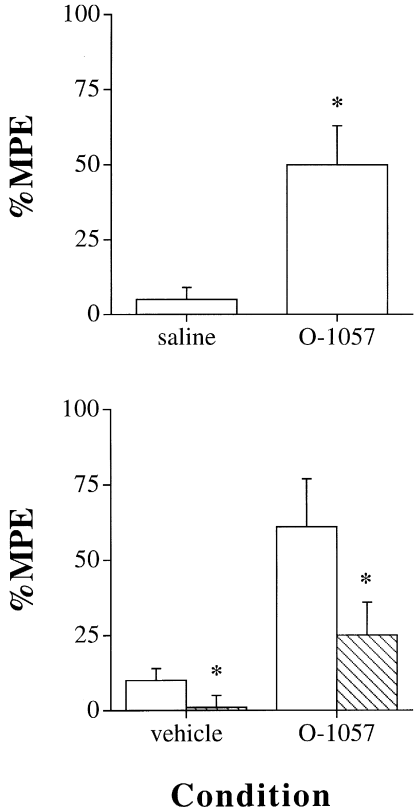

Fig. 6. Antinociceptive effects of the water soluble cannabinoid O-1057. Subjects were given a 10-min exposure to aerosolized saline or the water-soluble cannabinoid O-1057 (1 mg/ml) and assessed in the tail-flick test 20 min later. Top panel: O-1057 produced significantly more antinociception than exposure to saline ($p < 0.05$). Bottom panel: SR 141716A blocked the antinociceptive effects following inhalation exposure to the water soluble cannabinoid O-1057. Subjects were given an i.v. injection of vehicle (□) or SR141716A (▨) and 10 min later given a 10-min exposure to either aerosolized vehicle or aerosolized O-1057 (1 mg/ml). All results are presented as means ± S.E.M. of %MPE, $n = 6$ mice per group. * Significantly different from the respective placebo control group (Bonferonni t test, $p < 0.05$).

O-1057 was antagonized by SR 141716A (3 mg/kg), $F(1,44) = 20.4$, $p < 0.05$ (Fig. 6, bottom panel).

4. Discussion

The work described in this report indicates that the SPAG nebulizer can effectively generate aerosolized Δ^9-THC with a mass median aerodynamic diameter sufficient to be absorbed into the blood stream in mice upon inhalation. Inhalation exposure to aerosolized Δ^9-THC produced significant antinociceptive effects that depended on both the concentration of drug and the duration of exposure. SR 141716A blocked these antinociceptive effects indicating a CB_1 receptor mechanism of action. The water-soluble cannabinoid O-1057 produced an antinociceptive effect that was also blocked by SR 141716A. The AD_{50} value of SR 141716A found here in antagonizing Δ^9-THC-induced antinociception, 0.09 (0.02–0.26) mg/kg, is very similar to the 0.12 and 0.6 mg/kg AD_{50} values in antagonizing the antinociceptive effects following either i.v. administered Δ^9-THC (Compton et al., 1996) or inhalation of marijuana smoke (Lichtman et al., in press), respectively. Conversely, the opioid antagonist naloxone failed to attenuate the antinociception following inhalation exposure to aerosolized Δ^9-THC. Similarly, naloxone does not block the antinociceptive effects of cannabinoids administered intrathecally (Yaksh, 1981), intracerebroventricularly (Welch et al., 1995), or systemically (Ferri et al., 1986). These findings are consistent with the hypothesis cannabinoid-induced antinociception is not mediated by endogenous opioids acting at the μ opioid receptor.

An unexpected finding was that inhalation exposure to Δ^9-THC failed to produce any significant degree of hypothermia or locomotor activity suppression. Although separating the antinociceptive properties of cannabinoids from other cannabinoid effects is a desirable goal in developing cannabinoids for therapeutic use, this observation merits some discussion as all three indices are highly predictive of binding affinity to the CB_1 receptor (Martin et al., 1991). The potency of Δ^9-THC following i.v. injection in affecting each of these measures appears to vary as indicated in the literature. While in some experiments, Δ^9-THC was equipotent in producing antinociception and locomotor depression (Compton et al., 1992a,b), other studies reported that the ED_{50} value for antinociception was two or three fold lower than the ED_{50} for locomotor depression (Fan et al., 1994; Little et al., 1988; Martin, 1985). Similarly, Δ^9-THC's potency has been reported to be equivalent in producing antinociception and hypothermia (Compton et al., 1992b), but has also been found to be three to eight fold greater in producing antinociception than hypothermia (Compton et al., 1992a; Fan et al., 1994; Little et al., 1988). In addition, the antinociceptive effects following inhalation exposure to Δ^9-THC in the present study were not maximal. The estimated Δ^9-THC dose of 1.8 mg/kg is somewhat lower than either the 2.6 mg/kg ED_{50} dose we previously reported for Δ^9-THC administered intravenously (Lichtman et al., 1993) or the 2.4 mg/kg ED_{50} dose of Δ^9-THC that we calculated for marijuana inhalation (Lichtman et al., in press). Consequently, the relatively low dose of Δ^9-THC achieved and the tendency for Δ^9-THC to be more potent in producing antinociception than the other two indices may account for the apparent dissociation. Nonetheless, it would be advantageous to have a cannabinoid inhalation delivery system that is amenable for titrating the dose such that the therapeutic effects could be achieved while limiting the side-effects.

Unlike oral or i.v. routes of administration, the total absorbed dose of an inhaled drug is difficult to determine. Since mice are obligate nasal breathers and a nose-only exposure system was used, it is unlikely that any relevant amount of drug was swallowed. Upon exposure, the drug

can be deposited throughout the entire respiratory tract, including the upper respiratory tract (i.e., nose, nasopharynx, oropharynx, and larynx), tracheobronchial region, and the alveoli. Approximately 60% of the inhaled particles with a 2-μm mass median aerodynamic diameter, as found here, have been demonstrated to deposit across the entire respiratory tract of mice (Schlesinger, 1985). Factors influencing the systemic dose of Δ^9-THC upon inhalation would include deposition and absorbance into the bloodstream from each region of the respiratory tract. However, the body of knowledge of this information in mice is scant. Therefore, we opted to measure the concentration of Δ^9-THC in blood and infer the dose based on the drug blood levels following i.v. injection. Using this approach, we calculated a dose of 1.8 mg/kg Δ^9-THC. This estimated value should be interpreted with caution, however, because the degree to which it reflects the actual dose is dependent on the similarity of the biodisposition between the two routes of administration, information that is currently unknown. Nonetheless, strikingly similar time courses of both the subjective effects and blood levels of Δ^9-THC after smoking marijuana and i.v. injection of Δ^9-THC have been reported in human subjects (Hollister et al., 1981; Ohlsson et al., 1980).

Particle size is the most important parameter influencing the differential regional deposition of aerosols in the respiratory tract (Hinds, 1982). The small size of particles from marijuana smoke (Anderson et al., 1989; Hiller et al., 1984) and rapid absorption (Hollister et al., 1981; Ohlsson et al., 1980) indicates that smoking is an excellent mode of drug delivery. However, marijuana smoke also exposes users to polycyclic aromatic hydrocarbons, large amounts of carbon monoxide, and insoluble particulates (Lee et al., 1976; Wu et al., 1988). The ultrafine particles present in marijuana also increase the delivery of potentially toxic gases to the lung. Conversely, nebulizers or metered-dose inhalers deliver aerosolized Δ^9-THC rapidly, and in a safe and controlled manner, without the harmful effects of smoke constituents. The mass median aerodynamic diameter found here to be 2 μm with no particles larger than 5 μm are small enough to penetrate deeply into the small conducting airways in humans (Byron, 1987), and account for the drug's relatively good absorption in mice. Previous work using propylene glycol and water in a ratio of 9:1 as the vehicle and a nebulizer to generate aerosolized Δ^9-THC also reported a mean diameter of 2 μm and with no particles larger than 5 μm (Vachon et al., 1976).

The goal in the previous studies investigating aerosolized Δ^9-THC was to investigate its bronchodilator effects in human asthmatic patients (Hartley et al., 1978; Tashkin et al., 1977; Williams et al., 1976). Delivering the drug primarily to the lungs would limit the array of pharmacological effects. In contrast, the interest in aerosolized cannabinoids in the present study was to give a systemically active dose of Δ^9-THC via the respiratory tract. Two issues in developing a Δ^9-THC aerosol delivery device for medical use are that the drug must be in either a solution or suspension and the formulation must be acceptable for human inhalation. The insolubility of Δ^9-THC in aqueous solution (Garrett and Hunt, 1974) possesses a challenge in developing an aerosolized form of this drug. A vehicle consisting of ethanol/emulphor/saline in a concentration of 1:1:18 (Olsen et al., 1973) was successful in generating a Δ^9-THC aerosol through nebulization. While this formulation was sufficient to produce pharmacological effects in rodents upon inhalation, emulphor is not approved for human inhalation. Similarly, the propylene glycol vehicle employed in the nebulizer used by Hartley et al. (1978) is not suitable for human inhalation. The fact that O-1057 produced antinociception upon inhalation is encouraging and suggests that it or other water-soluble cannabinoids may have potential therapeutic benefits.

The results from the present study demonstrate that an aerosolized formulation of Δ^9-THC or the water-soluble analog O-1057 is pharmacologically active in mice. The observation that SR 141716A blocked the antinociceptive effects of both compounds indicates a CB_1 receptor mechanism of action. The lack of blockade by naloxone indicates that endogenous opioids are not involved. Further development of an aerosolized cannabinoid that is acceptable for human inhalation would circumvent the use of smoked marijuana as a Δ^9-THC delivery system.

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