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In vivo effects of synthetic cannabinoids JWH-018 and JWH-073 and phytocannabinoid Δ^9-THC in mice: Inhalation versus intraperitoneal injection

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ABSTRACT

Human users of synthetic cannabinoids (SCBs) JWH-018 and JWH-073 typically smoke these drugs, but preclinical studies usually rely on injection for drug delivery. We used the cannabinoid tetrad and drug discrimination to compare in vivo effects of inhaled drugs with injected doses of these two SCBs, as well as with the phytocannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC). Mice inhaled various doses of Δ^9-THC, JWH-018 or JWH-073, or were injected intraperitoneally (IP) with these same compounds. Rectal temperature, tail flick latency in response to radiant heat, horizontal bar catalepsy, and suppression of locomotor activity were assessed in each animal. In separate studies, mice were trained to discriminate Δ^9-THC (IP) from saline, and tests were performed with inhaled or injected doses of the SCBs. Both SCBs elicited Δ^9-THC-like effects across both routes of administration, and effects following inhalation were attenuated by pretreatment with the CB1 antagonist/inverse agonist rimonabant. No cataleptic effects were observed following inhalation, but all compounds induced catalepsy following injection. Injected JWH-018 and JWH-073 fully substituted for Δ^9-THC, but substitution was partial (JWH-073) or required relatively higher doses (JWH-018) when drugs were inhaled. These studies demonstrate that the SCBs JWH-018 and JWH-073 elicit dose-dependent, CB1 receptor-mediated Δ^9-THC-like effects in mice when delivered via inhalation or via injection. Across these routes of administration, differences in cataleptic effects and, perhaps, discriminative stimulus effects, may implicate the involvement of active metabolites of these compounds.

1. Introduction

Over the past 5 years, synthetic cannabinoids (SCBs) rapidly emerged as popular drugs of abuse in Europe and the US. Commercial preparations (typically branded as "K2" in the US or as "Spice" in Europe) are readily available online and in business establishments such as convenience stores and truck stops (Vardakou et al., 2010). Most of these preparations consist of inert plant materials laced with SCBs, typically from the aminoalkylindole (AAI) family (Fattore and Fratta, 2011), and are presumed to possess pharmacological properties similar to Δ^9-tetrahydrocannabinol (Δ^9-THC), the primary psychoactive constituent of marijuana (Gaoni and Mechoulam, 1964). The widespread over-the-counter availability of these products has led to the perception that they are safe to use, and this, combined with the fact that their active constituents are not detected in standard drug screens, has spurred use of SCBs to epidemic levels on many college campuses (Vandrey et al., 2012). Similarly, one in nine high school seniors admitted using SCBs over the past year, making these compounds the 2nd most frequently used recreational drug, after marijuana, in this population (Johnston et al., 2011). State and federal scheduling of some of the more common SCBs under the Controlled Substances Act has largely failed to curtail drug availability, and commercial preparations containing these drugs remain quasi-legal and easily obtainable (Seely et al., 2012).

Although structurally distinct from Δ^9-THC, the synthetic AAI cannabinoid compounds also bind and activate cannabinoid CB1 receptors (CB1Rs) (Estep et al., 1990; Eissenstat et al., 1990). The abuse liability of AAI SCBs therefore most likely results from their capability to potently and efficaciously activate these CB1Rs. While a plethora of different SCBs are reported to be present in various commercial preparations, two of the most commonly observed are JWH-018 [1-pentyl-3-(1-naphthoyl)indole] and JWH-073 [1-butyl-3-(1-naphthoyl)indole] (Logan et al., 2012; Seely et al., 2013). Previous studies revealed that these SCBs have high affinity for CB1Rs, and possess much higher efficacy at these receptors than Δ^9-THC (Lindigkeit et al., 2009; Atwood et al., 2010).

In this regard, although humans typically smoke commercial preparations of SCBs (Vandrey et al., 2012), almost all preclinical studies with

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these compounds have involved systemic injection. Drugs administered via inhalation largely bypass first-pass metabolism, whereas systemic injection allows for significant first-pass effects (Pond and Tozer, 1984). Importantly, we have recently reported that several phase I hydroxylated metabolites of JWH-018 and JWH-073 retain biological activity (Brents et al., 2011, 2012), which could have implications for human use. As such, it may be the case that laboratory animal models employing systemic injection of SCBs maximize formation of active phase I metabolites, whereas the human condition, i.e. smoking, would be expected to minimize metabolite formation. At the time of this writing, only a single study has evaluated the effects of a single inhaled SCB, JWH-018, in mice (Wiebelhaus et al., 2012), demonstrating dose-dependent effects on all measures of the cannabinoid tetrad, and reversal of these drug effects by prior administration of the CB1R antagonist/inverse agonist rimonabant. In the present studies, utilizing a whole-body exposure system, we extend these previous observations by directly comparing the effects of multiple doses of JWH-018 to its structural analogue JWH-073 and to Δ^9-THC using the cannabinoid tetrad (Martin et al., 1991) following inhaled exposure or intraperitoneal injection. The CB1R receptor antagonist/inverse agonist rimonabant was used to determine whether any observed drug effects in the tetrad assay following inhalation of cannabinoids were mediated via CB1R actions. Additional studies compared the interoceptive effects of inhaled or injected SCBs to those of intraperitoneal Δ^9-THC using drug discrimination. This further evaluation of the effects of SCBs via inhalation in mice may increase our understanding of their biological effects and may provide a more translational approach to the study of these compounds, as compared to systemic injection.

2. Materials and methods

2.1. Animals

All experiments were conducted in adult male Swiss Webster mice housed in the University of Arkansas for Medical Sciences (UAMS). Mice were maintained on a 12 h light:12 h dark cycle (lights on at 0700 h, off at 1900 h) in a temperature- and humidity-controlled room within the UAMS vivarium. Food and water were available ad libitum throughout the duration of all studies. All animals in the present studies were drug-naïve prior to initiation of experimental protocols. Mice in the tetrad studies were used only once, and were sacrificed immediately after testing, but mice in drug discrimination studies were repeatedly tested, with experimental observations taking place no more frequently than once per week. Experimental protocols were approved by the UAMS Institutional Animal Care and Use Committee, and complied with principles outlined in the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Apparatus

Inhaled cannabinoids were administered within a cylindrical glass chamber housed within a fume hood. Three mice (which were cagemates in the animal housing room) were placed in the chamber at the same time. The glass cylinder measured 30.48 cm × 45.72 cm (volume 30 L), and closure of the cylinder was ensured by a 1/2″ edge EPDM compression seal (product number 1120A45, McMaster-Carr, Aurora, OH) and a Plexiglas lid which was tightly screwed into three aluminum supports. A four blade fan attached to an electric motor (model OM87, Dayton Electric Manufacturing Co., Chicago, IL) was fixed to the inner surface of the Plexiglas lid to distribute the volatilized drug throughout the chamber, and a metal cage was fitted beneath the fan to contain the drug-impregnated nitrocellulose paper (see Drugs section, below) prior to ignition. An igniter was inserted through a hole in the Plexiglas lid to ignite the medium; once the medium was lit, the igniter was rapidly removed and a rubber stopper was used to plug the hole to prevent any loss of vaporized drug. A rubber tube connected to the chamber from an air source in the fume hood allowed air flow (applied at approximately 5 min into the experiment) to prevent hypoxia as a result of oxygen consumption in the chamber. For studies involving systemic administration of cannabinoids, mice were injected IP and then placed in the chamber 5 min later in order to rule out any non-specific effects of the inhalation procedure on subsequent measures in the tetrad test. The chamber was tested for air leaks periodically during the course of these studies by filling the chamber with colored smoke and sealing it tight. Colored smoke rapidly filled the chamber, and visual checks did not reveal any areas where smoke could escape. After these tests, the smoke was evacuated through the fume hood, and the chamber was disassembled for cleaning and sanitizing.

For drug discrimination experiments, mice were injected IP with cannabinoids, or exposed to vaporized drugs in the chamber as described above, then rapidly transported to an adjacent laboratory for behavioral testing in operant-conditioning chambers (model ENV-307A; MED Associates, St. Albans, VT) that were individually enclosed in larger lightproof Malaguard sound-attenuating cubicles (model ENV-022MD; MED Associates) modified to include retractable response levers (model ENV-312 M) for murine subjects. The right side wall of each chamber used in these studies was equipped with a dipper, centered between the two retractable levers, through which liquid reinforcement was delivered, and stimulus lights were present above each response lever. The left wall of each chamber contained a nose-poke aperture, which was not used in these studies.

2.3. Drugs

Trans-Blot Transfer Medium Pure Nitrocellulose Membrane (0.45 μm thick, BioRad Labs, Hercules, CA) was used as a matrix to volatilize cannabinoids in the chamber. Δ^9-THC, JWH-018 and JWH-073 were dissolved in 100% ethanol at a concentration of 100 mg/mL. Cannabinoid solutions were applied to nitrocellulose paper (8.0 × 8.0 cm squares), then left overnight in labeled beakers under the fume hood. During this time, ethanol evaporated off, leaving cannabinoids impregnated in the nitrocellulose paper, which was dry and ready for combustion the following morning. For all experiments with inhaled cannabinoids, doses are expressed as total mg of drug per 30 L of air in the chamber.

JWH-018, JWH-073, Δ^9-THC were also prepared for intraperitoneal injection, as was the CB1 antagonist/inverse agonist rimonabant. All compounds were dissolved in a solution of Tween 80 (8% of final volume) and 0.9% saline (92% of final volume). Injections were administered in a volume of 0.01 mL/g via 28 gauge needles. Rimonabant was administered via IP injection 15 min prior to cannabinoid exposure. After rimonabant injection, mice were placed back into their home cages during this 15-min period.

2.4. Cannabinoid tetrad

Immediately upon removal from the inhalation chamber, mice were sequentially tested for (1) hypothermia, (2) analgesia, (3) catalepsy and (4) suppression of locomotor activity, in that order. Hypothermia was measured using a digital thermometer (model BAT-12, PhysiTemp, Clifton, NJ) equipped with a Ret-3 mouse probe (model 50314, Stoelting Co., Dale, IL) inserted rectally approximately 2 cm; stable temperatures were obtained within ~6 s. Analgesia was measured as tail-flick latency using the EMDIE-TF6 radiant heat apparatus (Emdie Instrument Co., Montpelier, VA). For analgesia trials, mice were positioned on the stage of apparatus, while the tail was extended into a groove to break a photobeam. Beginning at t = 0, a button was depressed to begin a timer and illuminate a radiant heat source directed onto the dorsal surface of the tail, approximately 2 cm from its origin from the body. Movement of the tail at any point after the beginning of the trial broke the photobeam, stopped both the heat source and the timer, and ended the trial. The sensitivity of the photobeam detector was set at 150, and the light intensity was set to 369 for all trials in order to produce a tail