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The cannabinoid agonist HU-210: Pseudo-irreversible discriminative stimulus effects in rhesus monkeys

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ABSTRACT

Synthetic cannabinoid abuse and case reports of adverse effects have raised concerns about the pharmacologic mechanisms underlying in vivo effects. Here, a synthetic cannabinoid identified in abused products (HU-210) was compared to the effects of Δ^9-THC and two other synthetic cannabinoid agonists used extensively in pre-clinical studies (CP 55,940 and WIN 55,212-2). One group of monkeys discriminated Δ^9-THC (0.1 mg/kg i.v.); a separate group received chronic Δ^9-THC (1 mg/kg/12 h s.c.) and discriminated rimonabant (1 mg/kg i.v.). CP 55,940, HU-210, Δ^9-THC, and WIN 55,212-2 produced Δ^9-THC lever responding. HU-210 had a long duration (i.e., 1–2 days), whereas that of the other cannabinoids was 5 h or less. Rimonabant (1 mg/kg) produced surmountable antagonism; single dose-apparent affinity estimates determined in the presence of Δ^9-THC, CP 55,940, and WIN 55,212-2 did not differ from each other. In contrast, rimonabant (1 mg/kg) produced a smaller rightward shift in the HU-210 dose–effect function. In Δ^9-THC treated monkeys, the relative potency of CP 55,940, Δ^9-THC, and WIN 55,212-2 to attenuate the discriminative stimulus effects of rimonabant was the same as that evidenced in the Δ^9-THC discrimination, whereas HU-210 was unexpectedly more potent in attenuating the effects of rimonabant. In conclusion, the same receptor subtype mediates the discriminative stimulus effects of Δ^9-THC, CP 55,940 and WIN 55,212-2. The limited effectiveness of rimonabant to either prevent or reverse the effects of HU-210 appears to be due to very slow dissociation or pseudo-irreversible binding of HU-210 at cannabinoid receptors.

1. Introduction

The goal of this study was to compare the in vivo pharmacology of the synthetic cannabinoid HU-210, recently detected in Spice herbal blends, to Δ^9-tetrahydrocannabinol (Δ^9-THC) and other synthetic cannabinoids including CP 55,940 and WIN 55,212-2. Δ^9-THC is the natural product in Cannabis that exerts psychopharmacological effects that are primarily responsible for the widespread use of Cannabis. Δ^9-THC is an agonist at two G-protein coupled receptor subtypes designated CB_1 and CB_2. CB_1 receptors are abundant in the hippocampus, basal ganglia, cortex, amygdala and cerebellum (Herkenham et al., 1991; Gifford et al., 1999), and are also present in adipose tissue, skeletal muscle and liver (Lindborg et al., 2010; Wu et al., 2011). CB_2 receptors are expressed mainly in the immune system. Δ^9-THC is a tricyclic terpenoid derivative bearing a benzopyran moiety. Two other CB_1 and CB_2 receptor agonists of synthetic origin include CP 55,940, a bicyclic analog of Δ^9-THC lacking the pyran ring, and WIN 55,212-2, an aminoalkylindole (Palmer et al., 2002 for review). CP 55,940 and WIN 55,212-2 have been used extensively as reference compounds in pre-clinical studies involving relatively novel cannabinoids.

HU-210 is one synthetic cannabinoid that has been added to non-Cannabis plant material and marketed under a variety of trade names such as Spice or K2 in the United Kingdom, apparently in an attempt to circumvent laws banning Cannabis (Fattore and Fratta, 2011). HU-210 belongs to the same chemical class as Δ^9-THC and was initially synthesized in the laboratory of Raphael Mechoulam at the Hebrew University. HU-210 is a high-affinity CB_1 and CB_2 receptor agonist (Burkey et al., 1997; Howlett et al., 2002) and was reported to be highly potent in producing effects associated with cannabinoid agonism in rats (12.5–100 μg/kg i.p.) and pigeons (12.5–50 μg/kg, s.c.), including discriminative stimulus effects, decreased locomotor activity, rearing, and grooming, long-lasting hypothermia of a greater magnitude than that produced by Δ^9-THC, increased vocalization and circling, and sedative effects (Järbe et al., 1989; Ovadia et al., 1995; Ferrari et al., 1999). CB_1 receptors appear to mediate the in vivo effects of HU-210 as evidenced by attenuation of those effects by CB_1 receptor antagonists (e.g., rimonabant; Bosier et al., 2010; Janoyan et al., 2002) as well as in CB_1 receptor knockout mice (Zimmer et al., 1999).

Cannabinoid discrimination procedures in rhesus monkeys have been used previously to demonstrate that synthetic cannabinoids

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detected in Spice products, including JWH-018 and JWH-073, exert discriminative stimulus effects by acting at the same receptors as those mediating the effects of Δ^9-THC. In rhesus monkeys discriminating Δ^9-THC, both JWH-018 and JWH-073 substituted for Δ^9-THC (Ginsberg et al., 2012). Schild analysis with rimonabant was used to compare the receptor site(s) of action of Δ^9-THC, JWH-018, and JWH-073. Rimonabant appeared to be a simple, competitive, and reversible antagonist of each agonist, as evidenced by slopes of Schild plots that were not significantly different from unity. The apparent affinity (pA_2) or potency of rimonabant did not differ among agonists, suggesting that the same receptors mediated the discriminative stimulus effects of Δ^9-THC, JWH-018, and JWH-073. Moreover, each of these agonists attenuated rimonabant-induced Δ^9-THC withdrawal in a second drug discrimination assay with a relative potency similar to that for producing discriminative stimulus effects in the Δ^9-THC discrimination assay. Collectively, these drug discrimination data strongly suggest that Δ^9-THC, JWH-018, and JWH-073 act at the same receptor to produce subjective effects.

Here, the effects of HU-210, Δ^9-THC, CP 55,940, and WIN 55212-2 were compared in drug discrimination assays in rhesus monkeys that have documented utility for conducting quantitative analysis of drug interactions. One group of monkeys discriminated Δ^9-THC (0.1 mg/kg i.v.) from vehicle. A second group of monkeys discriminated rimonabant (1 mg/kg i.v.) while receiving 1 mg/kg of Δ^9-THC s.c. every 12 h. The Δ^9-THC discrimination assay was used to examine the potency and time course of HU-210, CP 55,940 and WIN 55,212-2, as well as antagonism of their effects by rimonabant. Δ^9-THC treated monkeys discriminating rimonabant were used to examine the capacity of rimonabant to reverse the effects of Δ^9-THC, CP 55,940, WIN 55,212-2, and HU-210. CP 55,940 and WIN 55,212-2 were shown to attenuate the rimonabant discriminative stimulus previously (Stewart and McMahon, 2010), although potency was only determined in the presence of a single dose of rimonabant. Here, the potency of agonists was determined from the relationship between agonist dose and magnitude of rightward shift in the rimonabant dose–effect function as described (Ginsburg et al., 2012). The results strongly suggest that HU-210 binds pseudo-irreversibly to CB_1 receptors, defined as a marked decrease in the rate of offset of receptor binding (Kenakin, 2009), which in turn interferes with the binding of rimonabant.

2. Material and methods

2.1. Subjects

Two female and two male adult rhesus monkeys (*Macaca mulatta*) discriminated Δ^9-THC from vehicle and two female and two male adult rhesus monkeys discriminated rimonabant during chronic Δ^9-THC (1 mg/kg s.c. every 12 h) treatment. Monkeys were housed individually on a 14-h light/10-h dark schedule. They were maintained at 95% free-feeding weight (range 6.0–11.3 kg) with a diet consisting of primate chow (High Protein Monkey Diet; Harlan Teklad, Madison, WI), fresh fruit, and peanuts; water was provided in the home cage. The monkeys had received cannabinoids and noncannabinoids in previous studies (Hruba et al., 2012; Ginsburg et al., 2012). Monkeys were maintained in accordance with the Institutional Animal Care and Use Committee, The University of Texas Health Science Center at San Antonio and the Guide for the Care and Use of Laboratory Animals (2011).

2.2. Surgery

Monkeys were anesthetized with ketamine (10 mg/kg i.m.) followed by isoflurane (1.5–3.0% inhaled via facemask). A catheter (heparin-coated polyurethane; o.d. = 1.68 mm; i.d. = 1.02 mm; Instech Laboratories, Plymouth Meeting, PA) was inserted into a subclavian or femoral vein and secured to the vessel with suture silk (coated vicryl; Ethicon Inc., Somerville, NJ). The catheter extended from the vessel to the midscapular region of the back and was attached to a vascular access port located s.c. (Mida-cbas-c50; Instech Laboratories).

2.3. Apparatus

Monkeys were seated in chairs (Model R001; Primate Products, Miami, FL) and were placed in ventilated, sound-attenuating chambers equipped with two levers; a light was positioned above each lever. Feet were placed in shoes containing brass electrodes to which a brief electric stimulus (3 mA, 250 ms) could be delivered from an A/C generator. The chambers were connected to a computer with an interface (MED Associates, St. Albans, VT); experimental events were controlled and recorded with Med-PC software (MED Associates).

2.4. Drug discrimination training

Four monkeys discriminated Δ^9-THC (0.1 mg/kg i.v.) from vehicle (1 part absolute ethanol, 1 part Emulphor-620, and 18 parts saline) while responding under a fixed ratio 5 (FR5) schedule of stimulus-shock termination. Four other monkeys received 1 mg/kg Δ^9-THC administered s.c. twice daily (at 6:15 AM and 6:15 PM) and discriminated rimonabant (1 mg/kg i.v.) from vehicle starting at 12:15 PM under an FR5 schedule of stimulus-shock termination.

Experimental sessions were divided into multiple, consecutive 10-min cycles. Each cycle began with a 5-min timeout and responding on a lever during the timeout had no programmed consequence. The timeout was followed by a 5-min schedule of stimulus-shock termination, the beginning of which was signaled by illumination of red lights. Five consecutive responses on the correct lever extinguished the red lights, prevented delivery of an electric stimulus, and initiated a 30-s timeout. Otherwise, an electric stimulus was delivered every 40 s in monkeys discriminating Δ^9-THC and 10 s in monkeys discriminating rimonabant. Responding on the incorrect lever reset the response requirement on the correct lever. Determination of correct levers varied among monkeys (e.g., left lever associated with the training dose; right lever associated with vehicle) and remained the same for that monkey for the duration of the study.

Training sessions consisted of a minimum of three and a maximum of six cycles. Drug training consisted of administration of Δ^9-THC (0.1 mg/kg i.v.) or rimonabant (1 mg/kg i.v) in the respective discrimination assays within the first min of three cycles; sham (dull pressure applied to the skin overlying the vascular access port) was administered within the first min of the second and third cycle. Vehicle training involved administration of vehicle within the first min of a cycle followed by vehicle or sham in subsequent cycles for a maximum of six cycles. Zero to three vehicle-training cycles immediately preceded three Δ^9-THC or rimonabant training cycles. Completion of the FR on the correct lever was required for reinforcement during each training cycle. Monkeys had previously satisfied the criteria for testing, i.e., at least 80% of the total responses occurred on the correct lever and fewer than five responses occurred on the incorrect lever before completion of the first FR on the correct lever within a cycle for all cycles during five consecutive or six of seven training sessions. Tests were conducted after performance satisfied the test criteria for consecutive training sessions including both vehicle and drug training sessions. The order of training with drug or vehicle was non-systematic.