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Effects of acute and repeated dosing of the synthetic cannabinoid CP55,940 on intracranial self-stimulation in mice☆

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ABSTRACT

Background: Synthetic cannabinoids have emerged as a significant public health concern. To increase the knowledge of how these molecules interact on brain reward processes, we investigated the effects of CP55,940, a high efficacy synthetic CB_1 receptor agonist, in a frequency-rate intracranial self-stimulation (ICSS) procedure.

Methods: The impact of acute and repeated administration (seven days) of CP55,940 on operant responding for electrical brain stimulation of the medial forebrain bundle was investigated in C57BL/6J mice.

Results: CP55,940 attenuated ICSS in a dose-related fashion (ED50 (95% C.L.) = 0.15 (0.12–0.18) mg/kg). This effect was blocked by the CB_1 receptor antagonist rimonabant. Tolerance developed quickly, though not completely, to the rate-decreasing effects of CP55,940 (0.3 mg/kg). Abrupt discontinuation of drug did not alter baseline responding for up to seven days. Moreover, rimonabant (10 mg/kg) challenge did not alter ICSS responding in mice treated repeatedly with CP55,940.

Conclusions: The finding that CP55,940 reduced ICSS in mice with no evidence of facilitation at any dose is consistent with synthetic cannabinoid effects on ICSS in rats. CP55,940-induced ICSS depression was mediated through a CB_1 receptor mechanism. Additionally, tolerance and dependence following repeated CP55,940 administration were dissociable. Thus, CP55,940 does not produce reward-like effects in ICSS under these conditions.

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1. Introduction

Cannabis sativa has been used both medicinally and recreationally for thousands of years (Mechoulam et al., 1991). The psychotropic effects of this plant are mainly due to its primary psychoactive constituent Δ^9-tetrahydrocannabinol (THC) (Mechoulam and Gaoni, 1965; Martin-Santos et al., 2012). THC falls within the class of drugs known as cannabinoids, which draw their moniker from the cannabis plant. Cannabinoids are primarily defined by their ability to bind and activate cannabinoid receptor 1 (CB_1) (Herkenham et al., 1990; Matsuda et al., 1990) and cannabinoid receptor 2 (CB_2) (Munro et al., 1993). Although CB_1 is well known to play a predominant role in mediating the behavioral effects of THC and other cannabinoids and to modulate the rewarding effects of other classes of drugs (Rinaldi-Carmona et al., 1994; Ledent et al., 1999; Zimmer et al., 1999; Forget et al., 2005), emerging evidence indicates that CB_2 plays opposing roles in the reinforcing effects of cocaine and nicotine (Xi et al., 2011; Ignatowska-Jankowska et al., 2013; Navarrete et al., 2013).

In addition to THC, hundreds of synthetic cannabinoids vary in structure and bind and activate cannabinoid receptors (for review, see Pertwee, 2006). These synthetic compounds were crucial for establishing the binding and distribution of cannabinoid receptors in brain (Devane et al., 1988; Herkenham et al., 1990). However, in recent years, synthetic cannabinoids such as CP-47,497 (Hudson et al., 2010), AM-2201 (Denooz et al., 2013), JWH-018, and JWH-073 (Brents and Prather, 2014), emerged as new drugs of abuse. Synthetic cannabinoids are generally abused by smoking plant material imbued with these compounds in much the same manner as marijuana, and are readily available as preparations commonly referred to as "Spice" or "K2" among other brand names (Fantegrossi et al., 2014). Synthetic cannabinoids are often markedly more potent and/or efficacious than THC (Griffin et al., 1998). Moreover, toxicological information is limited, and little is known about how these compounds affect brain reward circuitry *in vivo*. As synthetic cannabinoids have emerged as drugs of abuse (Maxwell, 2014),

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further research is needed to characterize their pharmacology and toxicology. The impact of chronic exposure to nonclassical cannabinoids also remains to be determined.

Similar to other drugs of abuse, cannabinoids can evoke dopamine release in the nucleus accumbens (NAc), a characteristic often indicative of drugs of abuse (Chen et al., 1993; Cheer et al., 2004). The NAc is one node in a neural circuit known as the mesolimbic dopamine pathway, which consists of dopaminergic neurons that originate in the ventral tegmental area (VTA) and project to NAc and more rostral targets such as prefrontal cortex (PFC). Intracranial self-stimulation (ICSS) of the medial forebrain bundle is one procedure that has been used to measure reinforced behavior mediated by the mesolimbic dopamine pathway (Carlezon and Chartoff, 2007) and to assess abuse potential of drugs (Negus and Miller, 2014). Although acute administration of synthetic cannabinoids generally suppresses ICSS (Arnold et al., 2001; Vlachou et al., 2003, 2005), the impact of repeated cannabinoid administration on ICSS has not been extensively studied but may be important. For example, repeated administration of mu opioid agonists evokes tolerance to their rate-decreasing effects and unmasks abuse-related ICSS facilitation (Altarifi and Negus, 2011; Altarifi et al., 2012). Additionally, ICSS has been used to detect withdrawal-related anhedonia for some drugs of abuse such as cocaine, nicotine and morphine (Altarifi and Negus, 2011; Stoker et al., 2012, 2014).

In the present study we tested the hypotheses that (a) repeated administration of a synthetic cannabinoid will facilitate ICSS in a similar fashion as other abused drugs, and (b) spontaneous or antagonist-precipitated withdrawal in mice repeatedly administered cannabinoids will produce an anhedonia-like depression of ICSS similar to that produced by withdrawal from other abused drugs. Because of the wide variety of synthetic cannabinoids and the ever changing composition of abused preparations, we chose to use a single, representative compound, CP55,940, to model acute and repeated effects of synthetic cannabinoids. Although CP55,940 has not emerged as a drug of abuse and has not been scheduled by the Drug Enforcement Agency, it has been extensively characterized in preclinical studies, and it is structurally similar to the abused and scheduled nonclassical cannabinoids CP47,497 and cannabicyclohexanol (Logan et al., 2012). Moreover, these compounds bind with similar affinity to CB_1 and CB_2 (Huffman et al., 2010; Atwood et al., 2011). Acute administration of CP55,940 depressed ICSS in rats (Arnold et al., 2001; Kwilasz and Negus, 2012), but its consequences on ICSS following repeated administration are unknown.

In initial experiments, we examined the dose-response relationship and time course of the effects of acute CP55,940 administration on ICSS. Rimonabant was used to infer CB_1 involvement. We then tested whether the acute effects of CP55,940 on ICSS would undergo tolerance following repeated administration. Because cannabinoids are well established to alter motor function, such as catalepsy, we also assessed the relationship between catalepsy and ICSS measures during repeated administration of CP55,940 (Little et al., 1988). Catalepsy was selected as a concurrent endpoint because the behavior may confound the ability of the mice to engage in operant responding, and CB_1-mediated depression of ICSS may reflect non-specific disruption of behavior rather than an ICSS specific effect. Finally, we examined whether mice treated repeatedly with CP55,940 displayed signs of either spontaneous or precipitated withdrawal in the ICSS procedure.

2. Materials and methods

2.1. Subjects

A total of 43 male C57Bl/6J mice were used (Jackson Laboratories, Bar Harbor, Maine). Mice were between 10 and 14 weeks of age at the start of each experiment and were individually housed and maintained on a 12 h light cycle, with lights on from 0600 to 1800 h, with free access to food and water. All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee (IACUC).

2.2. Drugs

CP55,940, rimonabant, and cocaine HCl were obtained from the National Institute of Drug Abuse Drug Supply Program (Rockville, MD). CP55,940 and rimonabant were dissolved in a vehicle (VEH) consisting of 5% ethanol, 5% Emulphor-620 (Rhone-Poulenc, Princeton, NJ), and 90% 0.9% saline. Cocaine was dissolved in 0.9% saline.

2.3. Intracranial Self-Stimulation (ICSS)

Apparatus: ICSS testing was conducted in eight mouse operant conditioning chambers ($18 \times 18 \times 18\ cm^3$; Med Associates Inc., St. Albans, VT). Each chamber was equipped with a retractable lever located on one wall, LED stimulus lights over the lever, a chamber house-light, a tone-generator, and an ICSS stimulator. The stimulator was connected to the electrode via bipolar cables routed through a swivel commuator and into the experimental chamber. Chambers were enclosed within sound- and light-attenuating chambers equipped with exhaust fans. Custom software was used to control manipulations in the operant chambers and to record data during training and testing sessions.

Stereotaxic surgery: Surgical procedures for implanting electrodes in mice for ICSS studies were similar to those previously reported (Carlezon and Chartoff, 2007; Wiebelhaus et al., 2014). Mice were anesthetized with isoflurane for implantation of bipolar twisted stainless steel electrodes (0.280 mm diameter and insulated except at the flat tips; Plastics One, Roanoke, VA) into the right medial forebrain bundle (2.0 mm posterior to bregma, 0.8 mm lateral from midline, and 4.8 mm below dura). The electrode was fixed to the skull with anchoring screws and dental cement. Mice were given acetaminophen (1–2 mg/ml) in their drinking water for one day before and five days after surgery. Training began one week after surgery.

Training: During initial training, lever-press responding under a fixed-ratio 1 (FR1) schedule produced both (a) delivery of a 0.5 s train of square-wave cathodal pulses (0.1 ms pulse duration) at a frequency of 141 Hz and amplitude of 150 μA and (b) 0.5 s onset of stimulus lights, house light, and tone cues. Responding during stimulation had no scheduled consequences. Amplitudes of stimulation were individually adjusted for each mouse to maximize response rates, and final amplitudes ranged from 45–300 μA. Training continued during daily 30–120 min sessions until response rates exceeded 30 responses per min for at least three days.

Once operant responding was established, mice were promoted to frequency-rate training as previously reported for mice (Wiebelhaus et al., 2014) and rats (Negus et al., 2010). Frequency-rate sessions were divided into multiple components, and each component consisted of 10 sequential frequency trials for presentation of a descending series of 10 stimulation frequencies (2.2–1.75 logHz in 0.05 log increments). Each frequency trial began with a 10 s time out period, during which, behavior had no scheduled consequences. During the last 5 s of the time out period, the lever was extended, and non-contingent stimulations were delivered once per second at a given frequency together with associated cues. The time out period was followed by a 60 s response period when responding under the FR1 schedule produced brain stimulation at the specified frequency together with associated cues. After the 60 s response period, the lever was retracted, the stimulation frequency was decreased by 0.05 log units, and the next frequency