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Structural analogs of pyrazole and sulfonamide cannabinoids: Effects on acute food intake in mice

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ABSTRACT

Obesity contributes to a multitude of serious health problems. Given the demonstrated role of the endogenous cannabinoid system in appetite regulation, the purpose of the present study was to evaluate structural analogs of two cannabinoids, rimonabant (cannabinoid CB_1 receptor antagonist) and O-2050 (sulfonamide analog of Δ^8-tetrahydrocannabinol), that showed appetite suppressant effects in previous studies. Structure–activity relationships of these two lead compounds were examined in several assays, including cannabinoid CB_1 and CB_2 receptor binding, food intake, and an in vivo test battery (locomotor activity, antinociception, ring immobility, and body temperature) in mice. Rimonabant and O-2050 reliably decreased feeding in mice; however, their analogs decreased feeding only at higher doses, even though some compounds had quite good cannabinoid CB_1 binding affinity. Results of the in vivo test battery were inconsistent, with some of the compounds producing effects characteristic of cannabinoid agonists while other compounds were inactive or were antagonists against an active dose of Δ^9-tetrahydrocannabinol. These results demonstrate that reduction of food intake is not a characteristic effect of pyrazole and sulfonamide cannabinoid analogs with favorable cannabinoid CB_1 binding affinity, suggesting that development of these classes of cannabinoids for the treatment of obesity will require evaluation of their effects in a broad spectrum of pharmacological assays.

1. Introduction

Obesity contributes to a multitude of serious health problems, including diabetes, stroke, and cardiovascular disease (Brown et al., 2009). Despite this substantial health impact, pharmacotherapy for treatment of obesity is largely limited to two drugs, phentermine (a sympathomimetic) and orlistat (oral lipase inhibitor) (for a review, see Powell et al., 2011). Current medication development efforts are focused on several avenues through which appetite may be modulated, including appetite-related peptides such as neuropeptide Y and AgRP (agouti-related protein), as well as combination drugs that modulate more than one system (Powell et al., 2011). The results of past studies strongly suggest that the endogenous cannabinoid system plays a role in appetite regulation (for a review, see Cota et al., 2003), and represents an additional target for medication development. Exogenous administration of cannabinoids also has been shown to affect feeding behavior. Agonists of cannabinoid CB_1 receptors, such as Δ^9-tetrahydrocannabinol, the primary psychoactive substituent of the marijuana plant, increase food intake in humans and rodents (Hart et al., 2002; Koch, 2001; Williams et al., 1998), whereas antagonists, such as the prototypic cannabinoid CB_1 receptor antagonist rimonbant, decrease food intake (McLaughlin et al., 2003; Rowland et al., 2001; Wiley et al., 2005). Interestingly, O-2050, a side chain analog of Δ^8-tetrahydrocannabinol, which has been described as a neutral cannabinoid CB_1 receptor antagonist (Gardner and Mallet, 2006, but see Wiley et al., 2011), also has been reported to decrease feeding behavior (Gardner and Mallet, 2006; Wiley et al., 2011).

In humans, rimonabant was originally marketed as an anti-obesity agent and as an aid to smoking cessation until its adverse psychiatric effects were revealed during advanced clinical trials after its release in Europe (Christensen et al., 2007); however, the extent to which the latter effects are related to rimonabant interaction with the cannabinoid CB_1 receptor is unknown. Although several of rimonabant's in vivo effects in rodents have been shown to be cannabinoid CB_1 receptor-mediated, including

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decreased feeding behavior (Wiley et al., 2005), not all of its effects are mediated by this mechanism, e.g., stimulation of locomotor activity (Bass et al., 2002). Similarly, the results of one study have suggested that rimonabant's effects on mood may be attenuated through blockade of kappa opioid receptors (Lockie et al., 2011). Given that conclusive evidence is lacking with regard to whether rimonabant's negative affective effects are mediated via its actions at central cannabinoid CB_1 receptors, investigation of cannabinoid-based medications for appetite regulation is still considered a viable alternative, particularly for peripherally restricted compounds (Alonso et al., 2012; Christopoulou and Kiortsis, 2011; Sasmal et al., 2011). One of the first steps in this type of investigation is evaluation of the activity and efficacy of promising compounds. To this end, the present study examined the effects of structural analogs of rimonabant and of the sulfonamide cannabinoid, O-2050, on food intake using a model previously used to show the appetite suppressant effects of these two lead compounds (Wiley et al., 2011; Wiley et al., 2005).

2. Materials and methods

2.1. Subjects

Some tests described herein were performed at Virginia Commonwealth University (VCU) and some were completed at Research Triangle Institute (RTI), as described in the drug section. Adult male ICR mice were obtained from Harlan (Dublin, VA) for testing at VCU or from Harlan (Frederick, MD) for testing at RTI. All animals at both institutions were individually housed in clear plastic cages in a temperature-controlled (20–24 °C) environment with a 12-h light–dark cycle. For the feeding experiments, each ICR mouse was tested with each dose of a single drug, presented in randomized Latin square order. Separate groups of mice were tested with each dose of each compound in the mouse test battery. All tests were conducted during the light part of the light–dark cycle. The studies reported in this manuscript were carried out in accordance with guidelines published in the guide for the care and use of laboratory animals (National Research Council, 1996) and were approved by the Institutional Animal Care and Use Committee at each institution.

2.2. Apparatus

Weight of food pellets was measured with a Mettler AT261 Delta Range scale (Toledo, OH) at 0.01 mg accuracy at VCU. At RTI, food weight was measured with an ACCULAB Model EC-211 scale (Bradford, MA) at 0.1 g accuracy. At VCU, assessment of spontaneous activity in mice occurred in standard activity chambers interfaced with a Digiscan Animal Activity Monitor (Omnitech Electronics, Inc., Columbus, OH). A standard tail-flick apparatus and a digital thermometer (Fisher Scientific, Pittsburgh, PA) were used to measure antinociception and rectal temperature, respectively. The ring immobility device consisted of an elevated metal ring (diameter = 5.5 cm, height = 16 cm) attached to a wooden stand. At RTI, locomotor sessions with heterodimer 6b were conducted in standard activity chambers interfaced with San Diego Instruments Photobeam Activity System software (San Diego, CA). Antinociception was measured with the use of a standard tail-flick apparatus (Stoelting, Wood Dale, IL). Rectal temperature was measured using a standard rodent rectal thermometer (Physitemp, Clifton, NJ).

2.3. Procedure

2.3.1. Membrane preparations

Chinese Hamster Ovary (CHO) cells stably expressing the human cannabinoid CB_1 or CB_2 receptor were cultured in a 50:50 mixture of DMEM and Ham F-12 supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 mg/ml G418, and 5% fetal calf serum. Cells were harvested by replacement of the media with cold PBS containing 0.4% EDTA followed by agitation. Membranes were prepared by homogenization of cells in 50 mM Tris-HCl, 3 mM $MgCl_2$, 1 mM EGTA, pH 7.4, centrifugation at 50,000 × *g* for 10 min at 4 °C, and resuspension in the same buffer at 1.5 mg/ml. Membranes were stored at −80 °C until use.

2.3.2. Radioligand binding

Membranes were diluted with assay buffer B (50 mM Tris-HCl; pH 7.4, 3 mM $MgCl_2$, 0.2 mM EGTA). Reactions containing membrane (10 μg protein) were incubated with 1 nM [^{3}H](-)-cis-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl)cyclohexanol [CP55,940] and varying concentrations of test compounds in assay buffer B containing 0.5% BSA. Nonspecific binding was measured in the presence of 1 μM unlabeled CP55,940. The assay was incubated for 60 min at 30 °C, and terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters that were pre-soaked in Tris buffer containing 5 g/L BSA (Tris-BSA), followed by five washes with cold Tris-BSA. Bound radioactivity was determined by liquid scintillation spectrophotometry at 45% efficiency for ^{3}H.

2.3.3. Feeding behavior

Twenty-four hours before the start of a feeding trial, all food was removed from the home cages of mice to be tested. The next day mice were transported to the laboratory at least one hour before the beginning of the feeding trial. They were injected with the test compound at the specified pre-session injection interval. Subsequently, they were placed in a clear plastic cage with paper towels lining the bottom and allowed access to a pre-measured amount of their regular lab chow. At the end of one hour, mice were removed from the test cage and placed back into their home cage. The amount of food left in the test cage, including crumbs, was measured, and amount consumed was calculated. Mice received up to two feeding trials per week, separated by at least 72 h.

2.3.4. In vivo pharmacology

The pyrazole and sulfonamide analogs were also evaluated in separate groups of mice in a battery of four tests, in which cannabinoid agonists produce a characteristic profile of in vivo effects (Martin et al., 1991): suppression of locomotor activity, antinociception in the tail flick assay, decreased rectal temperature and ring immobility. Prior to injection, rectal temperature and baseline latency in the tail flick test were measured in the mice. The latter procedure involved exposing the mouse's tail to an ambient heat source (i.e., bright light) and recording latency (in s) for tail removal. Typical control latencies were 2–4 s. A 10 s maximal latency was used in order to avoid damage to the mouse's tail. After measurement of temperature and baseline tail flick latency, mice were injected i.v. with vehicle or drug. Five minutes later they were placed into individual activity chambers for 10 min. Spontaneous activity was measured as the total number of beam interruptions during the entire session, which was expressed as percent inhibition of the control (vehicle) group's activity. Tail-flick latency was measured at 20 min post-injection. Antinociception was expressed as the percent maximum possible effect using a 10-s maximum test latency. Rectal temperature was measured at 30 min after injection and