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Phenotypic assessment of THC discriminative stimulus properties in fatty acid amide hydrolase knockout and wildtype mice

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ABSTRACT

A number of studies have examined the ability of the endogenous cannabinoid anandamide to elicit Δ^9-tetrahydrocannabinol (THC)-like subjective effects, as modeled through the THC discrimination paradigm. In the present study, we compared transgenic mice lacking fatty acid amide hydrolase (FAAH), the enzyme primarily responsible for anandamide catabolism, to wildtype counterparts in a THC discrimination procedure. THC (5.6 mg/kg) served as a discriminative stimulus in both genotypes, with similar THC dose–response curves between groups. Anandamide fully substituted for THC in FAAH knockout, but not wildtype, mice. Conversely, the metabolically stable anandamide analog O-1812 fully substituted in both groups, but was more potent in knockouts. The CB_1 receptor antagonist rimonabant dose-dependently attenuated THC generalization in both groups and anandamide substitution in FAAH knockouts. Pharmacological inhibition of monoacylglycerol lipase (MAGL), the primary catabolic enzyme for the endocannabinoid 2-arachidonoylglycerol (2-AG), with JZL184 resulted in full substitution for THC in FAAH knockout mice and nearly full substitution in wildtypes. Quantification of brain endocannabinoid levels revealed expected elevations in anandamide in FAAH knockout mice compared to wildtypes and equipotent dose-dependent elevations in 2-AG following JZL184 administration. Dual inhibition of FAAH and MAGL with JZL195 resulted in roughly equipotent increases in THC-appropriate responding in both groups. While the notable similarity in THC's discriminative stimulus effects across genotype suggests that the increased baseline brain anandamide levels (as seen in FAAH knockout mice) do not alter THC's subjective effects, FAAH knockout mice are more sensitive to the THC-like effects of pharmacologically induced increases in anandamide and MAGL inhibition (e.g., JZL184).

1. Introduction

The major psychoactive constituent of marijuana, Δ^9-tetrahydrocannabinol (THC; Gaoni and Mechoulam, 1964), exerts a range of pharmacological effects via activation of the endogenous cannabinoid (endocannabinoid) system. This system is comprised of two known receptors, cannabinoid type 1 (CB_1) and cannabinoid type 2 (CB_2), with notable differences in their distribution and function. Centrally located CB_1 receptors are involved in THC's psychoactivity (Wiley et al., 1995), as well as its effects on anxiety (Tambaro and Bortolato, 2012), cognition (Hart et al., 2001), appetite (Kirkham, 2009), and other physiological processes. A number of endogenous ligands have been identified for the endocannabinoid system, including the two best-characterized ligands: N-arachidonoylethanolamine (hereto referred as anandamide) and 2-arachidonoylglycerol (2-AG). These important neuromodulators are synthesized and released on demand (as opposed to being stored in vesicles), and they are short-lived in vivo, with anandamide rapidly degraded predominantly by fatty acid amide hydrolase (FAAH; Cravatt et al., 1996), and 2-AG metabolized primarily by monoacylglycerol lipase (MAGL; Blankman et al., 2007).

Development of pharmacological and genetic tools for probing the functions of the endocannabinoid system has aided understanding of its complex actions. For example, pharmacological inhibitors of FAAH (e.g., URB597, PF3845), MAGL (e.g., JZL184, KML29), and both enzymes (e.g. JZL195, SA57) have been generated and evaluated, as have transgenic mice lacking either of these enzymes (Cravatt et al., 2001; Schlosburg et al., 2010). Of these two

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knockout strains, mice lacking FAAH have been better characterized and were selected for study in the present set of experiments. FAAH knockout mice possess roughly 15-fold higher levels of anandamide than wildtype counterparts and display a characteristic set of cannabimimetic responses to anandamide, including antinociception, catalepsy, hypomotility, and hypothermia (Cravatt et al., 2001). Further, these mice display a hypoalgesic phenotype (Lichtman et al., 2004), and enhanced cognitive impairment in the Morris water maze following anandamide or JZL184 treatment (Varvel et al., 2006; Wise et al., 2012). FAAH knockout mice have been trained in THC drug discrimination, a pharmacologically selective animal model of marijuana's subjective effects (Balster and Prescott, 1992), but that study did not include characterization of the discrimination or direct comparison to THC-trained wildtype mice (Ignatowska-Jankowska et al., 2014). FAAH knockout mice (but not wildtype mice) also have been trained to discriminate anandamide from vehicle in drug discrimination (Walentiny et al., 2011).

The objectives of this study were to characterize the discriminative stimulus effects of THC in FAAH knockout mice compared to wildtype controls, and to determine potential phenotypic differences in responding with cannabinoid agonists and endocannabinoid metabolic enzyme inhibitors. Specifically, dose–response determinations were conducted with THC, anandamide, O-1812 (a metabolically stable anandamide analog; Di Marzo et al., 2001), JZL184 (MAGL inhibitor; Long et al., 2009a), and JZL195 (dual FAAH/MAGL inhibitor; Long et al., 2009b). Challenge tests with the CB_1 receptor antagonist rimonabant were conducted when appropriate.

2. Material and methods

2.1. Subjects

Adult male FAAH knockout (−/−; N = 16) and wildtype mice (+/+; N = 14) on a C57BL/6 background were derived from heterozygous breeding pairs at Virginia Commonwealth University and served as subjects in the THC discrimination study. An additional 23 FAAH knockout and 24 wildtype male mice were used to quantify anandamide and 2-AG levels following JZL184 administration. Mice were housed individually in a temperature controlled (20–22 °C) vivarium on a 12 h light/dark cycle. Mice in the discrimination study were maintained at 85–90% of their free-feeding body weights by restricting postsession feeding and had *ad libitum* access to water in their home cage. All methods were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University and adhered to the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 2003).

2.2. Apparatus

Experimental sessions were conducted in sound- and light-attenuated operant conditioning chambers (Med Associates, St. Albans, VT). Each chamber contained two nose-poke apertures on the front panel. A recessed food receptacle connected to a pellet hopper was centered in between the response apertures. Stimulus lights above each aperture were illuminated during experimental sessions. Fans provided ventilation and masking noise. A computer running Med-PC software (Med Associates) was used to control session parameters and record data.

2.3. Drugs

THC and rimonabant obtained from the National Institute on Drug Abuse (Bethesda, MD) and anandamide (Organix Inc., Woburn, MA) were dissolved in a solution of 0.78% Tween-80 (Fischer Scientific, Pittsburgh, PA) and 99.22% saline. JZL184 and JZL195 (provided by Dr. Benjamin Cravatt, The Scripps Research Institute, La Jolla, CA) and O-1812 (Organix Inc.) were dissolved in an ethanol, emulphor-620, (Rhone-Poulenc, Inc., Princeton, NJ) and saline mixture at a ratio of 1:1:18. THC, anandamide, and O-1812 were administered s.c. 30 min prior to the session. JZL184 and JZL195 were administered i.p. 2 h presession. For antagonism tests, rimonabant was administered i.p. 10 min before treatment with the respective agonist/enzyme inhibitor. All drugs were administered at a volume of 10 mL/kg. Doses were administered in ascending order.

2.4. Discrimination procedure

Mice were prompted to initiate nose poke behavior in an overnight operant session, during which each response on either aperture resulted in delivery of a 14 mg sweetened pellet (Bio-Serv, Frenchtown, NJ). Next, fixed ratio requirements were systematically increased during daily training sessions (15 min) until each subject was required to respond 10 times consecutively (i.e., fixed ratio 10; FR10) to receive a food pellet. Reliable responding on an FR10 schedule was obtained on both apertures before proceeding to discrimination training.

For discrimination training, drug and vehicle apertures (i.e., left or right) were randomly assigned to each subject. During sixteen daily training sessions, subjects were administered 5.6 mg/kg THC or vehicle according to a double alternation sequence of drug delivery (i.e., DDVVDDVV …). Responses during these training sessions were restricted to the condition-appropriate aperture (i.e., were errorless) by inserting a rubber stopper into the inactive aperture.

Following errorless training, the double alternation sequence of drug and vehicle administration continued and subjects were allowed to respond on either aperture. However, only responses on the condition-appropriate aperture resulted in the delivery of reinforcement according to the FR10 schedule. A response on the incorrect aperture reset the counter on the condition-appropriate aperture. During these training sessions, three criteria were used as indices of successful acquisition of the task: 1) first completed ratio on the condition-appropriate aperture, 2) ≥80% of total responses made on the condition-appropriate aperture, and 3) response rate ≥10 responses/min. Testing began once a subject met all three of these criteria during 7 out of 8 consecutive training sessions.

During testing, session parameters were identical to training sessions, except that responses on either aperture resulted in reinforcement presentation according to an FR10 schedule. Prior to the initiation of each dose–response evaluation, control tests were conducted with the training drug and vehicle to demonstrate stimulus control. Tests were conducted up to twice per week, provided subjects met the three training criteria described above during the most recent training sessions with THC and vehicle. A minimum 72 h washout period was implemented between test sessions for most compounds, and test sessions with JZL184 or JZL195 were conducted at least one week apart due to their longer half-lives.

First, a THC dose-effect determination was conducted in all subjects, followed by a rimonabant challenge against the THC training dose. Next, systemic anandamide and its metabolically stable analog O-1812 were assessed for their ability to produce THC-like discriminative stimulus effects in each genotype. Rimonabant challenge against 10 mg/kg anandamide was assessed in FAAH knockout mice, but not in FAAH wildtype mice. Lastly, we determined whether either MAGL inhibition (JZL184) or dual inhibition of MAGL and FAAH (JZL195) would result in substitution for THC's discriminative stimulus. Rimonabant antagonism of the effects of 40 mg/kg JZL195 was also evaluated.

2.5. Quantification of brain concentrations of 2-AG and anandamide

Adult male FAAH knockout and wildtype mice were injected i.p. with vehicle (1:1:18) or JZL184 (3–30 mg/kg) two hours before decapitation. After decapitation, the cerebellum was harvested and rapidly cooled by immersion in liquid nitrogen. Tissue was stored at −80 °C until use. Anandamide and 2-AG were then extracted using a methanol/chloroform extraction (Burston et al., 2008; Hardison et al., 2006). Samples were homogenized on ice in 2 mL chloroform: methanol (2:1, v/v). The internal standards, 1 pmol anandamide-d8 and 2 nmol 2-AG-d8, were added to each sample, calibrator or control. Samples were mixed and centrifuged after the addition of 0.2 mL of a 0.73% sodium chloride solution. The chloroform was collected and evaporated to dryness with nitrogen. The extracts were reconstituted with 100 μL methanol and placed in autosampler vials for analysis. The injection volume was 20 μL and the auto sampler temperature was set at 5 °C. The chromatographic separation of anandamide and 2-AG was performed on a Discovery® HS C18 column 15 cm × 2.1 mm, 3 μm (Supelco: Bellefonte, PA) kept at 40 °C. The mobile phase was 10% water with 1 g/L ammonium acetate and 0.1% formic acid, and 90% methanol with 1 g/L ammonium acetate and 0.1% formic acid. The flow rate was 0.3 mL/min. The following transition ions (m/z) were monitored in positive multiple reaction monitoring (MRM) mode: anandamide, 348 > 62 & 348 > 91; anandamide-d8, 356 > 62; 2-AG, 379 > 287 & 279 > 269; and 2-AG, 387 > 296. Total run time was 10 min.

2.6. Data analysis

The percentage of responses made on the THC-paired aperture for each subject was calculated. Full substitution for THC was defined as ≥80% THC-appropriate responding. ED_{50} values (and 95% confidence intervals) were calculated for drugs that fully substituted for THC using least squares linear regression. Response rates were expressed as responses per min. Response rates (for all compounds) and % THC-appropriate responding (for rimonabant and THC combination tests) were analyzed using split-plot analysis of variance (ANOVA), with genotype as the between subjects variable and dose as the within subjects variable. Rimonabant antagonism of anandamide in FAAH knockout mice was analyzed with repeated measures ANOVA across dose. Brain levels of anandamide and 2-AG after JZL184 administration were analyzed via factorial (genotype × dose) between-subjects ANOVA. For all ANOVAs, significant main effects and/or interactions were further evaluated with Tukey's post hoc tests ($\alpha = 0.05$). Discrimination data for subjects that made fewer than 10 responses during a test session for a particular dose were excluded from analysis, but the corresponding response rate data were included.