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Just Add Water: Cannabinoid Discrimination in a Water T-Maze with FAAH^(-/-) and FAAH^(+/+) Mice

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Abstract

Incomplete overlap in the discriminative stimulus effects of ⁹-tetrahydrocannabinol (THC) and the endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), has been reported in food-reinforced tasks. The objective of this study was to examine cannabinoid discriminative stimulus effects in a non-appetitive procedure. Adult male mice lacking the gene for AEA's major metabolic enzyme, fatty acid amide hydrolase (FAAH), and FAAH^(+/+) mice were trained to discriminate THC or AEA in a water T-maze, in which the response was swimming to an escape platform on the injection-appropriate side. JZL184, a monoacylglycerol lipase (MAGL) inhibitor, was also tested. FAAH^(-/-) mice exhibited speedier acquisition than FAAH^(+/+) mice. THC and AEA fully substituted, with only minor cross-procedure potency variations. Incomplete substitution of JZL184 was observed in THC-trained FAAH^(-/-) mice in the water maze task, as contrasted with full substitution in a food-reinforced nose-poke procedure. Stress-induced changes in AEA and/or 2-AG concentrations in the brain may have mediated this attenuation. JZL184 also partially substituted in AEA-trained FAAH^(-/-) mice in the water maze, suggesting incomplete overlap in the stimulus effects of AEA and JZL184. Through use of a novel water maze procedure, the present study supports the work of previous behavioral pharmacologists in demonstrating robustness of the discrimination paradigm.

Keywords

Aversion; Anandamide; 2-Arachidonoyl glycerol; Cannabinoid; Discriminative stimulus; Endocannabinoid; FAAH; JZL184; MAGL; THC; mouse

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Conflicts of Interest

None declared.

Introduction

Two major individual endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), have been identified. Tools to manipulate endocannabinoid levels include inhibitors of fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), the primary metabolic enzymes of AEA and 2-AG, respectively, as well as FAAH^(-/-) and MAGL^(-/-) mice, which exhibit enhanced brain concentrations of AEA and 2-AG, respectively (Chanda et al., 2010; Cravatt et al., 2001). One purpose of this study was to examine the effects of increases in AEA and/or 2-AG in a procedure related to cannabinoid abuse.

To this end, cannabinoid discrimination represents a selective animal model of the subjective effects of ⁹-tetrahydrocannabinol (THC) (Balster and Prescott, 1992). While a few studies have reported that AEA substitutes in THC or CP55,940 discrimination when administered alone in rodents or nonhuman primates (McMahon, 2009; Wiley et al., 1995), most studies have found substitution for THC or CP55,940 occurred when AEA metabolism was inhibited (Ignatowska-Jankowska et al., 2015; Vann et al., 2009; Walentiny et al., 2015; Wiley et al., 2014). AEA substitution in THC discrimination has also been reported in FAAH^(-/-) mice (Walentiny et al., 2015). Further, FAAH^(-/-) mice have been trained to discriminate AEA from vehicle, with cross-substitution of THC for AEA (Walentiny et al., 2011).

All mouse cannabinoid discriminations to date have used an appetitive reinforcer with associated chronic food restriction. Because AEA and 2-AG serve as neuromodulators in complex metabolic and hormonal regulatory pathways (Cota, 2008; Cota et al., 2003) and also enhance the pleasure associated with eating (Kirkham, 2009), appetitive and/or hedonic variables associated with food reinforcement could conceivably be part of the internal state experienced during discrimination testing with endocannabinoids (e.g., Lotfizadeh et al., 2012). To avoid this potential confound, mice were trained to discriminate cannabinoids in an ethologically relevant behavioral task (i.e., swimming to escape water immersion). An additional advantage of this novel procedure is that it also allows for discrimination training without the expense of a commercially available operant system.

Methods

Subjects

FAAH^(-/-) and FAAH^(+/+) mice on a C57BL/6 background (Jackson Laboratories, Bar Harbor, ME) were individually housed in a temperature-controlled (20–22°C) environment with a 12 h light-dark cycle (lights on at 6 am) and free access to water and rodent chow. All experiments were carried out in accordance with federal regulations and were approved by RTI's IACUC.

Procedure

Experiments were conducted in two T-shaped water mazes, filled with room temperature water to a depth of 1cm above the escape platforms (Fig. 1). Following orientation sessions in the maze, discrimination training was initiated, in which mice received 10 trials in one daily session. Thirty min prior to being placed into the maze for the first trial, mice were

injected with vehicle or their training drug (5.6 mg/kg THC or 6 mg/kg AEA). A single escape platform was located at the end of one arm of the T-maze, dependent upon the injection (i.e., “drug-associated” vs. “vehicle/no drug-associated” platforms). The mouse was confined to the chosen side for 30 s, regardless of whether or not a platform was present. Failure to swim the end of either arm within 60 s resulted in removal from the maze. Between trials, mice were placed into their home cages. Vehicle and drug injections were administered on a double alternation schedule on weekdays. Choice training continued until the mice reached the following acquisition criteria on 8 of 10 consecutive sessions: (1) overall 80% choice for the injection-appropriate platform; (2) completion of at least 8 of the 10 daily trials within the 60-s maximal latency period; and (3) injection-appropriate choice on the first trial. After acquisition criteria were met, mice began the testing phase of the experiment. During test sessions (2 / week), session parameters were identical to training, except that both escape platforms were available. Training sessions continued during the intervening days.

Drugs

THC [National Institute on Drug Abuse (NIDA), Bethesda, MD], rimonabant (NIDA), AEA (NIDA), and JZL184 (synthesized in Dr. Cravatt's lab) were suspended in a vehicle of 7.8 % Polysorbate 80 N.F. (VWR, Radnor, PA) and sterile saline USP (Butler Schein, Dublin, OH) and were administered to the mice i.p. at a volume of 10 ml/kg. Pre-session injection times were 30 min for THC and AEA and 2 h for JZL184. Rimnabant was injected immediately before THC or AEA.

Data Analysis

Percent drug-associated platform choice and latency (s) to reach a platform were the dependent variables. ED₅₀s were calculated for drugs that produced more than 50% drug-associated platform choice. Repeated measures ANOVAs were used for analyses of substitution and latency data, with Tukey's post hoc tests ($\alpha=0.05$), as appropriate. One-way ANOVA was used to analyse acquisition data across the three groups, with Dunnett's post hoc test ($\alpha=0.05$) to compare each group to FAAH^(+/+) group.

Results

To meet the acquisition criteria, FAAH^(+/+) mice required 37.9 ± 8.47 choice sessions for THC discrimination and FAAH^(-/-) mice required 18.9 ± 4.45 and 14.6 ± 1.61 choice sessions for THC and AEA discrimination, respectively [$F(2,27)=5.32$, $p<0.05$ for each FAAH^(-/-) compared to FAAH^(+/+) group]. THC substituted for the training drug in THC-trained FAAH^(+/+) and FAAH^(-/-) mice (Fig. 2, panels A and B, respectively) and in AEA-trained FAAH^(-/-) mice (Fig. 2, panel C) and rimnabant (1 mg/kg) attenuated this effect in all groups [$F(2,18)=19.3$, $p<0.05$, panel A; $F(2,16)=104.9$, $p<0.05$, panel B; $F(2,18)=15.4$, $p<0.05$, panel C]. In the FAAH^(-/-) mice, AEA increased THC-like responding in the THC- and AEA-trained mice (panels B and C, respectively), with rimnabant attenuation in each instance [$F(2,16)=7.6$, $p<0.05$, panel B and $F(2,18)=23.5$, $p<0.05$, panel C, respectively]. JZL 184 produced maximums of 39% and 47% THC-associated platform choice in FAAH^(+/+) and FAAH^(-/-) mice (panels A and B, respectively) and 59% AEA-associated

platform choice (panel C). Potencies for all drugs are shown in Table 1. Compared to vehicle, latencies to swim to the platform did not differ for any drug dose in THC-trained mice of either genotype (panels D and E), but 10 mg/kg THC and 10 and 40 mg/kg JZL 184 increased latencies in AEA-trained mice [$F(5,45)=3.5$, $p<0.05$ and $F(3,24)=5.8$, $p<0.05$, respectively; panel F].

Discussion

Acquisition of the maze discrimination task occurred more quickly for FAAH^(-/-) mice than for FAAH^(+/+) mice. In contrast, when a procedure involving nose-poke for food reinforcement was used, both genotypes acquired THC discrimination in a similar number of trials (Walentiny et al., 2015). These results are consistent with those of previous studies which reported that FAAH^(-/-) and MAGL^(-/-) mice showed accelerated acquisition of tasks motivated by aversive (vs. appetitive) stimuli (Pan et al., 2011; Varvel et al., 2007; Wise et al., 2009). Given the increased brain AEA and 2-AG concentrations that have been observed in FAAH^(-/-) and MAGL^(-/-) mice, respectively (Chanda et al., 2010; Cravatt et al., 2001), combined behavioral results of the present and previous studies suggest that stimulation of the endocannabinoid system may facilitate acquisition of aversively, but not appetitively, motivated tasks.

Despite differences in acquisition rates, potency differences in THC and AEA across procedure and genotype were relatively minor in magnitude (Table 1). Further, rimonabant attenuation of THC and AEA substitution confirmed CB₁ receptor mediation in all discrimination groups. In FAAH^(+/+) mice, maximal substitution of JZL184 (47–49%; Table 1) was similar across procedures and occurred within a dose range that increased brain 2-AG concentrations in mice (Walentiny et al., 2015; Wiley et al., 2014). These results are consistent with those of previous studies showing that most MAGL inhibitors produce little substitution for direct CB₁ receptor agonists in rats and wildtype mice in traditional procedures in operant chambers (Hrubá et al., 2015; Ignatowska-Jankowska et al., 2014; Wiley et al., 2014). Because the primary action of JZL184 is to inhibit MAGL, THC-like discriminative stimulus effects of this compound would be presumed to result from its enhancement of 2-AG in the brain, a process that likely occurs over a different time course and in different brain regions than THC absorption and distribution after i.p. injection. Lack of substitution may be related to these differences. Interestingly, however, JZL184 produced other effects in mice that have been observed with exogenous cannabinoid agonist administration, including suppression of locomotor activity, antinociception, and hypothermia (Long et al., 2009a). Nevertheless, present and previous data do not support the hypothesis that JZL184-induced increases in 2-AG concentrations in the brain are sufficient to engender THC-like responding in FAAH^(+/+) mice, regardless of procedure/type of reinforcement (negative in water maze or positive in operant chamber). Previous research has demonstrated that reinforcer type (food or shock termination) also did not affect discrimination of a benzodiazepine (Gerak and France, 1999), which (like cannabinoids) also may affect food intake or sensitivity to shock (Anderson-Baker et al., 1979; Howard et al., 1982). Mild food restriction also does not affect the discriminative stimulus effects of morphine in rats trained in a shock-avoidance procedure (Ukai and Holtzman, 1988).

Together with the present results, these findings suggest robustness of the discriminative stimulus effects of psychoactive drugs across procedural variations.

Results from previous discrimination studies suggest that endogenous increases in both AEA and 2-AG are required to engender THC-like effects (Hrubá et al., 2015; Long et al., 2009b; Wiley et al., 2014), as also exemplified by full substitution of JZL184 in FAAH^(-/-) mice trained to discriminate THC in a food-reinforced nose-poke procedure (Walentiny et al., 2015). In contrast, maximal substitution in FAAH^(-/-) mice trained to discriminate THC or AEA in the water T-maze was somewhat less (39% and 59%, respectively). Repetitive exposure of rodents to the water escape procedure may have contributed to this attenuation of substitution of JZL184 for THC through its effects on the HPA axis (Connor et al., 1998), as substantial work has shown that one of the many roles of the endocannabinoid system is modulation of the stress response (reviewed in Morena et al., 2015). In particular, repeated exposure to a stressor (e.g., restraint or water immersion) decreases AEA and increases 2-AG across several brain areas (Hill et al., 2010, 2011; McLaughlin et al., 2012; Patel et al., 2005; Rademacher and Hillard, 2007). If perception of THC-like discriminative stimulus effects requires increases in both AEA and 2-AG (as previous work suggests), a stress-induced decrease in the genetically enhanced AEA concentrations in the brains of FAAH^(-/-) mice may have contributed to the lower level of THC-like responding observed here as compared to full substitution seen in the nose-poke procedure. Neither JZL184 nor other MAGL inhibitors have been assessed previously in AEA-trained mice in any discrimination procedure. Hence, the reason for incomplete substitution of JZL184 for AEA is unknown. One possible explanation is that the increased 2-AG produced by JZL184 produces stimulus effects that overlap with, but also differ from, those produced by AEA. Additional empirical work is required to support this hypothesis as well as to delineate further the role of stress-related changes in brain endocannabinoid levels on the pharmacological effects of FAAH and MAGL inhibitors.

In summary, the results show that FAAH^(-/-) mice acquired aversive cannabinoid discrimination tasks faster than FAAH^(+/+) mice whereas speed of acquisition was similar across genotypes for appetitively motivated tasks. THC and AEA substituted in all groups of mice, regardless of the task. Further, potencies were similar for each training drug across genotype and across procedures. Full substitution for JZL184 also was observed FAAH^(-/-) mice trained to discriminate THC in the nose-poke procedure. This effect was attenuated in the water maze procedure, with JZL184 showing only partial substitution in THC-trained FAAH^(-/-) mice. Stress-induced changes in AEA and/or 2-AG concentrations in the brain due to repeated water immersion are posited to mediate the lower level of substitution in the water maze procedure. JZL184 also partially substituted in AEA-trained FAAH^(-/-) mice in the water maze procedure, which may suggest incomplete overlap in the stimulus effects of AEA and JZL184. Further research is needed to confirm these hypotheses. Finally, through the use of a novel water maze cannabinoid discrimination procedure that requires minimal equipment purchase, the present study supports the work of previous behavioral pharmacologists in demonstrating robustness of the discrimination paradigm.

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Figure 1.
Diagram of water T-maze used for cannabinoid discrimination.

Figure 2.

Shown are the effects of THC (filled squares), AEA (unfilled squares), and JZL184 (filled triangles) on % THC platform choice in FAAH^(+/+) and FAAH^(-/-) mice trained to discriminate 5.6 mg/kg THC (panels A and B, respectively) and on % AEA platform choice in FAAH^(-/-) mice trained to discriminate 6 mg/kg AEA (panel C). Latencies for each discrimination group to reach the escape platform are shown in the corresponding lower [Paul Will1]panels (D–F). In each panel, the dashed line represents results for the training drug. Results of antagonism tests with 1 mg/kg rimonabant in combination with THC and AEA (filled and unfilled circles, respectively) are also shown (panels A–C). In all panels, points above VEH and THC or AEA represent the results of control tests with vehicle and the cannabinoid agonist, respectively, on % agonist platform choice (panels A–C) and latencies (panels D–F) conducted prior to the dose-effect curve determination for each test compound. Values represent the mean ($\pm$ S.E.M.) of data from 8–10 FAAH^(-/-) mice and 10 FAAH^(+/+) mice for all substitution dose-effect curves and for the rimonabant antagonism tests. Asterisks (*) designate a significant effect of the compound dose on latency to swim to a platform ($p < 0.05$), as compared to vehicle. Number signs (#) designate a significant antagonism effect of 1 mg/kg rimonabant ($p < 0.05$), as compared to the effect of THC or AEA alone.

Table 1

Cannabinoid potencies across genotype (FAAH^(-/-) or FAAH^(+/+)) and training drug (5.6 mg/kg THC or 6 mg/kg AEA) in a water-maze cannabinoid discrimination in mice

Compound	ED ₅₀ (95% confidence interval)		
	THC FAAH ^(+/+)	THC FAAH ^(-/-)	AEA FAAH ^(-/-)
THC	3.4 mg/kg (2.6 – 4.5)	1.0 mg/kg (0.5 – 2.3)	1.3 mg/kg (0.8 – 2.2)
AEA	Not tested	4.8 mg/kg (3.3 – 7.1)	1.1 mg/kg (0.7 – 1.6)
JZL184 [*]	47%	39%	59%

* None of the groups tested with JZL184 showed > 80% choice / responding on the side associated with the training drug. For each group, % maximal responding on the drug-associated side occurred at the 40 mg/kg dose and is shown in the table.