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Cannabinoid agonists differentially substitute for the discriminative stimulus effects of Δ^9 -tetrahydrocannabinol in C57BL/6J mice

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Abstract

Rationale—A variety of behavioral procedures have been developed to assess cannabinoid activity in mice; however, the feasibility of establishing Δ^9 -THC as a discriminative stimulus in mice has not been documented.

Objective—One goal was to establish Δ^9 -THC as a discriminative stimulus in mice; after having done so, another goal was to examine the in vivo mechanism of action of Δ^9 -THC with other cannabinoids and noncannabinoids.

Materials and methods—C57BL/6J mice ($n=8$) were trained to discriminate Δ^9 -THC (10 mg/kg i.p.) from vehicle while responding under a fixed ratio 30 schedule of food presentation.

Results—Mice satisfied the discrimination criteria in 18–98 (median=67) sessions and the discriminative stimulus effects of Δ^9 -THC were dose-dependent ($ED_{50}=2.6$ mg/kg). CP 55940 and WIN 55212-2 dose-dependently increased Δ^9 -THC-appropriate responding to 100% ($ED_{50}=0.032$ and 0.45 mg/kg, respectively), whereas methanandamide and a variety of noncannabinoids (cocaine, ethanol, and ketamine) produced a maximum of 34% Δ^9 -THC-appropriate responding. The cannabinoid CB_1 antagonist SR 141716A (rimonabant) surmountably antagonized the discriminative effects of Δ^9 -THC, CP 55940, and WIN 55212-2; methanandamide did not significantly modify the Δ^9 -THC discriminative stimulus.

Conclusions—The discriminative stimulus effects of Δ^9 -THC, CP 55940, and WIN 55212-2 are mediated by the same (i.e., CB_1) receptors, whereas the effects of methanandamide or a metabolite of methanandamide are mediated at least in part by non- CB_1 receptors. The discriminative stimulus effects of Δ^9 -THC in mice could be used to evaluate mechanisms of cannabinoid activity with approaches (e.g., inducible knockouts) currently unavailable in nonmurine species.

Keywords

Antagonist; Cannabinoid; Δ^9 -THC; Drug discrimination; Efficacy; Mouse; Rimonabant; SR 141716A

Introduction

Drug discrimination can be a highly sensitive, quantitative, and pharmacologically selective approach for evaluating the mechanism of action of drugs in vivo, and this approach has well-documented utility for examining the in vivo effects of cannabinoids. For example, cannabinoid CB₁ agonists can have discriminative stimulus effects at doses smaller than doses producing some other in vivo effects (e.g., De Vry et al. 2004) and CB₁ receptor agonists and not other drugs typically substitute for the discriminative stimulus effects of Δ^9 -THC (Balster and Prescott 1992; McMahon 2006a). Cannabinoid agonist discriminations appear to be selectively mediated by CB₁ and not CB₂ receptors insofar as the discriminative stimulus effects of nonselective agonists (e.g., Δ^9 -THC) are blocked by selective CB₁ antagonists (SR 141716A or rimonabant; Wiley et al. 1995; Järbe et al. 2001; McMahon 2006b) and not by selective CB₂ antagonists (Järbe et al. 2006; McMahon 2006a). In addition to providing a selective measure of CB₁ receptor activity in vivo, the discriminative stimulus effects of cannabinoid agonists in nonhumans are highly predictive of cannabis-like effects in humans (Balster and Prescott 1992 for review).

Cannabinoid agonists have been trained as discriminative stimuli in a variety of species including rats, pigeons, and primates (Järbe et al. 1977; Chait et al. 1988; Balster and Prescott 1992; Gold et al. 1992). However, it does not appear that cannabinoid discriminations have been established in mice. There are a variety of indices that have been used to evaluate cannabinoid agonist activity in mice including hypothermia, catalepsy, antinociception, and hypoactivity (Martin et al. 1991). While efficient, these measures are not necessarily selective for cannabinoid agonists (Wiley and Martin 2003) and drug discrimination in mice could provide greater pharmacologic selectivity relative to other behavioral procedures. Mice also provide the opportunity to examine the molecular determinants of behavioral effects with genetic manipulations (i.e., knockouts) that are currently unavailable in rats, pigeons, or primates. In particular, temporal control over the expression of a gene and its products with inducible knockouts (Morozov et al. 2003) might be particularly useful for drug discrimination studies in which the presence of the gene is desired under one condition (drug discrimination training) and not another (drug discrimination testing). The first goal of this study was to establish Δ^9 -THC as a discriminative stimulus in C57BL/6J mice; after having done so, the pharmacologic mechanisms responsible for the Δ^9 -THC discriminative stimulus were examined with a variety of cannabinoids (CP 55940, WIN 55212-2, methanandamide, and rimonabant). To examine pharmacologic specificity, cocaine, ketamine, and ethanol were chosen for study because their behavioral activity has been indexed with operant procedures in mice (Shelton 2004) and these and related compounds (i.e., phencyclidine) were shown to not substitute for the discriminative stimulus effects of Δ^9 -THC in rats (Järbe and Henriksson 1974). The C57BL/6J strain of mouse was chosen because this strain is frequently used for genetic manipulation.

Materials and methods

Subjects

Eight male C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) were purchased at 6 weeks of age (approximately 15 g) and were housed individually on a 12/12-h light/dark

cycle. Mice received approximately 1.5 cc of 50% condensed milk during experimental sessions (Monday through Friday) and 2.5 g of food (Dustless Precision Pellets 500 mg, Rodent Grain-Based Diet, Bio-Serv, Frenchtown, NJ) per day after sessions and on the weekend; water was available ad libitum in the home cage. Mice were habituated to the experimental room for 7 days before the first experimental session, and testing was conducted during the light period. Mice were maintained and experiments were conducted in accordance with the Institutional Animal Care and Use Committee, The University of Texas Health Science Center at San Antonio and with the “Principles of Laboratory Animal Care” and the “Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research” (National Research Council 2003).

Apparatus

Discrimination experiments were conducted in commercially available mouse operant conditioning chambers (MedAssociates, St. Albans, VT) that were placed in ventilated, sound-attenuating enclosures. The ceiling of each operant conditioning chamber contained a light (i.e., house light), and the side of one wall contained two recessed holes (2.2-cm diameter) spaced 12.7 cm apart from center to center; the center of each hole was positioned 1.6 cm from the floor. Each hole contained a photobeam, a light, and a dipper to which 0.01 cc of condensed milk could be delivered from a tray positioned outside the operant conditioning chamber. An interface (MedAssociates) connected the operant conditioning chambers to a computer, and experimental events were controlled and recorded with Med-PC software.

Discrimination training

Sessions were conducted once daily Monday through Friday. Vehicle (a mixture of 1 part absolute ethanol, 1 part Emulphor-620, and 18 parts of physiologic saline) was administered in the home cage, and 30 min later, mice were placed inside the operant conditioning chambers. During 30-min sessions, each recessed hole was illuminated and mice could insert their snouts to disrupt a photobeam. A single disruption of the photobeam (continuous reinforcement) in either hole resulted in 10-s access to 0.01 cc of condensed milk diluted to 50% with tap water, during which time the lights in each hole were turned off and the house light was illuminated. Disruptions of a photobeam during the 10-s period of milk availability had no programmed consequence. Training under continuous reinforcement continued until a minimum of 100 reinforcers was obtained in three consecutive sessions. Thereafter, the number of photobeam disruptions required to obtain a reinforcer (i.e., fixed ratio or FR) was increased every two sessions (i.e., to an FR3, FR10, and FR30). To minimize odor traces between sessions, holes were wiped with absolute ethanol and milk dippers and trays were washed with a dilute soap solution and then rinsed with tap water.

Once responding was maintained at an FR30, mice received injections (vehicle or Δ^9 -THC) and were placed into the darkened operant chamber without schedule contingencies for 30 min (i.e., timeout). Thereafter, during a 30-min response period, lights in each hole were illuminated and mice could respond under the FR30 schedule of milk presentation described above except that responding in only one of the holes (i.e., correct hole) was reinforced depending on whether vehicle or Δ^9 -THC was administered before the session. Determination of correct holes (e.g., left, vehicle; right, Δ^9 -THC) varied among mice and remained the same for that mouse for the duration of the study. Training with vehicle was alternated daily with Δ^9 -THC and dose was increased from 1 to 10 mg/kg in 1/2 log unit. A training dose of 10 mg/kg of Δ^9 -THC was used for the remainder of the study and, thereafter, two consecutive vehicle training sessions were alternated with two consecutive Δ^9 -THC training sessions (i.e., double-alternation sequence). The first test was conducted when, for 5 consecutive or for 6 out of 7 days, at least 80% of the total responses occurred

on the correct hole and fewer than 30 responses occurred in the incorrect hole before completion of the first FR on the correct hole (i.e., fewer than 59 total responses were made before delivery of the first reinforcer).

Discrimination testing

Test sessions were identical to training sessions except that 30 responses in either hole resulted in milk presentation and mice received vehicle or a dose of Δ^9 -THC or another drug. Test sessions were conducted Wednesday, Thursday, or Friday, and a maximum of two tests was conducted per week. Tests subsequent to the first test were conducted only after mice satisfied the criteria specified above during consecutive vehicle and Δ^9 -THC training sessions earlier in the week, and a second test in the same week was conducted only after mice satisfied the test criteria during either a vehicle or Δ^9 -THC training session on an intervening day. Training conditions (vehicle or Δ^9 -THC) preceding test sessions were nonsystematic.

Δ^9 -THC, CP 55940, WIN 55212-2, and methanandamide were studied by administering a dose of drug at the beginning of a 1-h session (beginning with a 30-min timeout followed by a 30-min response period). Rimobant and methanandamide were studied in combination with other cannabinoid agonists by administering a dose of rimobant or methanandamide immediately before a dose of cannabinoid agonist. For tests with drugs requiring pretreatment intervals shorter than 30 min, mice were placed in the chamber without injection at the beginning of the 30-min timeout. A dose of ketamine was administered 10 min before the response period, and a dose of cocaine or ethanol was administered 5 min before the response period; the response period was 10 min for each of these tests. The order of testing among drugs was nonsystematic except that tests with different doses of Δ^9 -THC were conducted at the beginning of the study and again with the same doses of Δ^9 -THC at end of the study, and tests involving combination of rimobant or methanandamide with cannabinoid agonists were conducted after establishing the individual dose-response functions for methanandamide and other cannabinoid agonists.

Drugs

The levo enantiomer of Δ^9 -THC (100 mg/ml in absolute ethanol; The Research Technology Branch, National Institute on Drug Abuse, Rockville, MD) was prepared as a concentration of 5 mg/ml in a mixture of 1:1:18 of absolute ethanol, Emulphor-620 (Rhone-Poulenc, Princeton, NJ), and physiologic saline; smaller concentrations (1 mg/ml used for the training dose of 10 mg/kg) of Δ^9 -THC were diluted with physiologic saline. Rimobant base (The Research Technology Branch, National Institute on Drug Abuse), WIN 55212-2 (Sigma, St. Louis, MO), and CP 55940 (Tocris, Ellisville, MO) were dissolved in a 1:1:18 mixture of Tween 80 (Sigma), propylene glycol (Sigma), and physiologic saline. (*R*)-methanandamide (in Tocrisolve vehicle; Tocris) and ketamine hydrochloride (100 mg/ml in physiologic saline; Fort Dodge Laboratories, Fort Dodge, IA) were diluted with physiologic saline. Cocaine hydrochloride (The Research Technology Branch, National Institute on Drug Abuse) was dissolved in physiologic saline. Drugs were administered i.p. in a volume of 0.1 ml/kg at doses (milligrams per kilogram) expressed as the weight of the forms listed above. Ethanol was administered in concentrations of 5–20% (w/v).

Data analyses

Discrimination data were expressed as an average ($\pm$ S.E.M.) percentage of Δ^9 -THC-appropriate responding (i.e., number of Δ^9 -THC-appropriate responses divided by the total responses made during the duration of the test) and were plotted as a function of dose. All data for a particular drug treatment were collected in six to eight mice. For each mouse contributing to a particular drug treatment, the dose-effect curve for that mouse was defined

by one ineffective dose and larger doses up to a dose producing greater than 80% Δ^9 -THC-appropriate responding or decreasing rate of responding to less than 20% of the control response rate. The potencies of cannabinoid agonists for producing discriminative stimulus effects were estimated by analyzing the dose–effect curves by simultaneously fitting straight lines to the individual dose–response data by means of GraphPad Prism version 4.03 for Windows (San Diego, CA), using the following equation: $\text{effect} = \text{slope} \times \log(\text{dose}) + \text{intercept}$. Straight lines were fitted to the linear portion of dose–effect curves, defined by doses producing 20–80% of the maximum effect, including not more than one dose producing less than 20% of the maximum effect. Other doses were excluded from the analyses. The same approach was used to establish the potency of an agonist alone and in combination with various doses of an antagonist.

The slopes of dose–effect curves were compared with an F-ratio test using GraphPad. If the slopes were not significantly different, then a common, best-fitting slope was used for further analyses (for detailed examples of this approach, see Kenakin 1997). Doses corresponding to the 50% level of the effect (ED_{50} value), potency ratios, and their 95% confidence limits (CL) were calculated by parallel line analyses of data from individual subjects (Tallarida 2000). The potencies of different agonists were considered significantly different when the 95% confidence limits of their potency ratio did not include 1.

Response rate was averaged ($\pm$ S.E.M.) among mice and plotted as a function of dose. The effects of a drug on response rate were considered significant when the mean rate of responding was not contained within the 95% confidence limits of the mean control response rate, defined in individual mice as mean response rate calculated from ten consecutive vehicle training sessions in which the criteria for testing were satisfied and that were conducted after the first test. Discrimination data were not included for analysis when response rate for an individual mouse was less than 20% of the vehicle control rate for that mouse; however, all response rate data were included in the group average.

Results

Discriminative stimulus and rate effects of Δ^9 -THC and other cannabinoid agonists

The criteria for testing were satisfied in 18–98 sessions (median=67 sessions) including both vehicle and Δ^9 -THC training sessions. During tests, Δ^9 -THC dose-dependently increased drug-appropriate responding with the training dose (10 mg/kg) producing a mean ($\pm$ S.E.M.) of 97 ($\pm$ 1)% of Δ^9 -THC-appropriate responses (Fig. 1, top left, circles). Administration of vehicle produced 1% Δ^9 -THC-appropriate responding (Fig. 1, top left, VEH). CP 55940 and WIN 55212-2 also dose-dependently increased Δ^9 -THC-appropriate responding to a mean of 100% at doses of 0.1 and 3.2 mg/kg, respectively (Fig. 1, top left, triangles and squares, respectively). The dose–effect curves for the discriminative stimulus effects of CP 55940, WIN 55212-2, and Δ^9 -THC did not deviate from parallelism; ED_{50} values (95% confidence limits) were 0.032 (0.018–0.056) mg/kg for CP 55940, 0.45 (0.27–0.74) mg/kg for WIN 55212-2, and 2.6 (1.8–3.7) mg/kg for Δ^9 -THC. CP 55940 was significantly more potent than WIN 55212-2 and Δ^9 -THC (14- and 82-fold, respectively; Table 1, top), and WIN 55212-2 was significantly more potent than Δ^9 -THC (5.8-fold; Table 1, top). The mean control response rate (95% confidence limits) calculated from ten vehicle training sessions was 4.4 (3.2–5.6) responses per second. Some doses of CP 55940 and WIN 55212-2 increased rate of responding, whereas rate of responding was not significantly modified by Δ^9 -THC up to a dose of 10 mg/kg (Fig. 1, bottom left).

Methanandamide, in contrast to the other cannabinoid agonists, produced limited Δ^9 -THC-appropriate responding up to doses that significantly decreased rate of responding. For example, maximum Δ^9 -THC-appropriate responding was a mean ($\pm$ S.E.M.) of 25 ($\pm$ 12)% at

a dose of 10 mg/kg of methanandamide, and larger doses (32 and 56 mg/kg) significantly decreased rate of responding (Fig. 1, second from left). Rate of responding at 56 mg/kg of methanandamide was 24% of the vehicle control rate, and six out of eight mice responded at rates below 20% of the vehicle control rate. A variety of noncannabinoids also produced limited Δ^9 -THC-appropriate responding. For example, maximum Δ^9 -THC-appropriate responding was a mean ($\pm$ S.E.M.) of 34 ($\pm$ 18)% at a dose of 10 mg/kg of ketamine, and larger doses (17.8 and 32 mg/kg) of ketamine significantly decreased rate of responding (Fig. 1, second from right, closed squares). A dose of 17.8 mg/kg of cocaine produced a mean ($\pm$ S.E.M.) of 9 ($\pm$ 8)% of Δ^9 -THC-appropriate responses and larger doses (32 and 56 mg/kg) significantly decreased response rate (Fig. 1, second from right, open diamonds). Ethanol produced a maximum average ($\pm$ S.E.M.) of 25 ($\pm$ 12)% of Δ^9 -THC-appropriate responses and response rate was markedly decreased at larger doses (1,000 and 3,200 mg/kg; Fig. 1, right, closed diamonds).

Re-determination of the dose–response curve for Δ^9 -THC at the end of the study (data not shown) demonstrated the potency of Δ^9 -THC (ED_{50} value of 3.4 mg/kg; 95% confidence limits of 1.8–6.4 mg/kg) to be not significantly different from its potency established at the beginning of the study.

Antagonism of the discriminative stimulus effects of Δ^9 -THC by rimonabant and not methanandamide

Rimonabant (0.32 and 1 mg/kg) produced a mean of 0% Δ^9 -THC-appropriate responses and surmountably and dose-dependently antagonized the discriminative stimulus effects of Δ^9 -THC (Fig. 2, top left). For example, doses of 0.32 and 1 mg/kg of rimonabant significantly increased the ED_{50} of Δ^9 -THC by 2.0- and 3.9-fold, respectively (Table 1, middle). Rimonabant alone, at doses of 0.32 and 1 mg/kg, did not significantly decrease rate of responding (Fig. 2, bottom left, VEH) and did not appear to attenuate decreases in response rate produced by larger doses (greater than 10 mg/kg) of Δ^9 -THC (Fig. 2, bottom left).

Methanandamide, in contrast to rimonabant, did not significantly modify the discriminative stimulus effects of Δ^9 -THC (Fig. 2, top right). For example, a dose of 10 mg/kg of methanandamide did not significantly modify the ED_{50} of Δ^9 -THC (Table 1, middle). The combined effects of methanandamide and Δ^9 -THC on rate of responding appeared to be additive. For example, response rate was significantly decreased when methanandamide and Δ^9 -THC were combined at doses (10 mg/kg for both) that separately did not significantly modify response rate (Fig. 2, bottom right). Moreover, methanandamide (32 mg/kg) slightly decreased response rate when administered alone yet markedly decreased response rate when combined with Δ^9 -THC at doses (3.2 and 10 mg/kg) that by themselves did not significantly modify response rate.

Antagonism of the discriminative stimulus effects of CP 55940 and WIN 55212-2 by rimonabant

Rimonabant (0.32 mg/kg) surmountably antagonized the Δ^9 -THC-like discriminative stimulus effects of CP 55940 and WIN 55212-2 (Fig. 3, top left and right, respectively), as evidenced by significant 2.9- and 3.0-fold increases in their ED_{50} values, respectively. Rate of responding was not significantly altered by rimonabant (0.32 mg/kg) in combination with CP 55940 and WIN 55212-2 up to doses of 0.32 and 3.2 mg/kg, respectively (Fig. 3, bottom left and right, respectively).

The potency of rimonabant as an antagonist of discriminative stimulus effects did not vary as a function of cannabinoid agonist. Thus, a dose of 0.32 mg/kg of rimonabant produced the same magnitude of increase in the ED_{50} values of Δ^9 -THC, CP 55940 and WIN 55212-2

(2.0-, 2.9-, and 3.0-fold, respectively), as evidenced by overlap in the 95% confidence limits for each of these potency ratios.

Discussion

The behavioral effects of many cannabinoid agonists (e.g., Δ^9 -THC, CP 55940, and WIN 55212-2) are strikingly similar across different species and procedures including drug discrimination (Wiley 1999 for review; McMahon 2006a); the current study extends these observations to discriminative stimulus effects in C57BL/6J mice discriminating Δ^9 -THC. CP 55940 and WIN 55212-2 substituted for the discriminative stimulus effects of Δ^9 -THC, whereas noncannabinoids (cocaine, ethanol, and ketamine) did not substitute for Δ^9 -THC. The CB₁ receptor-selective antagonist rimonabant surmountably antagonized the discriminative stimulus effects of not only Δ^9 -THC but also the Δ^9 -THC-like discriminative stimulus effects of CP 55940 and WIN 55212-2, whereas methanandamide did not substitute for or otherwise modify the discriminative stimulus effects of Δ^9 -THC. Thus, CB₁ receptors appear to mediate the discriminative stimulus effects of Δ^9 -THC, CP 55940, and WIN 55212-2, whereas CB₁ and other receptors appear to mediate the effects of methanandamide in mice.

CP 55940 and WIN 55212-2 substituted for the discriminative stimulus effects of Δ^9 -THC in mice, consistent with previous studies in rats and rhesus monkeys (Gold et al. 1992; Compton et al. 1992; Wiley et al. 1995; McMahon 2006a). The relative potency of cannabinoids to produce discriminative stimulus effects is similar in rats and mice, with Δ^9 -THC being approximately 100-fold and sixfold less potent than CP 55940 and WIN 55212-2, respectively. Differences in relative potency are smaller in rhesus monkeys, with Δ^9 -THC being somewhat more potent than WIN 55212-2, perhaps reflecting differences in metabolism of these cannabinoids as a function of species. The qualitatively similar discriminative stimulus effects of Δ^9 -THC, CP 55940, and WIN 55212-2 across species suggests that their shared mechanism of action (i.e., CB₁ receptor agonism; Howlett et al. 2002) is conserved across species. An advantage of drug discrimination is its pharmacologic selectivity relative to other behavioral assays, and the discriminative stimulus effects of Δ^9 -THC in rats are highly selective for cannabinoid agonist activity (Balster and Prescott 1992). The discriminative stimulus effects of Δ^9 -THC in mice appear also to be pharmacologically selective insofar as noncannabinoids (cocaine, ethanol, and ketamine) failed to substitute for the Δ^9 -THC discriminative stimulus up to doses producing other behavioral effects (i.e., decreases in rate of fixed ratio responding).

Rimonabant binds reversibly to cannabinoid CB₁ receptors and has little or no efficacy in vitro (Childers 2006), and the effects of rimonabant in mice discriminating Δ^9 -THC are consistent with this pharmacologic profile. Rimonabant dose-dependently and surmountably antagonized the discriminative stimulus effects of Δ^9 -THC and also surmountably antagonized the Δ^9 -THC-like discriminative stimulus effects of CP 55940 and WIN 55212-2. The potency of rimonabant as an antagonist was similar among the agonists, suggesting that the same receptors mediated the discriminative stimulus effects of Δ^9 -THC, CP 55940, and WIN 55212-2. Rimonabant also has similar potency as an antagonist of the hypothermic and cataleptic effects of Δ^9 -THC and WIN 55212-2 in C57BL/6J mice (McMahon and Koek 2007). Collectively, the cannabinoid antagonist activity of rimonabant in mice suggests that rimonabant-sensitive receptors have a similar role in mediating the various in vivo effects of Δ^9 -THC, CP 55940, and WIN 55212-2.

The endogenous cannabinoid CB₁ agonist anandamide does not always substitute for the discriminative stimulus effects of Δ^9 -THC (Wiley et al. 1997; 1998; Järbe et al. 2001). Anandamide is rapidly metabolized by fatty acid amide hydrolase and appears to have

limited activity in vivo (Deutsch and Chin 1993). Metabolism of anandamide can be decreased with fatty acid amide hydrolase inhibitors (e.g., URB 597; Fegley et al. 2005) and URB 597 was reported to increase the potency of anandamide to substitute for a Δ^9 -THC discriminative stimulus in rats (Solinas et al. 2007). Modification of the chemical structure of anandamide has yielded high affinity CB₁ agonists with greater metabolic stability than anandamide (e.g., methanandamide; Abadji et al. 1994), and methanandamide has been shown to substitute for the discriminative stimulus effects of Δ^9 -THC in rats and monkeys (Burkey and Nation 1997; Järbe et al. 1998; McMahon 2006a). Nevertheless, substitution of methanandamide for the discriminative stimulus effects of Δ^9 -THC is not unanimous (present results).

Intrinsic activity (agonist efficacy) can determine the extent to which agonists acting at the same receptors share behavioral effects and, under conditions requiring relatively high efficacy to achieve a particular behavioral effect, a low-efficacy agonist is less likely to produce the effect obtained with a high efficacy agonist. According to receptor theory, a low-efficacy agonist that does not mimic the effect of a high efficacy agonist can block or antagonize the effect of the high-efficacy agonist (Bergman et al. 2000 for review). The efficacy required for agonist activity in drug discrimination assays apparently can be increased by increasing the training dose of an agonist and a previous study showed that substitution of methanandamide for a Δ^9 -THC discriminative stimulus decreases as a function of increasing the training dose of Δ^9 -THC (Järbe et al. 1998). Failure of methanandamide to substitute for Δ^9 -THC might indicate that methanandamide has lower agonist efficacy than Δ^9 -THC at CB₁ receptors. However, methanandamide did not block the discriminative stimulus effects of Δ^9 -THC in the current study, indicating that differences in CB₁ agonist efficacy were not responsible for the qualitatively different effects of methanandamide and Δ^9 -THC in mice.

The mechanism by which methanandamide exerts behavioral activity in C57BL/6J mice appears to involve non-CB₁ receptors in both the present and previous studies. For example, Δ^9 -THC was shown to decrease rate of responding for food in wildtype C57BL/6J mice and not transgenic mice lacking CB₁ receptors, whereas methanandamide produced similar decreases in rate of responding in both wildtype and CB₁ knockout mice (Baskfield et al. 2004). These results do not appear to reflect inability of methanandamide to bind to CB₁ receptors in mouse brain inasmuch as a previous study (Petitet et al. 1999) reported that methanandamide binds to cannabinoid receptors in mouse brain after i.p. administration at the doses and interval of pretreatment chosen for the present study. Aside from CB₁ receptors, vanilloid VR₁ receptors appear to mediate some of the effects of methanandamide (Ross et al. 2001); however, a VR₁ receptor antagonist did not attenuate the Δ^9 -THC-like discriminative stimulus effects of methanandamide in rats (Solinas et al. 2007). Differences in the effects of methanandamide in rats and mice might be due to metabolic factors. For example, methanandamide might be converted to a metabolite that exerts behavioral activity at non-CB₁ receptors in mice and the same metabolite might be absent or have less prevalent activity in rats.

In summary, this study shows that Δ^9 -THC can be readily trained as a discriminative stimulus in mice, and further shows the pharmacologic profile of this discrimination to be similar to that obtained in other mammalian species inasmuch as other cannabinoid agonists (CP 55940 and WIN 55212-2) substitute for the Δ^9 -THC discriminative stimulus and noncannabinoids do not. The same receptors appear to mediate the discriminative stimulus effects of Δ^9 -THC, CP 55940, and WIN 55212-2 in mice, as evidenced by a similar potency of rimonabant to antagonize the effects of these agonists. Methanandamide or a behaviorally active metabolite of methanandamide, on the other hand, appears to act at receptors in addition to or instead of CB₁ receptors to produce behavioral effects in mice, and it is not

clear whether non-CB₁ receptors also contribute to the behavioral effects of methanandamide in other mammalian species (e.g., primates). The Δ^9 -THC discriminative stimulus in mice appears to have utility not only for predicting cannabis-like activity in humans but also for evaluating molecular mechanisms of cannabinoid activity with genetic manipulations such as inducible knockouts.

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Fig. 1.

Discriminative stimulus effects of cannabinoid agonists and noncannabinoids in C57BL/6J mice discriminating Δ^9 -THC. *Abscissae*: dose in milligram per kilogram body weight; *VEH* vehicle. *Ordinates*: mean ($\pm$ S.E.M.) percentage of Δ^9 -THC-appropriate responding (*top*) and mean ($\pm$ S.E.M.) response rate expressed in responses per second. *Dashed lines* show the 95% confidence limits of the vehicle control response rate determined from ten vehicle training sessions; *symbols* outside the confidence limits are considered significantly different from control

Fig. 2. Effects of Δ^9 -THC, alone (*circles*) and in combination with rimonabant (*left; squares and triangles*) or methanandamide (*right; triangles*), in C57BL/6J mice discriminating Δ^9 -THC. *Circles*, control dose–effect functions for Δ^9 -THC were re-plotted from Fig. 1. See legend of Fig. 1 for further details

Fig. 3. Effects of CP 55940 (*left*) and WIN 55212-2 (*right*), alone (*circles*) and in combination with rimonabant (*squares*), in C57BL/6J mice discriminating Δ^9 -THC. See legend to Fig. 1 for details

Table 1

ED₅₀ values, potency ratios (expressed as most divided by least potent), and 95% confidence limits (95% CL) for drugs that produced Δ⁹-THC-appropriate responding (top), for Δ⁹-THC in combination with rimonabant or methanandamide (middle), and for CP 55940 or WIN 55212-2 in combination with rimonabant (bottom)

Drug(s)	ED ₅₀ (95% CL) in mg/kg	Dose ratio (95% CL) versus Δ ⁹ -THC	Dose ratio (95% CL) versus CP 55940	Dose ratio (95% CL) versus control
Cannabinoid agonists alone				
Δ ⁹ -THC	2.6 (1.8–3.7)	–	82 (45–150) ^a	
CP 55940	0.032 (0.018–0.056)	82 (45–150) ^a	–	
WIN 55212-2	0.45 (0.27–0.74)	5.8 (3.2–10) ^a	14 (7.0–29) ^a	
Δ ⁹ -THC alone and in combination with rimonabant or methanandamide				
Δ ⁹ -THC	2.6 (1.8–3.7)			–
+ 0.32 Rimonabant	5.3 (3.1–8.9)			2.0 (1.1–3.6) ^a
+ 1 Rimonabant	10 (5.2–20)			3.9 (2.0–7.6) ^a
+ 10 Methanandamide	4.5 (2.4–8.6)			1.7 (0.9–3.3)
CP 55940 or WIN 55212-2 alone and in combination with rimonabant				
CP 55940	0.031 (0.017–0.058)			–
+0.32 Rimonabant	0.091 (0.053–0.16)			2.9 (1.4–6.2) ^a
WIN 55212-2	0.35 (0.22–0.56)			–
+0.32 Rimonabant	1.0 (0.59–1.8)			3.0 (1.5–5.7) ^a

^a Dose ratio significantly different from 1, i.e., significant difference in potency