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Differentiation between low- and high-efficacy CB₁ receptor agonists using a drug discrimination protocol for rats

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Abstract

Rationale—The “subjective high” from marijuana ingestion is likely due to Δ^9 -tetrahydrocannabinol (THC) activating the central cannabinoid receptor type 1 (CB₁R) of the endocannabinoid signaling system. THC is a weak partial agonist according to in vitro assays, yet THC mimics the behavioral effects induced by more efficacious cannabinoids. This distinction may be important for understanding similarities and differences in the dose–effect spectra produced by marijuana/THC and designer cannabimimetics (“synthetic marijuana”).

Objective—We evaluated if drug discrimination is able to functionally detect/differentiate between a full, high-efficacy CB₁R agonist [(±)AM5983] and the low-efficacy agonist THC in vivo.

Materials and methods—Rats were trained to discriminate between four different doses of AM5983 (0.10 to 0.56 mg/kg), and vehicle and dose generalization curves were determined for both ligands at all four training doses of AM5983. The high-efficacy WIN55,212-2 and the lower-efficacy (R)-(+)-methanandamide were examined at some AM5983 training conditions. Antagonism tests involved rimonabant and WIN55,212-2 and AM5983. The separate (S)- and (R)-isomers of (±)AM5983 were tested at one AM5983 training dose (0.30 mg/kg). The in vitro cyclic adenosine monophosphate (cAMP) assay examined AM5983 and the known CB₁R agonist CP55,940.

Results—Dose generalization ed_{50} values increased as a function of the training dose of AM5983, but more so for the partial agonists. The order of potency was (R)-isomer > (±)AM5983 > (S)-isomer and AM5983 > WIN55,212-2 > THC > (R)-(+)-methanandamide. Surmountable antagonism of AM5983 and WIN55,212-2 occurred with rimonabant. The cAMP assay confirmed the cannabinergic nature of AM5983 and CP55,940.

Conclusions—Drug discrimination using different training doses of a high-efficacy, full CB₁R agonist differentiated between low- and high-efficacy CB₁R agonists.

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Parts of these data were presented at the annual meeting of the International Cannabinoid Research Society symposium held last June 25 to 29, 2008, at the MacDonald Aviemore Highland Resort, Aviemore, Scotland (Tai et al. 2008).

Keywords

Drug discrimination; Intrinsic activity; Cannabinergics; AM5983; AM5760; AM4971; WIN55,212-2; Δ^9 -Tetrahydrocannabinol; (*R*)-(+)-Methanandamide; Rimonabant; Rats

Introduction

The best known messenger lipids of the endocannabinoid signaling network (ECS) are arachidonylethanolamine (anandamide; AEA) and 2-arachidonoylglycerol (2-AG) acting on two cloned G protein-coupled cannabinoid type 1 and 2 (CB₁ and CB₂) receptors. The cannabis constituent Δ^9 -tetrahydrocannabinol (THC) is an exogenous molecule that binds to these receptors (CB₁R/CB₂R) with moderate affinity and with no apparent receptor subtype selectivity. Other commonly studied exogenous ligands for the ECS include the bicyclic CP55,940, the aminoalkylindole WIN55,212-2 (WIN) and the metabolically stable AEA analog methanandamide (mAEA). Thus, the above ligands act as agonists exhibiting varying degrees of receptor affinity and efficacy for the ECS and are thought to primarily recruit G_{i/o} proteins as the signaling pathway (Mackie 2008). Agonist activity often is described in terms of receptor coupling efficiency, ranging from low to high (intrinsic activity) alternate descriptors are partial and full agonists. THC is considered a weak partial cannabinoid receptor agonist, whereas AEA and mAEA were classified as somewhat more efficacious partial agonists using agonist-stimulated [³⁵S]guanylyl-5'-O-(γ -thio)-triphosphate ([³⁵S]GTP γ S) binding; WIN and CP55,940 acted as full agonists in the [³⁵S]GTP γ S binding assay, with the implication being that not all CB₁R agonists produce the same level of cannabinoid receptor activation. Whether or not a partial agonist can elicit a maximal response will depend on the system requirement(s) (Paronis et al. 2012). For example, only a fractional occupation by WIN of CB₁R in hippocampal slices was accompanied by a marked hypo-locomotion in mice (Gifford et al. 1999), suggesting a large receptor pool (spare receptors) for the ECS circuitry subserving this effect. THC also produced hypo-locomotion in mice, but the phytocannabinoid was less potent than WIN (Compton et al. 1992) and was 13 times less efficacious than WIN in the [³⁵S]GTP γ S binding assay examining rat cellular membranes (Breivogel et al. 1998). Yet, it has been commonly observed that THC mimics the effects of full CB₁R agonists in a variety of in vivo behavioral procedures, including drug discrimination. Thus, THC will substitute completely for rats trained to discriminate between vehicle and the full CB₁R agonists CP55,940, WIN, BAY 38-7271 and AM5983 (De Vry and Jentsch 2002; Järbe et al. 2012; Péroio et al. 1996; Wiley et al. 1995).

There are ways by which the system requirements can be altered to enhance the likelihood of in vivo detection of differences between low- vs. high-efficacy agonists. Drug discrimination studies with dopaminergics, serotonergics, cholinergics, opioids, and benzodiazepines have provided examples suggesting possible in vivo differentiation between high- and low-efficacy agonists. Thus, although a low-efficacy agonist may substitute fully for a high-efficacy agonist, raising the training dose of a high-efficacy, full agonist requires disproportionately raising the test doses of the low-efficacy, partial agonist to achieve substitution (Jutkiewicz et al. 2011; Lelas et al. 2000; Li et al. 2011; Picker and Dykstra 1989; Wolff and Leander 1997; Woolverton and Schuster 1983). Other approaches to alter the pharmacological sensitivity are by reducing the receptor pool through receptor inactivation by an irreversible ligand or through tolerance induction (Bergman et al. 2000; Walker and Young 2002). Examples of reducing the behavioral sensitivity to THC through tolerance induction by repeated administration of THC to mice and monkeys were described by McMahon and colleagues using schedule-controlled responding as well as hypothermia testing in mice (Hrubá et al. 2012; McMahon 2011; Singh et al. 2011).

Current studies are based on previous observations (De Vry and Jentsch 2003) where rats were trained to discriminate between vehicle and two doses of CP55,940 (0.014 and 0.03 mg/kg) and subsequently tested for generalization with CP55,940 and THC. The change of the CP55,940 training dose from 0.014 to 0.03 mg/kg resulted in a threefold shift in the median effective dose (ED)₅₀ value for CP55,940, whereas the change of the ED₅₀ for THC was close to sixfold, indicative of higher intrinsic activity for the CP55,940 induced, as opposed to the THC-induced, discriminative stimulus effects (De Vry and Jentsch 2003). Current studies employed four training doses (range 0.10 to 0.56 mg/kg) of the high-efficacy aminoalkylindole cannabinergic AM5983 and generalization tests were carried out with AM5983 and THC under all four training conditions. The ECS ligands WIN and mAEA were examined under some of the training conditions. Antagonism testing of the two aminoalkylindoles used the CB₁R antagonist/inverse agonist rimonabant, and the results are contrasted with previous studies where rats had been trained to discriminate between different doses of THC and vehicle (Järbe et al. 2011a, 2010). AM5983 is a racemic compound, and therefore, it was important to assess the activity of the separate *S*- and *R*-isomers to determine if both isomers were active, and if so, whether both isomers confer cannabinergic activity or not (Glennon and Young 2011b). Receptor binding affinities (CB₁R/CB₂R) and cyclic adenosine monophosphate (cAMP) assay data for AM5983 are provided and, in the case of the cAMP assay, also compared to the effects of CP55,940.

Materials and methods

Animals

Twenty-four male Sprague–Dawley rats (Taconic Farms, Germantown, NY, USA) were individually housed in a colony room with an average temperature of 20 °C and a 12-h light/dark cycle (lights on at 7:00 etc) with free access to food and water during a 1-week acclimation period before implementing below described protocols (training and testing occurred during the light phase). Bedding was provided twice weekly to each cage using SoftZorb® (Northeastern Products Corp., Warrensburg, NY, USA). Animals were approximately 90 days old at the time of purchase and experimentally naïve at the time of shaping the lever-pressing response (see below). After the acclimation period, the animals were accustomed to being handled and injected during the subsequent week, and access to water was gradually limited to 0.5 h/day. Animals had free access to tap water from Friday afternoon until Sunday. All procedures were approved by the Animal Care and Use Committee of Northeastern University, Boston, MA, USA. The guidelines in “Principles of animal laboratory care” (National Institutes of Health 1996) were followed.

Apparatus

Drug discrimination training and testing utilized eight operant conditioning chambers (Camden Instruments, Ltd., London, UK), enclosed in sound-attenuating cubicles and connected to the IBM-compatible PC via an LVB interface. Behavioral sessions and data collection were conducted using the Med-PC software program (v. 1.16, Med Associates, St. Albans, VT, USA). The chambers were equipped to deliver liquid reinforcement and also had two retractable response levers. The levers were separated by a receptacle in which fluid could be presented by a retractable drinking cup. The reinforcer has 5-s access to sweetened (saccharin 0.1 %) water. The cup delivered 0.1 ml fluid per presentation.

Procedure

Rats were trained to drink from a cup accessible through a receptacle located midway between the two response levers. The animals were shaped by successive approximation to lever press for fluid until they responded 10 times for each reinforcer (fixed ratio 10 (FR-10) schedule of reinforcement). The position of drug-appropriate levers was randomly assigned

among subjects so that it was to the right of the receptacle for half the subjects and left for the other half. Throughout the session, the aforementioned FR-10 schedule of reinforcement was in effect. When the house light was off, and the stimulus lights above the response levers were lit, completion of 10 presses on the active lever resulted in the delivery of one reinforcer. The house light went off simultaneously with a 5-s cup presentation and illumination of the cup by a receptacle light. At the end of the 5-s reinforcement period, the stimulus lights above the levers were lit, the house light was turned off, and the FR-10 schedule of reinforcement contingency reinstated. Sessions ended by all lights in the box being turned off. Post-session supplemental drinking for 0.5 h took place in the afternoon/evening. Pellet food (Harlan Rat Chow[®], no. 2018) was freely available except during the operant conditioning sessions.

Discrimination training and testing—We used four training doses of the full cannabinergic ligand AM5983 (0.10, 0.18, 0.30, and 0.56 mg/kg) in two separate groups of rats. Group 1 ($n = 12$) was initially trained to discriminate between 0.18 mg/kg AM5983 and vehicle for 84 sessions, and the training dose then was lowered to 0.10 mg/kg in successive steps of 0.16, 0.14, and 0.12 mg/kg during 20 additional sessions before dose generalization testing began with AM5983 and THC (group 1a). Once these tests were completed, the animals were retrained with 0.18 mg/kg AM5983 vs. vehicle for 15 sessions before initiating testing with different cannabinergics as well as rimonabant (group 1b). Group 2 ($n = 12$) initially received 79 discrimination sessions using 0.30 mg/kg AM5983 as the training drug dose vs. vehicle before initiating testing with various cannabinergics (group 2a). After completing these tests, the animals were retrained for 18 sessions using 0.56 mg/kg AM5983 vs. vehicle before further testing (group 2b). The training drug was administered i.p. (2 ml/kg), 20 min before session onset. Rats were trained for 20-min sessions, 5 days a week (Monday through Friday). The schedule of drug or vehicle training sessions was nonsystematic, with no more than two sessions under the same training condition occurring consecutively. Which lever was correct depended upon whether AM5983 or its vehicle had been administered before the session. Responses on the inappropriate manipulandum were recorded but had no programmed consequences. To avoid potential inter-animal cues, the daily order of drug or vehicle sessions for animals trained in the same chamber was varied (Extance and Goudie 1981). The criterion for the acquisition of the discrimination was the completion of the first FR on the correct, injection-appropriate lever on at least 8 out of 10 consecutive training sessions. Correct selection was defined as the number of responses for receiving the daily first reward being equal to or less than 14, i.e., not having pressed the state-inappropriate lever more than four times before accumulating 10 responses on the state-appropriate lever.

Upon reaching the above criterion, testing began with different doses and drugs occurring 20 min post-administration (i.p.). Test (T) sessions were conducted on average three times every 2 weeks; on interim days, regular drug (D) or vehicle (V) training sessions of 20-min duration took place. Such scheduling assured that each test was preceded by at least one D and one V maintenance session. Typically, the order of sessions was as follows: D, V, T, V, D (week 1); V, T, V, D, T (week 2); V, D, T, D, V (week 3); and D, T, D, V, T (week 4). Tests were conducted only if responding during the preceding training sessions had been corrected during the initial six FR-10 cycles of the session. If incorrect, animals were retrained for at least three sessions where the number of responses for receiving the daily first reward being equal to or less than 14 before additional testing took place. In test sessions, fluid was delivered for 10 presses on either lever for six reinforcement cycles or until 20 min had elapsed, whichever occurred first. There was one session per test day. Doses and drugs were examined in a mixed order. For each dose tested, the percentage of responding on the drug-appropriate lever was calculated from the ratio of the number of presses on the AM5983-associated lever to the total number of lever presses in a test session

(excluding responding during the time-out periods). Only data for animals receiving at least one reinforcer during the test session were considered for this measure, i.e., animals must have made a minimum of 10 presses on one of the two levers. Additionally, response rate (responses per second) across all subjects was calculated. This measure was based on the performance of all animals, including nonresponders.

cAMP assay

Human embryonic kidney (HEK)-293 cells transfected with rat CB₁R were used with the PerkinElmer's Lance Ultra cAMP kit following the protocol of the manufacturer. Briefly, the assays were carried out in 384-well format using 1,000–1,500 cells/well. The cells were grown for 24 to 48 h, harvested using the nonenzymatic cell dissociation reagent Versene[®] (ethylenediamine tetra-acetic acid), washed twice with Hank's balanced salt solution, and were resuspended in the stimulation buffer. Test compounds in forskolin (2 μM) solution were added to wells containing stimulation buffer followed by the cell suspension. After 30 min of stimulation, the Eu-cAMP tracer and ULight-anti-cAMP working solution were added to the plate and incubated at room temperature for 1 h. The TR-FRET signal was measured using the PerkinElmer Envision microplate reader; for details, see Sink et al. (2008).

Statistics

Nonlinear regression analyses of mean drug discrimination dose generalization data after log-X transformation were performed using the Prism software (v. 5, GraphPad Software, San Diego, CA, USA; www.graphpad.com) to provide estimates of the independent variable when the coordinates of X intersected with $Y=50$ and their 95 % confidence limits [$ED_{50} \pm 95\% \text{ C.L.}$; regression model, log dose vs. response-variable slope ($Y = \text{bottom} + (\text{top} - \text{bottom}) / (1 + 10^{((\log EC_{50} - X) \times \text{Hill slope}))})$) with the top and bottom of the curves constrained to 100 and 0, respectively]. Linear regression was applied to the ED_{50} values for THC and AM5983 as a function of the AM5983 training dose to determine if (a) the slopes differed significantly from zero and if (b) the slopes were significantly different for the two drugs. For the cAMP assay, data were normalized prior to log-X transformation followed by the above nonlinear equation.

Results are presented as the mean ($\pm$ standard error of the mean (SEM)). Significant differences regarding response rate were calculated by means of one-way analyses of variance, followed by Holm–Sidak post hoc statistical test procedure using the SigmaStat software (v. 11, Systat Software, Chicago, IL, USA; www.systat.com). Differences were considered significant at the $P = 0.05$ level.

Drugs

mAEA (AM356) [(5Z,8Z,11Z)-*N*-((*R*)-1-hydroxypropan-2-yl) eicosa-5,8,11,14-tetraenamide; K_i (CB₁)=28 nM; K_i (CB₂)= 867 nM], synthesized according to (Abadji et al. 1994), was dissolved in ethanol appropriate amounts withdrawn, the ethanol evaporated under a stream of nitrogen, and the residue then was dissolved (v/v) in a solution of dimethyl sulfoxide (DMSO), propylene glycol (PG), and Tween-80 (T-80) and stored at -20°C . Shortly before being used, the solute was diluted with normal (0.9 %) saline in a step-wise fashion after the solute had been sonicated for 20–30 min. This procedure was followed for preparing suspensions of all the other drugs as well. The levo-isomer of Δ^9 -THC (6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6H-benzo[*c*]chromen-1-ol), dissolved in ethanol (200 mg/ml), was provided by the National Institute on Drug Abuse (NIDA; Bethesda, MD, USA) and also stored at -20°C until used. Rimobant, as the base (*N*-(piperidin-1-yl)-5-(4-chloro-phenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide), was also provided by NIDA and was stored refrigerated at 4°C before being dissolved in the DMSO/

PG/T-80 (v/v) mixture (final suspension of 2/4 and 4 % for all ligands) before being diluted with saline (90 %). Racemic AM5983 [(1-((1-methylpiperidin-2-yl)methyl)-1*H*-indol-3-yl)(naphthalen-1-yl)methanone, K_i (CB₁)=2.1 nM, K_i (CB₂)= 2.3 nM] as well as the isomers [AM5760 (*S*), K_i (CB₁)=6.3 nM, K_i (CB₂)=14.8 nM and AM4971 (*R*), K_i (CB₁)=0.75 nM, K_i (CB₂)=0.31 nM] were synthesized at the Center for Drug Discovery, Northeastern University, Boston, MA, USA, and K_i values were determined as described before (Lan et al. 1999; Sink et al. 2008). (*R*)-(+)-WIN55,212-2 mesylate [(*R*)-(5-methyl-3-(morpholinomethyl)-2,3-dihydro-[1,4]oxazino[2,3,4-*hi*]indol-6-yl)(naphthalene-1-yl)methanone; K_i (CB₁)=1.9 nM; K_i (CB₂)=0.3 nM; purity 98 %; purchased from Biomol (Plymouth Meeting, PA, USA)]; k_i values for WIN were based on the published literature (Thakur et al. 2005). Doses were administered 2 ml/kg i.p., except for the highest mAEA dose where 3 ml/kg was used. Suspensions were prepared fresh daily just prior to administration. Doses are expressed as the forms indicated. Structures of the cannabinergics used for the discrimination studies are shown in Fig. 1.

Results

cAMP assay

In rat CB₁R-transfected HEK-293 cells, AM5983 acted as an agonist with an half maximal inhibitory concentration (IC₅₀) of 2.85±0.15 (95 % C.L. of ±1.36–5.96) nM with a maximum inhibition of forskolin-stimulated cAMP by 55 % at 100 nM; r^2 =0.83. In comparison, the known CB₁R agonist, CP55,940, inhibited cAMP with an IC₅₀ of 2.84±0.19 (95 % C.L. of ±1.15–7.03) nM, and the maximal inhibition was 58 % at 500 nM; r^2 =0.87 (Fig. 2).

Drug discrimination

Dose generalization and response rate data for AM5983 and THC for rats discriminating between vehicle and 0.10 mg/kg AM5983 are illustrated in Fig. 3, top panel (group 1a). The ED₅₀ (±95 % C.L.) values and goodness of fit (r^2) of the generalization curves here and elsewhere in the “Results” section are listed in Table 1. The ratio of the two ED₅₀ values (THC/AM5983) suggests that AM5983 is approximately 3.4 times more potent than THC and the Hill slopes for the curves were not significantly different [F (1, 4)=5.52; P >0.05]. With one exception (0.03 mg/kg AM5983), response rates associated with these tests did not differ significantly from the vehicle rate (Fig. 3, bottom panel).

Dose generalization and response rate data for four cannabinergics (AM5983, THC, mAEA, and WIN) for rats discriminating between vehicle and 0.18 mg/kg AM5983 are illustrated in Fig. 4 (group 1b). The order of potency was as follows: AM5983 > WIN = THC > mAEA (Fig. 4, top), and the Hill slopes did not differ significantly among the four curves [F (3, 10)=0.45; P >0.05]. Thus, AM5983 was 3 and 3.8 times more potent than WIN and THC, respectively, and 49 times more potent than mAEA. Rates significantly exceeding the vehicle rate occurred in testing with THC (0.056 to 0.18 mg/kg) and WIN (0.1 and 0.3 mg/kg), as illustrated in the lower portion of Fig. 4.

The generalization curves for AM5983 and WIN (group 1b) in the presence of 1 mg/kg rimonabant are illustrated in Fig. 5 (top). Both curves were shifted to the right (5.8 and 5.5 times for AM5983 and WIN, respectively) compared to the curves obtained with the two cannabinergic aminoalkylindoles when examined singly as shown in Fig. 4. Hill slopes did not differ significantly among the four curves [F (3, 10)=2.07; P >0.05]. Response rates associated with the combination tests did not differ significantly from the vehicle rate (Fig. 5, bottom).

Animals trained to discriminate between vehicle and 0.30 mg/kg AM5983 were tested with various doses of AM5983, THC, mAEA, as well as the separate *S*-isomer (AM5760) and *R*-isomer (AM4971) of AM5983 (group 2a). The generalization curves are illustrated in the top portion of Fig. 6, and the associated response rates, in the lower portion of Fig. 6. The order of potency was as follows: AM4971 (*R*-isomer) > AM5983 > AM5760 (*S*-isomer) = THC > mAEA. Thus, the *R*-isomer was 1.9 times more potent than the racemic AM5983 and 12.6 times more potent than the *S*-isomer. Some of these tests were associated with response rates significantly lower than the corresponding vehicle rate as indicated by asterisks in Fig. 6, bottom panel.

Animals trained to discriminate between vehicle and 0.56 mg/kg AM5983 were tested with various doses of AM5983, THC, mAEA, and WIN, and the generalization curves are depicted in the top portion of Fig. 7 (group 2b). The order of potency was the following: AM5983 > WIN > THC > mAEA; Hill slopes did not differ significantly among the curves [$F(3, 11)=3.09$; $P>0.05$]. Thus, AM5983 was approximately 3.2, 6.7, and 75 times more potent than WIN, THC, and mAEA, respectively. Response rates which are significantly lower than the corresponding vehicle rate are indicated by asterisks, as depicted in the lower portion of Fig. 7.

When challenged by 1 mg/kg rimonabant, the two aminoalkylindole curves exhibited rightward shifts indicating surmountable antagonism, as illustrated in the top portion of Fig. 8 (group 2b). Hill slopes did not differ significantly between data sets [$F(3, 11)=0.87$; $P>0.05$], and the order of shifts was 10.7 and 3.9 for AM5983 and WIN, respectively. Response rates, as shown in the lower portion of Fig. 8, did not differ significantly from the corresponding vehicle rate for any of the dose combinations examined.

Combining AM5983 with THC and mAEA in group 2b resulted in essentially no change at the 0.03 mg/kg dose of AM5983 or an enhancement of AM5983 responding at the dose of 0.1 mg/kg AM5983; the doses of THC and mAEA were 0.56 and 5.6 mg/kg, respectively. The mean ($\pm$ SEM) AM5983 appropriate responding was 32 % (16) and 74 % (14) and 38 % (18) and 74 % (16) with the addition of THC and mAEA, respectively ($n=8$ to 9). The effects of the separate drug doses are shown in Fig. 7. There were no significant changes in response rates (range, 0.42 to 0.80 resp./s) compared to vehicle during these tests; the mean ($\pm$ SEM) vehicle rate preceding the combination tests was 0.60 ($\pm$ 0.06) responses per s (data not shown).

Figure 9 graphically displays the changes in the ED₅₀ values (in milligram per kilogram) of the four study cannabinoids as a function of the training dose of AM5983 (the ED₅₀ estimate for mAEA obtained under the 0.30 mg/kg AM5983 training condition was omitted from this display because of the very wide 95 % C.L. interval; see Table 1). Applying a linear regression analysis to the AM5983 and the THC data sets suggested that both slopes differed significantly from zero and that the slopes differed significantly from one another with THC exhibiting the steeper slope of the two CB₁R ligands ($F(1, 4)=22.66$; $P<0.009$).

Discussion

To recapitulate, rats were trained to discriminate between i.p. injected vehicle and four doses (range, 0.10 to 0.56 mg/kg) of the full, high-efficacy CB₁R agonist AM5983, and dose generalization curves were generated for the training drug and the partial, low-efficacy CB₁R agonist THC. Dose generalization curves were also determined for the cannabinoids WIN and mAEA under some of the AM5983 training conditions. A synopsis of the outcome(s) is depicted in graph 9, showing the ED₅₀ value(s) as a function of the AM5983 training dose. Although the ED₅₀ values for all the four cannabinoids increased as the

training dose of AM5983 increased, the magnitude of the increase appeared more pronounced for the presumed partial CB₁R agonists THC and mAEA compared to the two aminoalkylindoles, both of which are considered to be full CB₁R agonists. Additional findings were the following: (1) the (*R*)-isomer of racemic AM5983 was considerably more potent than the (*S*)-isomer, and both isomers confer cannabinergic activity; (2) rimonabant shifted the dose generalization curves of the two aminoalkylindoles to the right in a surmountable manner; and (3) low doses of THC and mAEA either produced no change or acted additively when examined together with low doses of AM5983. The cAMP assay confirmed the cannabinergic profile of AM5983 in vitro.

The change in ED₅₀ values as a function of the training dose is consistent with previous drug discrimination data irrespective of the training drug, species, or procedure used (for reviews, see Colpaert 1999; Glennon and Young 2011a; Järbe 1986; Stolerman et al. 2011). However, of more interest is the observation that the relative change was larger for THC and mAEA compared to the two aminoalkylindoles. Previous research with the other drug classes as discriminative stimuli (see “Introduction”) suggested that intrinsic activity (efficacy) can be a determinant such that the higher the training dose of a full agonist, a disproportionate amount of a low-efficacy, partial agonist is required for substitution. This may be the underlying reason why the potency ratios between THC as well as mAEA displayed more upward shifts compared to the two full CB₁R agonists as a function of the AM5983 training dose in this study. Similarly, rats trained to sequentially discriminate between the CB₁R full agonist CP55,940 at two dose levels (0.014 and 0.03 mg/kg) and vehicle produced dose generalization curves that differed by a factor of 3 (CP55,940) and 5.75 (THC). Thus, the increase in the ED₅₀ value of THC nearly doubled that of CP55,940 as a function of the increased CP55,940 training dose. Although the magnitude of the shifts in the latter study (De Vry and Jentsch 2003) was larger than those in the current study, the direction of the ED₅₀ changes from both studies is in agreement. Thus, the three high-efficacy CB₁R agonists CP55,940, WIN, and AM5983 exhibited lesser relative increases in the ED₅₀ values as a function of an increase in the full agonist training dose compared to the partial, lower-efficacy CB₁R agonist THC and, in the current study, also mAEA. Using a different approach to address the issue of functional in vivo partial vs. full CB₁R agonism, rhesus monkeys discriminating 0.1 mg/kg THC from vehicle disclosed a rightward shift of the THC dose generalization curve after periods of noncontingent exposure to 1 mg/kg THC (“tolerance”); the position of the dose–response curves for CP55,940 and WIN, however, did not change from those obtained prior, as compared to the THC tolerance induction phase (McMahon 2011; see also Hrubá et al. 2012). Similar findings were described also for THC-tolerant monkeys and mice employing different types of operant schedule-maintained responding and, additionally, in mice hypothermia testing (McMahon 2011; Singh et al. 2011).

An alternative explanation is that as the training dose of AM5983 increased, nonoverlapping effect spectra also increased, making the discriminative stimulus effects of THC and mAEA qualitatively less similar compared to those induced by the two aminoalkylindoles, rather than merely requiring the activation of a larger receptor pool. Note that when testing a dose higher than the training dose of AM5983, maximum drug discriminative responding was retained, albeit the response rates decreased. Thus, the higher test doses of AM5983 did not result in considerable changes in the qualitative aspects of the discriminative stimulus effects of the training drug. If the quality of the discriminative stimulus effects had changed, one would have expected a significant downward change in the percentage of drug appropriate responding (Järbe 1989; Järbe and Swedberg 1982; Overton 1988; Stolerman 2011).

Assuming that the relative shifts in the ED₅₀ values primarily reflect the receptor occupancy requirement for eliciting the AM5983-like stimulus effects, one would not have predicted the relatively large potency difference between mAEA and THC in eliciting AM5983 appropriate responding based on the [³⁵S]GTPγS binding assay as both AEA and mAEA were more efficacious than THC in that *in vitro* system (Childers 2006), as well as in their ability to inhibit adenylyl cyclase (Breivogel and Childers 2000). Although published *in vivo* functional potency estimates between THC and mAEA have varied, most drug discrimination data in rats and monkeys are congruent with the present results (Järbe et al. 2000, 2001, 2006, 1998a, 2009, 2010; Kangas et al. 2013; McMahon 2006; Solinas et al. 2007), with exceptions being reported by Alici and Appel (2004) as well as by Burkey and Nation (1997), where the potency differences (THC > mAEA) were considerably less for rats. Apart from potency considerations (Carriero et al. 1998), additional data suggest that even though THC and mAEA cross-generalize in rats (Järbe et al. 2001, 2010), other behavioral end-points suggest that these two CB₁R agonists produce different effect spectra. For example, rather than attenuating mAEA-induced changes of open-field behaviors in rats, rimonabant exaggerated those behaviors (Järbe et al. 2003), which was in contrast to similar behavioral alterations following THC administration (Järbe et al. 2002; see also Järbe et al. 1998b and Adams et al. 1998). Moreover, unlike for rats and monkeys, mAEA did not generalize in C57BL/6J mice trained to discriminate between 10 mg/kg Δ⁹-THC and vehicle, nor did coadministration of mAEA significantly alter the THC dose generalization curve, but the drug combination acted in an additive manner to suppress response rates (McMahon et al. 2008; see also Giuffrida and McMahon 2010; Wiley et al. 2011). In the current study, both mAEA and THC combined with relatively low doses of the training drug resulted in either no change or an increased level of AM5983 appropriate responding without significantly affecting the response rates. Whether higher doses of the partial agonists would have attenuated the discriminative stimulus effects of AM5983 awaits examination. Such a possibility would be in accord with current receptor theory (Kenakin 1993). Regarding the potency difference between THC and mAEA, it is possible that *in vivo* mAEA is not activating the ECS as broadly as might THC. Such an idea may be supported by studies indicating that dual simultaneous inhibition of the two enzymes primarily responsible for the degradation of AEA and 2-AG is required to produce the whole spectrum of THC-like effects *in vivo* using both drug discrimination and the tetrad functional assays (Long et al. 2009). Thus, it appears that 2-AG and AEA exert complimentary but different signaling roles in the ECS (Falenski et al. 2010; Schlosburg et al. 2010). Moreover, mAEA and the related AEA analog AM1346 did not attenuate the discriminative stimulus effects of the training drug rimonabant in a discriminated drinking aversion procedure to the same extent as did THC and AM5983 (Järbe et al. 2011b, 2008), again pointing to differences among CB₁R agonists in their interaction with rimonabant.

The commonly used cannabinergic research ligand WIN substituted for AM5983 but was less potent than AM5983. Coadministration of 1 mg/kg rimonabant with either of the two aminoalkylindoles resulted in rightward shifts of their respective dose curves, signifying a surmountable antagonism and, hence, supporting CB₁R mediation as the primary signaling pathway for their discriminative stimulus effects. These findings are not surprising in view of previous drug discrimination data using cannabinergics as the training drugs (for review, see Järbe 2011). Yet, in our hands, WIN substituted less readily for THC at training doses of 1.8 and 3 mg/kg compared to the aminoalkylindoles AM678 (also known as JWH018; “Spice”) and AM5983. Similarly, the rightward displacements of the generalization curves by rimonabant (1 mg/kg) were more pronounced in the cases of AM678 and AM5983 (as well as for THC) compared to the displacements observed with WIN plus rimonabant combinations in the THC-trained animals (Järbe et al. 2011a, 2001, 2010; see also Stewart and McMahon 2010). Response rates also appeared to be differentially affected in the above tests with the three aminoalkylindoles, i.e., WIN, AM678, and AM5983. Further work is

needed to determine the reason for such varied effects, e.g., if this represents a case of so-called selectively biased trafficking or not (Bosier et al. 2010; Kenakin 2011) or if they are due to off-target effects.

Collectively, current results and previous data (Järbe et al. 2011a, 2010) tentatively imply that for the discriminative stimulus effects of CB₁R agonists, (a) the potency ratio will remain approximately the same between low- and high-efficacy test agonists when subjects are trained with a low-efficacy CB₁R agonist (see also Walker and Young 1993) regarding opioids, and (b) the potency ratio will vary between low- and high-efficacy test agonists as a function of training dose, as in this study, when subjects are trained with a high-efficacy CB₁R agonist (see also Järbe et al. 2012) regarding the high-efficacy CB₁R agonist AM2389 where the potency ratio between AM5983 and AM2389 was around 20 for the two AM5983 training conditions (0.18 and 0.56 mg/kg). These tentative conclusions require further experimental validation to determine their generality and limitations imposed by factors such as, e.g., biased trafficking wherein the ligand/receptor coupling can preferentially activate different proteins that result in different downstream events. Notwithstanding, the distinction between full and partial CB₁R agonism likely is important for understanding similarities and differences in the pharmacology resulting from exposure to so-called designer cannabimimetics (Spice), which customarily have utilized full CB₁R agonists, and marijuana/THC (Järbe and Gifford 2013).

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Fig. 1.
Chemical structures of cannabinoids used in the drug discrimination studies

Fig. 2. Effects of the cannabinoids (±)-AM5983 and (-)-CP55,940 on cAMP levels in HEK-293 cells expressing rat CB₁R. Cells were stimulated in the presence of 2 μ M forskolin (*FSK*) for 30 min. The data are the average of five experiments performed in triplicates for each ligand

Fig. 3.

Generalization test data for AM5983 and Δ^9 -THC (*top*) and the corresponding response rate data (*bottom*) for rats trained to discriminate between vehicle and 0.10 mg/kg AM5983 (group 1a). Data points are based on one to two observations for each rat ($n=12-18$, AM5983, and $n=11-22$, Δ^9 -THC) and were obtained on separate test days. Vehicle response rate (mean $\pm$ SEM) was 0.42 ± 0.05 responses per s (*horizontal lines*), based on the initial six reinforcement cycles of the nondrug maintenance sessions immediately preceding the above tests. Asterisk indicates a significant difference from the vehicle rate at $P < 0.05$ (Holm-Sidak post hoc multiple comparison procedure involving a control mean)

Fig. 4.

Generalization test data for four cannabinoids (*top*) and the corresponding response rate data (*bottom*) for rats trained to discriminate between vehicle and 0.18 mg/kg AM5983 (group 1b). Data points are based on one observation for each rat ($n = 11-12$, AM5983, and $n = 9-12$, Δ^9 -THC; mAEA; and WIN55,212-2) and were obtained on separate test days. Vehicle response rate (mean $\pm$ SEM) was 0.61 ± 0.04 responses per s (*horizontal lines*), based on the initial six reinforcement cycles of the nondrug maintenance sessions immediately preceding the above tests. Asterisk indicates significant difference from the vehicle rate at $P < 0.05$ (Holm–Sidak post hoc multiple comparison procedure involving a control mean)

Fig. 5.

Antagonism test data for AM5983 and WIN55,212-2 (*top*) and the corresponding response rate data (*bottom*) for rats trained to discriminate between vehicle and 0.18 mg/kg AM5983 (group 1b). Tests were conducted 20 min after concurrent i.p. administrations of 1 mg/kg rimonabant (SR) and AM5983 or WIN55,212-2. Data points are based on one observation for each rat ($n = 8-10$ for both drug combinations) and were obtained on separate test days. Vehicle response rate (mean $\pm$ SEM) was 0.50 ± 0.03 responses per s (*horizontal lines*), based on the initial six reinforcement cycles of the nondrug maintenance sessions immediately preceding the above tests. Asterisk indicates a significant difference from the vehicle rate at $P < 0.05$ (Holm-Sidak post hoc multiple comparison procedure involving a control mean)

Fig. 6.

Generalization test data for five cannabinoids (*top*) and the corresponding response rate data (*bottom*) for rats trained to discriminate between vehicle and 0.30 mg/kg AM5983 (group 2a). Data points are based on one observation for each rat ($n = 11-12$, AM5983 and AM4971; $n = 9-11$, Δ^9 -THC; $n = 10-12$, mAEA; and $n = 10-11$, AM5760) and were obtained on separate test days. Vehicle response rate (mean $\pm$ SEM) was 0.46 ± 0.03 responses per s (*horizontal lines*), based on the initial six reinforcement cycles of the nondrug maintenance sessions immediately preceding the above tests. Asterisk indicates a significant difference from the vehicle rate at $P < 0.05$ (Holm-Sidak post hoc multiple comparison procedure involving a control mean)

Fig. 7.

Generalization test data for four cannabinoids (*top*) and the corresponding response rate data (*bottom*) for rats trained to discriminate between vehicle and 0.56 mg/kg AM5983 (group 2b). Data points are based on one observation for each rat ($n=8-12$, AM5983; $n=8-9$, Δ^9 -THC; $n=8-10$, mAEA; and $n=8-9$, WIN55,212-2) and were obtained on separate test days. Vehicle response rate (mean $\pm$ SEM) was 0.84 ± 0.18 responses per s (*horizontal lines*), based on the initial six reinforcement cycles of the nondrug maintenance sessions immediately preceding the above tests. Asterisk indicates a significant difference from the vehicle rate at $P = 0.05$ (Holm-Sidak post hoc multiple comparison procedure involving a control mean)

Fig. 8.

Antagonism test data for AM5983 and WIN55,212-2 (*top*) and the corresponding response rate data (*bottom*) for rats trained to discriminate between vehicle and 0.56 mg/kg AM5983 (group 2b). Tests were conducted 20 min after concurrent i.p. administrations of 1 mg/kg rimonabant (SR) and AM5983 or WIN55,212-2. Data points are based on one observation for each rat ($n = 8-9$ for both drug combinations) and were obtained on separate test days. Vehicle response rate (mean $\pm$ SEM) was 0.57 ± 0.04 responses per s (*horizontal lines*), based on the initial six reinforcement cycles of the nondrug maintenance sessions immediately preceding the above tests. Asterisk indicates a significant difference from the vehicle rate at $P < 0.05$ (Holm-Sidak post hoc multiple comparison procedure involving a control mean)

Fig. 9.

Graphical display of the changes in milligram per kilogram of the ED₅₀ values (Y-axis) for the four study cannabinoids as a function of the training dose (range, 0.10 to 0.56 mg/kg) of AM5983 (X-axis)

Table 1

Summary of substitution test results

Test drug	ED ₅₀ (±95 % C.L.), mg/kg	r ²
Training condition: AM5983 0.10 mg/kg (group 1a)		
AM5983	0.0465 (0.0270–0.0800)	0.929
Δ ⁹ -THC	0.156 (0.1548–0.1575)	1.000
Training condition: AM5983 0.18 mg/kg (group 1b)		
AM5983	0.0693 (0.0413–0.1163)	0.918 ^a
Δ ⁹ -THC	0.2635 (0.1328–0.5230)	0.916 ^a
mAEA	3.365 (2.311–4.899)	0.956
WIN	0.2076 (0.1347–0.3197)	0.981
AM5983 + SR	0.4038 (0.2318–0.7035)	0.973
WIN + SR	1.138 (0.4706–2.752)	0.925
Training condition: AM5983 0.30 mg/kg (group 2a)		
AM5983	0.0780 (0.0693–0.0878)	0.993
Δ ⁹ -THC	0.6811 (0.6717–0.6907)	1.000
mAEA	3.095 (0.1461–65.58)	0.981
AM5760 (<i>S</i>)	0.5204 (0.3940–0.6874)	0.999
AM4971 (<i>R</i>)	0.0413 (0.0298–0.0573)	0.970
Training condition: AM5983 0.56 mg/kg (group 2b)		
AM5983	0.1258 (0.0836–0.1893)	0.967 ^a
Δ ⁹ -THC	0.8438 (0.8133–0.8755)	0.999 ^a
mAEA	9.445 (4.965–17.97)	0.863
WIN	0.3946 (0.2690–0.5790)	0.958
AM5983 + SR	1.352 (0.8885–2.057)	0.962
WIN + SR	1.539 (1.119–2.118)	0.965

SR = rimonabant (1 mg/kg). (*S*) and (*R*) refer to the separate isomers of (±)-AM5983

^aBased on data from Järbe et al. (2012)