Published in final edited form as:

Psychopharmacology (Berl). 2008 July ; 198(4): 467–478. doi:10.1007/s00213-008-1076-0.

Discriminative stimulus effects of the cannabinoid CB₁ receptor antagonist rimonabant in rats

Torbjörn U. C. Järbe

Department of Psychology, Temple University, 265-67 Weiss Hall, 1701 North 13th Street, Philadelphia, PA 19122, USA

Center for Drug Discovery (CDD), Department of Pharmaceutical Sciences, Northeastern University, 116 Mugar Hall, 360 Huntington Ave, Boston, MA 02115, USA t.jarbe@neu.edu

Chen Li

Center for Drug Discovery and Departments of Pharmaceutical Sciences and Molecular and Cell Biology, University of Connecticut, U-92, Storrs, CT 06269, USA

Subramanian K. Vadivel

Center for Drug Discovery and Departments of Pharmaceutical Sciences and Molecular and Cell Biology, University of Connecticut, U-92, Storrs, CT 06269, USA

Center for Drug Discovery (CDD), Department of Pharmaceutical Sciences, Northeastern University, 116 Mugar Hall, 360 Huntington Ave, Boston, MA 02115, USA

Alexandros Makriyannis

Center for Drug Discovery and Departments of Pharmaceutical Sciences and Molecular and Cell Biology, University of Connecticut, U-92, Storrs, CT 06269, USA

Center for Drug Discovery (CDD), Department of Pharmaceutical Sciences, Northeastern University, 116 Mugar Hall, 360 Huntington Ave, Boston, MA 02115, USA

Abstract

Objective—To examine the discriminative stimulus effects of the cannabinoid CB₁ receptor (CB₁R) antagonist/inverse agonist rimonabant (SR141716A) using a discriminated taste aversion (DTA) procedure.

Materials and methods—Groups of rats were trained to discriminate between drug (5.6 or 3 mg/kg) and vehicle in DTA ($t' = 20$ min). The 30-min drinking opportunity after rimonabant pretreatment was followed by injection of lithium chloride (120 mg/kg) in the experimental (EXP) animals. When offered fluid after vehicle pretreatment, EXP animals subsequently were given intraperitoneal saline (NaCl, 10 ml/kg). Post-drinking treatment for controls (CONT) was NaCl irrespective of the pretreatment condition (rimonabant or vehicle). Tests examined other doses and drugs ($t' = 20$ min).

Results—The rimonabant analog AM251 (1 to 5.6 mg/kg) substituted for rimonabant. AM281 also appeared to substitute, but interpretation is complicated by unconditioned effects (drinking suppressed also in the CONT group). The CB₂R antagonists SR144528 (18 and 30 mg/kg), AM630 (1 to 10 mg/kg), and the CB₁R agonist methanandamide (mAEA, 3 and 10 mg/kg) did not substitute. There was a dose-related attenuation of the rimonabant-induced suppression of saccharin drinking when Δ^9 -tetrahydrocannabinol (Δ^9 -THC; 0.3 to 5.6 mg/kg), but not mAEA (1

to 10 mg/kg), was given together with rimonabant (3 mg/kg). Unconditioned effects occurred with the mAEA–rimonabant combination, not evident for combinations of rimonabant and Δ^9 -THC. mAEA (10 mg/kg) plus AM251 (5.6 mg/kg) resulted in strong unconditioned effects.

Conclusion—Rimonabant induces a discriminative stimulus in DTA that continues to show potential for further examination of cannabinoid receptor antagonism.

Keywords

Drug discrimination; Cannabinoid antagonists; Rimonabant–AM251–AM281–SR144528–AM630; Cannabinoid agonists; Δ^9 -THC; Methanandamide–rats

Introduction

Drug discrimination (DD) is a powerful behavioral assay for discerning similarities and differences among central nervous system (CNS) active drugs. In such preparations, the vast majority of which has relied on operant conditioning methodology (<http://www.sspd.org.uk/>); animals or humans are trained to respond differently between the presence and absence of drug effects. When reliably discriminating between the training conditions, other drugs and doses may be evaluated to characterize the receptor mechanisms involved (e.g., Järbe 1989; Solinas et al. 2006). Many such studies have been carried out with various cannabinoid receptor subtype 1 (CB₁R) agonists (see Balster and Prescott 1992; Järbe and Mathis 1992; Wiley 1999 for overviews). Characterization of receptor mechanisms is greatly facilitated by the availability of receptor selective antagonists. The first selective CB₁R antagonist was rimonabant, previously known as SR141716A (Rinaldi-Carmona et al. 1994). However, attempts to establish rimonabant as a discriminative stimulus in pigeons, rats, and monkeys using operant conditioning were unsuccessful (Mansbach et al. 1996; Pério et al. 1996; McMahon 2006b). Another approach for evaluating drug discriminative control is the discriminated taste aversion (DTA; Lucki 1988; Mastropaolo and Riley 1990). Taste aversion presumably results from an association of ingesting novel food or flavored fluid resulting in nausea/malaise (<http://www.ctalearning.com/>). DTA is sensitive to, for example, the discriminative stimulus effects of the opiate antagonist naloxone (Kautz et al. 1989) and the neuro(octa) peptide cholecystokinin (Melton et al. 1993) at low doses (see Riley 1997 for overview). More recently, implementing DTA was successful in establishing rimonabant as a discriminative cue for rats (Järbe et al. 2004). Intriguingly, given the previous failures with operant conditioning methodology (vide supra), rimonabant (5.6 mg/kg) induced stimulus control in our DTA procedure was rapid. A clear-cut separation between drug and vehicle was evident after only 12 training sessions (six drug and six vehicle sessions) in the experimental (EXP) group. In contrast, the control (CONT), nonconditioned group consumed approximately equal amounts of fluid irrespective of rimonabant or vehicle pretreatment. In substitution tests, only the rimonabant derivative AM251 resulted in a drinking pattern similar to that of rimonabant. Other cannabinoid ligands such as Δ^9 -tetrahydrocannabinol (Δ^9 -THC) at doses ranging from 1 to 10 mg/kg and the CB₂R selective antagonist/inverse agonist SR144528 (3 and 10 mg/kg) did not substitute. Various non-cannabinoid compounds (naloxone, flumazenil, morphine, and *d*-amphetamine) likewise did not substitute, indicating pharmacological specificity. Moreover, drinking increased monotonically in EXP when increasing doses of Δ^9 -THC was combined with the rimonabant training dose. At 10 mg/kg Δ^9 -THC, fluid intake was significantly above the level induced by rimonabant alone. Fluid intake essentially was flat and at a level comparable to that induced by rimonabant alone when the CONT animals were tested with this drug combination. We interpreted these results as evidence of CB₁R blockade of the rimonabant-induced discriminative stimulus

control (see also Stevenson et al. 1992). Collectively, these data suggest that CB₁R mediation is important for the discriminative stimulus effects of rimonabant.

The current studies were aimed at replicating and expanding previous findings with the CB₁R antagonist/inverse agonist rimonabant as a discriminative stimulus using DTA as the baseline for DD [employing lithium chloride (LiCl) as the “toxin”]. To that end, we expanded the test dose range of SR144528 (Rinaldi-Carmona et al. 1998) and also included another CB₂R antagonist/inverse agonist AM630 (Pertwee et al. 1995) as well as a third CB₁R antagonist/inverse agonist AM281 (Lan et al. 1999). The CB₁R agonist methanandamide (mAEA; Abadji et al. 1994) was examined alone and in combination with rimonabant and AM251. Our previous findings suggested that such interaction(s) may be different compared to that of, for example, Δ^9 -THC-rimonabant combinations (see Järbe et al. 2006a, b for discussion). We also explored the possibility of using doses lower than 5.6 mg/kg rimonabant to maintain the DD, thereby potentially reducing unconditioned effects associated with the higher training dose. Another aim was to assess the importance of saccharin as a contextual element in our DTA (systematic replication; Sidman 1960). Our previous rimonabant DD study did not use saccharin as part of the conditioning context, but rather, plain tap water was offered during the morning drug/nondrug drinking sessions (Järbe et al. 2004). In all other respects, the two studies were carried out similarly.

Materials and methods

Subjects

Forty-eight male Sprague–Dawley rats (Taconic Farms, Germantown, NY), weighing between 275 and 300 g upon arrival to the laboratory, were used. Rats were individually housed in polycarbonate cages in a colony room with an average temperature of 20°C and a non-reversed 12-h light/dark cycle (lights on at 7 A.M.) at the University's vivarium. The animals had unlimited access to pellet food, but access to fluid was limited (see below). All procedures were approved by the Animal Care and Use Committee of Temple University, Philadelphia, PA, USA. The “Principles of animal laboratory care” (National Institutes of Health 1996, publication NO. 85-23, revised 1996) was followed.

General procedure for DTA acquisition and testing

During the week, before the start of the experiment proper, tap water was freely available. Thereafter, access to fluid (0.1% saccharin) was restricted to 30 min in the morning (Nalgene®, 250-ml plastic bottles with #7 rubber stoppers). In the afternoon, the animals had access to tap water for 30 min in 500-ml glass bottles (consumption not recorded during the afternoon sessions). All experimental procedures were implemented during the morning sessions.

For discrimination training, intraperitoneal (i.p.) administrations of the training drug rimonabant or vehicle (2 ml/kg) occurred 20 min before the 30-min morning saccharin presentation (see Järbe and Lamb 1995). After rimonabant pretreatment, the EXP animals were injected i.p. with 10-ml/kg LiCl (120 mg/kg) immediately after the 30-min morning saccharin presentation (“danger days”), whereas vehicle controls (CONT) received 10-ml/kg of 0.9% saline (NaCl) post-session. The unconditioned group (CONT) is used to assess potential unconditioned from conditioned drug effects relative to the conditioned group (EXP). Drug sessions alternated with sessions, during which, administration of the drug vehicle was followed by a 30-min saccharin presentation and subsequent i.p. injection of 10-ml/kg NaCl for all animals (“safe days”). There was an equal number of drug and nondrug discrimination training sessions during acquisition (see “Results” below). The initial training condition examined was 5.6 mg/kg rimonabant (Study 1; $N=32$; $n=16$). After completing

testing, half the animals from Study 1 were subjected to training with a maintenance dose of 3 mg/kg rimonabant, which, subsequently, was followed by 1.8 mg/kg rimonabant (Study 2; $N=16$; $n=8$). In Study 3, experimentally naive animals were subjected to 3 mg/kg rimonabant and vehicle from the very beginning of training ($N=16$; $n=8$).

Animals were trained and tested Monday through Friday. Once saccharin consumption was reduced for the EXP rats during rimonabant discrimination days, test sessions with different drugs and doses occurred on average three times in a two-week period. Different drug doses were tested in a mixed order. After test sessions, neither LiCl, nor NaCl were given. To maintain the discriminations, training continued on interim days. At least two training sessions under each of the two training conditions followed one test day before new tests resumed. Training, testing, as well as the afternoon drinking sessions took place in the rats' home cages in the vivarium. The animals had free access to tap water offered in glass bottles from Friday afternoon until Sunday afternoon in the vivarium (consumption not recorded).

Drugs

(-)- Δ^9 -THC, dissolved in ethanol (200 mg/ml), was kindly provided by the National Institute on Drug Abuse (NIDA), Bethesda, MD, and stored at -20°C until used. To prepare suspensions, appropriate amounts of Δ^9 -THC were with-drawn, the ethanol evaporated under a stream of nitrogen, the residue then dissolved in a solution of 5% propylene glycol and 5% Tween-80 (cc), and stored at -20°C . Shortly before being used, the solute was diluted with normal (0.9%) saline after the solute had been sonicated for 20–30 min. This was also the procedure for mAEA (i.e., AM356). Rimonabant, as the base [N -(piperidin-1-yl)-5-(4-chloro-phenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; NIDA], was dissolved in a propylene glycol (5%)/Tween-80 (5%) mixture before being diluted with saline. Suspending AM251 [N -(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; 2 ml/kg] and 18 mg/kg SR144528 [N -(1S)-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)pyrazole-3-carboxamide; 3 ml/kg} followed the procedure outlined for rimonabant. AM281 [N -(morpholin-4-yl)-1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-1H-pyrazole-3-carboxamide], AM630 [6-iodo-2-methyl-1-[2-(4-morpholinyl)-ethyl]-1-Hindol-3-yl](4-methoxyphenyl)-methanone] as well as the 30-mg/kg dose of SR144528 were dissolved in pure dimethyl sulfoxide (DMSO) and given i.p., 1 ml/kg, because of solubility problems. For comparison, one set of tests also used pure DMSO to dissolve and administer rimonabant. Ligands pre-fixed AM were synthesized in Dr. A. Makriyannis' laboratory. Saccharin and LiCl were obtained from Sigma (St. Louis, MO, USA). LiCl was dissolved in normal saline. With the exception of LiCl and NaCl, all drugs were administered i.p. 20 min before session onset.

Analysis

Overall analyses were performed with (1) three-way (discrimination acquisition) and (2) two-way (all other data) repeated measures (RM) analysis of variance (ANOVA) using the software packages SPSS (v. 14.0) and SigmaStat (v. 3.1), respectively. When ANOVA was significant ($p < 0.05$), post hoc analyses were carried out with the Sidak test with alpha, two-tailed, set at 0.05 experiment-wise, i.e., for the collection of comparisons. To better meet the assumptions of homogeneity of error variances and normality of treatment-level distributions, all saccharin intake data were square-root transformed for statistical analysis (Kirk 1968). Median effective dose (ED_{50}) estimates ($\pm 95\%$ confidence level [CL]) for the DTA data were calculated with the software package Prism (Graphpad v. 5) after log dose transformation and data normalization (0% to 100%) using equation $Y = 100 / (1 + 10^{-(\text{LogIC}_{50} - X) \times \text{HillSlope}})$.

Results

Study 1: discrimination between 5.6 mg/kg rimonabant and vehicle

Figure 1 shows the average fluid intake in EXP (top a) rats and CONT (bottom b) on consecutive drug (rimonabant, 5.6 mg/kg) and nondrug (vehicle, 2 ml/kg) sessions. Saccharin intake was higher after vehicle compared to drug [$F(1, 30)=300.99$; $p<0.001$ for treatment]; the effect being more marked in EXP relative to CONT [$F(1, 30)=87.62$; $p<0.001$ for treatment $\times$ group]. The effect over time [$F(17, 510)=p<0.001$ for time] is because of a greater separation between D/V sessions among EXP compared to CONT [$F(17, 510)=p<0.001$ for time $\times$ group]. The change over time is also reflected in the treatment $\times$ time interaction [$F(17, 510)=19.48$; $p<0.001$] as well as the three-way ANOVA, i.e., drug or vehicle (D/V), time (Sessions 1–18) and group (EXP/CONT) [$F(17, 510)=14.22$; $p<0.001$]. Results of post hoc analyses are detailed in Fig. 1. Significant separation of fluid intake between vehicle and drug in EXP was evident from the fifth rimonabant session and onward (represented by “x” in Fig. 1a). A significant difference in intake after rimonabant treatment between EXP and CONT was apparent from the fourth drug session and onward (indicated by “*” in Fig. 1a). Furthermore, intake during nondrug sessions tended to be higher in EXP compared to CONT with increasing number of training sessions (symbol “+” in Fig. 1a). Fluid intake tended to be reduced among the CONT after rimonabant compared to vehicle (significant differences indicated by symbol “#” in Fig. 1b), indicating unconditioned effects on fluid consumption.

Figure 2 shows the average ($\pm$ SEM) fluid intake in the EXP (squares) and CONT (hexagons) groups when tested with different doses of rimonabant (Fig. 2a and b), AM281 (Fig. 2c), and SR144528 and AM630 (Fig. 2d).

In tests with rimonabant administered as a suspension (Fig. 2a), ANOVA was significant for group (EXP/CONT) [$F(1, 14)=18.53$; $p<0.001$], dose [$F(4, 56)=37.83$; $p<0.001$], as well as for the group $\times$ dose interaction [$F(4, 56)=5.90$; $p<0.001$]. Selected pair-wise comparisons are indicated in Fig. 2a. EXP consumed less fluid after 5.6 mg/kg rimonabant (D) compared to being nondrugged (V). No significant differences between drug (D) and nondrug (V) emerged for CONT. Compared to CONT, EXP consumed less fluid at higher rimonabant doses, indicative of generalization ($ED_{50}=0.44\pm0.37$ – 0.52 mg/kg; $r^2=0.91$).

In tests with rimonabant administered in pure DMSO (Fig. 2b), ANOVA indicated significance for group (EXP/CONT) [$F(1, 14)=13.46$; $p=0.003$], dose [$F(5, 69)=16.11$; $p<0.001$], as well as for the group $\times$ dose interaction [$F(5, 69)=11.02$; $p<0.001$]. Pair-wise comparisons indicated that EXP consumed less fluid after 5.6 mg/kg rimonabant (D) compared to vehicle (V). No significant differences between drug (D) and vehicle (V) emerged for CONT. Compared to CONT, EXP showed more suppression at higher rimonabant doses, indicating a dose-dependent generalization ($ED_{50}=0.55\pm0.35$ – 0.87 mg/kg; $r^2=0.63$). Tests with DMSO alone (1 ml/kg) resulted in EXP ($n=7$) and CONT ($n=8$) drinking an average ($\pm$ SEM) of $14.0 (\pm 3.06)$ g and $14.5 (\pm 2.15)$ g of fluid, respectively (not shown). Two-way ANOVA comparing fluid intake during vehicle baseline conditions (EXP= 21.38 ± 1.56 g; CONT= 18.19 ± 1.36 g) and DMSO alone suggested reduced intake in the test with DMSO [$F(1, 13)=7.61$; $p=0.016$].

In tests with AM281 (administered in pure DMSO), ANOVA was significant for group (EXP/CONT) [$F(1, 14)=9.96$; $p=0.006$], dose [$F(5, 55)=42.90$; $p<0.001$], as well as for the group $\times$ dose interaction [$F(5, 55)=8.85$; $p<0.001$]; $n=8$ except for 10 mg/kg where $n=4$ (Fig. 2c). The only significant pair-wise comparison for factor dose between the groups occurred with 3 mg/kg AM281. Intake after 0.3 and 1 mg/kg (but not 3 and 10 mg/kg) was significantly different from the intake associated with the training dose condition (D) in

EXP, and all drug conditions resulted in less intake compared to vehicle (V). In CONT, intake was lower after 3 and 10 mg/kg compared to 5.6 mg/kg rimonabant (D), and these doses as well as 1 mg/kg AM281 resulted in less intake relative to vehicle (V). Thus, these tests were associated with considerable unconditioned effects as revealed by the drinking pattern in the CONT group.

In tests with SR144528 (Fig. 2d), ANOVA was significant for dose [$F(3, 33)=39.94$; $p<0.001$] as well as for the interaction [$F(3, 33)=27.78$; $p<0.001$] but not for group [$F(1, 14)=0.001$; $p>0.05$]; $n=8$ except for 30 mg/kg where $n=4$. Pair-wise comparisons revealed significantly higher fluid intake for EXP compared to CONT at the dose of 18 mg/kg SR144528. Furthermore, intake associated with the two doses examined suggested less intake with 30 mg/kg compared to 18 mg/kg SR144528 as well as vehicle (V) in the EXP, but not in the CONT group. In addition, both doses of SR144528 were associated with higher intakes compared to the training dose condition (D) in the EXP group (but not in the CONT group).

In tests with AM630 (Fig. 2d), ANOVA was significant for group (EXP/CONT) [$F(1, 14)=6.05$; $p=0.023$], dose [$F(5, 69)=29.76$; $p<0.001$], as well as for the group $\times$ dose interaction [$F(5, 69)=6.90$; $p<0.001$]. There were no differences in fluid intake on a dose by dose basis between the two groups. In EXP, all AM630 doses resulted in higher intakes compared to that during the drug (rimonabant) training condition (D). With the exception of 3 mg/kg AM630, there were no significant differences regarding intake on vehicle sessions (V) and AM630 in EXP. In CONT, intake was higher after 5.6 and 10 mg/kg AM630 compared to the rimonabant maintenance sessions (D), and none of the test drug doses resulted in an intake that differed significantly from vehicle (V). Thus, the effects of CB₂R blockade induced by SR142845 or AM630 did not mimic the discriminative stimulus effects of rimonabant.

Study 2: discrimination between 3 mg/kg rimonabant and vehicle (retraining)

Figure 3 shows the average ($\pm$ SEM) saccharin intake in EXP rats (graph a; $n=8$) and CONT (graph b; $n=8$) during discrimination reacquisition for consecutive drug (rimonabant, 3 mg/kg) and nondrug (vehicle, 2 ml/kg) drinking sessions in rats previously trained to differentiate 5.6 mg/kg rimonabant from vehicle. Consumption of saccharin fluid tended to be higher after vehicle compared to drug [$F(1, 14)=82.44$; $p<0.001$ for treatment], the effect being more marked in EXP compared to CONT [$F(1, 14)=82.44$; $p<0.001$ for treatment $\times$ group]. The effect over time [$F(8, 112)=3.23$; $p=0.002$ for time] is because of a greater separation between D/V sessions among EXP compared to CONT [$F(8, 112)=2.05$; $p=0.047$ for time $\times$ group]. The change over time is also reflected in the treatment $\times$ time interaction [$F(8, 112)=3.21$; $p<0.003$] as well as the three-way ANOVA, i.e., drug or vehicle (D/V), time (Sessions 1–9) and group (EXP/CONT) [$F(8, 112)=3.21$; $p<0.003$]. The results of the post hoc analyses are detailed in Fig. 3.

Testing different doses of rimonabant after retraining indicated significance for group (EXP/CONT) [$F(1, 14)=13.39$; $p=0.003$], dose [$F(5, 70)=16.69$; $p<0.001$], as well as for the group $\times$ dose interaction [$F(5, 70)=8.12$; $p<0.001$]; data shown in Fig. 4. CONT consumed more fluid overall compared to EXP, and consumption in EXP, but not in CONT, was dose related. The major difference between the groups concerned consumption after treatment with 1.8 mg/kg rimonabant. Thus, the results from EXP were indicative of a dose-dependent generalization ($ED_{50}=1.15\pm 1.05$ – 1.26 mg/kg; $r^2=0.94$) with no indication of unconditioned effects on fluid consumption as evidenced by the drinking pattern of CONT.

Once the above data collection was completed, the EXP ($n=8$) and CONT ($n=8$) rats were retrained with 1.8 mg/kg rimonabant for 12 equally divided alternating drug and vehicle

sessions with an injection pre-session interval of 20 min. The average ($\pm$ SEM) amount of fluid consumed during the last two drug and vehicle sessions were 10.9 ($\pm$ 2.5) and 19.8 ($\pm$ 1.2) g for EXP and 17.4 ($\pm$ 1.1) and 17.2 ($\pm$ 1.2) g for CONT, respectively (not shown). ANOVA suggested significance for dose [$F(1, 14)=7.76$; $p=0.015$] as well as for the interaction dose $\times$ group [$F(1, 14)=5.72$; $p=0.031$]. Pair-wise comparisons suggested significantly less fluid intake during drug (1.8 mg/kg rimonabant) compared to vehicle in EXP as well as compared to drug and vehicle in the CONT group. Hence, some drug (rimonabant) discriminative control was evident with no suggestion of rimonabant-induced unconditioned effects.

Study 3: discrimination between 3 mg/kg rimonabant and vehicle in naïve rats

Figure 5 shows the average ($\pm$ SEM) fluid intake in EXP rats (graph a; $n=8$) and CONT (graph b; $n=8$) during discrimination acquisition for consecutive drug (rimonabant 3 mg/kg) and nondrug (vehicle, 2 ml/kg) drinking sessions in experimentally naïve rats. Consumption of saccharin tended to be higher after vehicle compared to drug [$F(1, 14)=514.93$; $p<0.001$ for treatment], the effect being more marked in EXP compared to CONT [$F(1, 14)=268.89$; $p<0.001$ for treatment $\times$ group]. The effect over time [$F(29, 406)=4.77$; $p<0.001$ for time] is because of a greater separation between D/V sessions among EXP compared to CONT [$F(29, 406)=1.51$; $p=0.047$ for time $\times$ group]. The change over time is also reflected in the treatment $\times$ time interaction [$F(29, 406)=9.48$; $p<0.001$] as well as in the three-way ANOVA, i.e., drug or vehicle (D/V), time (Sessions 1–30) and group (EXP/CONT) [$F(29, 406)=9.33$; $p<0.001$]. The results of selected post hoc analyses are detailed in Fig. 5. Thus, a significant separation of fluid intake between vehicle and drug sessions in the EXP group was evident from the 11th rimonabant session and onward (as represented by the symbol “x” in Fig. 5a). There were few instances of unconditioned effects of rimonabant on fluid intake in the CONT group (symbol “#” in Fig. 5b). Clear-cut discrimination between drug and vehicle in the EXP group is also supported by intakes after drug being consistently lower in EXP compared to CONT from the 11th rimonabant session and onward (indicated by symbol “*” in Fig. 5a).

Figure 6 shows the average ($\pm$ SEM) intake in EXP (squares) and CONT (hexagons) after treatment with different doses of rimonabant (a), AM251 (b), AM281 (c), and mAEA (d).

For rimonabant (Fig. 6a), ANOVA was significant for group (EXP/CONT) [$F(1, 14)=27.49$; $p<0.001$], dose [$F(5, 69)=34.10$; $p<0.001$], as well as for the group $\times$ dose interaction [$F(5, 69)=17.70$; $p<0.001$]. Thus, CONT consumed more fluid overall compared to EXP, and consumption in EXP, but not in CONT, was related to dose; none of the doses in CONT differed significantly from the intake levels associated with the maintenance dose of 3 mg/kg rimonabant (D). The major difference between the groups concerned fluid consumption after 1.8 mg/kg rimonabant, although intake at the level of 1 mg/kg rimonabant also significantly differentiated EXP and CONT. Thus, rimonabant disclosed a dose-related generalization pattern in EXP ($ED_{50}=0.82\pm1.55$ –8.03 mg/kg; $r^2=0.85$) without significantly affecting fluid intake in CONT.

Regarding AM251 (Fig. 6b), ANOVA was significant for group (EXP/CONT) [$F(1, 14)=27.49$; $p<0.001$], dose [$F(5, 69)=34.10$; $p<0.001$], as well as for the group $\times$ dose interaction [$F(5, 69)=17.70$; $p<0.001$]. CONT drank more fluid overall compared to EXP, and intake in EXP, but not in CONT, was related to dose; none of the doses in the CONT group differed significantly from the intake levels associated with the maintenance dose of 3 mg/kg rimonabant (D). Thus, the effects of AM251 on fluid intake generalized to rimonabant without being accompanied by significant changes in fluid intake of the CONT group.

For AM281 (Fig. 6c), ANOVA was significant for group (EXP/CONT) [$F(1, 13)=8.52$; $p=0.012$], dose [$F(5, 64)=42.32$; $p<0.001$], as well as for the group $\times$ dose interaction [$F(5, 64)=13.79$; $p<0.001$]. CONT drank more fluid overall compared to EXP. The major difference between the groups concerned fluid intake after 0.3, 1.0, and 1.8 mg/kg AM281. Intake in EXP, but not in CONT, was related to dose; none of the doses in CONT differed significantly from the intake associated with the maintenance dose of 3 mg/kg rimonabant (D), although intake after 3 mg/kg AM281 was significantly lower than that of vehicle (V) in CONT.

In tests with mAEA alone (Fig. 6d), EXP consumed significantly more fluid compared to CONT at both doses of mAEA (3 and 10 mg/kg), and intake did not differ from the corresponding vehicle (V) condition in either group. This is reflected in ANOVA by significance for dose [$F(3, 36)=29.21$; $p<0.001$], as well as for the group $\times$ dose interaction [$F(3, 36)=23.78$; $p<0.001$], but not for group (EXP/CONT) [$F(1, 12)=1.26$; $p>0.05$].

Figure 7 shows the average ($\pm$ SEM) fluid intake in EXP (squares) and CONT (hexagons) after Δ 9-THC (graph a) and mAEA (graphs b and c) combined with rimonabant and AM251.

Figure 7a shows the results of combining Δ 9-THC (0.3 to 5.6 mg/kg) with 3 mg/kg rimonabant. ANOVA was significant for group (EXP/CONT) [$F(1, 11)=9.82$; $p=0.011$], dose [$F(5, 50)=5.97$; $p<0.001$], as well as for the group $\times$ dose interaction [$F(5, 50)=5.69$; $p<0.001$]. Thus, CONT consumed more fluid than EXP, and intake was dose related in EXP, but not for CONT. EXP and CONT differed at the 0.3 and 1 mg/kg dose levels of Δ 9-THC (but not at the 3 and 5.6 mg/kg dose levels of Δ 9-THC). Moreover, fluid intake after 1, 3, and 5.6 mg/kg Δ 9-THC was significantly higher than the intakes noted with 0.3 mg/kg Δ 9-THC and the rimonabant training condition (D) in EXP. Although treatments with 1 and 3 mg/kg Δ 9-THC resulted in significantly less fluid intake compared to EXP vehicle (V), the difference between 5.6 mg/kg Δ 9-THC and EXP vehicle was not significant. There were no significant differences in intake for CONT. Thus, blockade of the effects of rimonabant by Δ 9-THC in the EXP group occurred without unconditioned effects as revealed by the fluid intake in CONT.

Figure 7b shows the results of combining mAEA (1 to 10 mg/kg) with 3 mg/kg rimonabant. ANOVA was significant for dose [$F(5, 63)=11.36$; $p<0.001$], as well as for the group $\times$ dose interaction [$F(5, 63)=5.65$; $p<0.001$] but not for group (EXP/CONT) [$F(1, 13)=3.49$; $p>0.05$]. There was a significant difference between EXP and CONT at 1 but not at 3, 5.6, and 10 mg/kg mAEA. None of the dose combinations differed significantly from the respective rimonabant maintenance conditions (D). All test doses resulted in less fluid intake in the EXP group compared to vehicle (V); such comparisons were not significant in the CONT group.

Figure 7c shows the results of combining mAEA (3 and 10 mg/kg) with 5.6 mg/kg AM251. ANOVA was significant for group (EXP/CONT) [$F(1, 12)=11.20$; $p=0.006$], dose [$F(3, 36)=57.52$; $p<0.001$], as well as for the group $\times$ dose interaction [$F(3, 36)=5.60$; $p=0.003$]. EXP consumed less fluid than CONT at both dose levels of mAEA (in combination with AM251). Both doses of mAEA resulted in an intake different from the rimonabant training condition (D) in the EXP group; that is, 3 mg/kg resulted in a slightly higher intake, and 10 mg/kg mAEA resulted in a lower intake. In the CONT group, combination of 10 mg/kg mAEA and AM251 resulted in significantly less intake compared to both the rimonabant (D) and the vehicle (V) conditions, implicating strong unconditioned effects for the drug combination.

Discussion

We replicated establishing 5.6 mg/kg rimonabant as a discriminative stimulus, and, as previously reported (Järbe et al. 2004), acquisition of DD was rapid (Study 1). Thus, a clear-cut separation of fluid intake was evident after only five rimonabant sessions and five vehicle sessions in the EXP group. As before, the rimonabant (5.6 mg/kg) DD was associated with unconditioned effects. Thus, in many instances, CONT animals tended to consume less fluid after rimonabant compared to vehicle pretreatment. Reacquisition of 3 mg/kg rimonabant was also rapid for animals transitioned from previously being trained to discriminate between 5.6 mg/kg rimonabant and vehicle (Study 2). Significant discriminative control was also evident for the animals subsequently transitioned from 3 to 1.8 mg/kg rimonabant (Study 2). As expected, naive animals subjected to discrimination training with 3 mg/kg rimonabant from the very beginning of training (Study 3) required more sessions until a stable separation was evident (session block 11) compared to training with 5.6 mg/kg rimonabant (session block 5 in Study 1). As intended, the unconditioned effects of rimonabant on drinking appeared reduced as revealed by the drinking pattern of the corresponding CONT group in Study 3.

Acquisition of rimonabant DD was dose dependent (revealed by the DD acquisition data from Studies 1 and 3), which is consistent with previous demonstrations using different species, tasks, and drugs (e.g., Colpaert et al. 1980; Järbe and Lamb 1999; Järbe and Swedberg 1998; Overton et al. 1986). This was accompanied by significant differences in the ED₅₀ values between the 5.6 and 3 mg/kg rimonabant-training drug-dose generalization gradients. That prior DD training with a higher drug dose, which facilitates subsequent acquisition of a DD based on lower training doses (Studies 2 and 3), has also been demonstrated before with different drugs and species using operant conditioning (e.g., Greenberg et al. 1975; Overton 1979; Swedberg and Järbe 1982). The close similarity in DD acquisition with 5.6 mg/kg rimonabant in this and our previous study (Järbe et al. 2004; see also Järbe and Lamb 1999), where only tap water was offered, suggests that the use of saccharin as a context in DTA is less crucial than previously thought (Mastropalo and Riley 1990). Yet, saccharin (taste) can be a salient target both in its own right as well as when embedded with morphine and/or drinking nozzle type (Järbe and Lamb 1995, 1999; Lamb and Järbe 1997).

AM251 induced a drinking pattern similar to that induced by a rimonabant that is consistent with our previous data (Järbe et al. 2004). AM251 has slightly higher affinity in vitro for the cannabinoid CB₁R sub-type (Lan et al. 1999) but comparatively less brain permeability (J. A. Wood, personal communication) and shares with rimonabant the ability to attenuate responses induced by cannabinoid agonists (summarized by Howlett et al. 2002). For example, in rats trained to discriminate between Δ^9 -THC (1.8 and 3 mg/kg) and vehicle (using operant methodology), AM251 was approximately equipotent with rimonabant in antagonizing in vivo the agonist-induced discriminative response when either antagonist was administered concurrently with the agonist 30 min before test session onset (Järbe et al. 2006a, b; see also McMahon 2006a). AM251 also substituted for rimonabant with similar potency in monkeys trained to discriminate between 1 mg/kg rimonabant (after Δ^9 -THC pretreatment) and vehicle (McMahon 2006b). However, although the two CB₁R ligands share discriminative stimulus effects, their pharmacological profiles may not be identical (e.g., Håjos and Freund 2002; Rodgers et al. 2005; Vlachou et al. 2005; McMahon 2006a; McMahon and Koek 2007).

The strong unconditioned effects (particularly in Study 1 and less in Study 3) in tests with AM281 complicate interpretation of these data. Because of solubility problems, this CB₁R antagonist was administered in pure DMSO. By comparison, test results with rimonabant in

DMSO did not seem to differ substantially from tests where rimonabant was delivered as a suspension (as revealed by similar and nonsignificant ED₅₀ values for the two sets of tests). However, it is possible that enhanced uptake from the peritoneal cavity coupled with the drug's ability to more readily penetrate the blood-brain barrier, resulting in a comparatively rapid increase in brain drug concentrations (Gatley et al. 1998), might explain the unconditioned effects. DMSO alone exerted a modest but statistically significant reduction in fluid intake. Of the three CB₁R antagonists/inverse agonists examined, AM281 has received the least attention. In our hands (Järbe et al. unpublished), AM281 (0.1 to 1 mg/kg in pure DMSO, i.p. 1 ml/kg; $n=8-9$) antagonized the discriminative stimulus effects of the training dose of 1.8 mg/kg Δ^9 -THC (i.p., 2 ml/kg; $t=20$ min) in a two-lever choice operant task. The ED₅₀ value was 0.59 ($\pm 95\%$ CL=0.41–0.84) mg/kg estimated by linear regression (Prism, v. 5). This estimate is similar to those for rimonabant and AM251 in rats discriminating 1.8 mg/kg Δ^9 -THC from vehicle (Järbe et al. 2006a). Compared to the average vehicle rate preceding the tests, 0.56 and 1 mg/kg AM281 (plus 1.8 mg/kg Δ^9 -THC) resulted in a significant response-rate decrease (oneway RM ANOVA followed by Sidak post hoc test involving a control mean; $p < 0.05$).

The drinking pattern resulting from tests with the two CB₂R antagonists/inverse agonists SR144528 and AM630 is consistent with lack of generalization to rimonabant and reinforces the notion that blockade of the two cannabinoid receptor subtypes represents distinct phenomena. Congruently, 1 to 10 mg/kg SR144528 did not block stimulus control by 1.8 mg/kg Δ^9 -THC or 10 mg/kg mAEA using operant methodology in rats (Järbe et al. 2006a; see also Vann et al. 2007). McMahon (2006a) reported similar results for combinations of Δ^9 -THC and SR144528 (3.2 mg/kg, intravenous [i.v.]) in monkeys discriminating between Δ^9 -THC (0.32 mg/kg, i.v.) and vehicle. Conversely, administration of the CB₂R selective agonist AM1241 (3.2 mg/kg, i.v.) did not mimic Δ^9 -THC in the same monkeys (McMahon 2006a). Prominent behavioral effects (e.g., reduced bar pressing) did not occur in these tests, although a downward trend for reduced drinking with 30 mg/kg SR144528 appeared in the current study. Other studies (Malan et al. 2001) show elevated thermal pain thresholds in rats pretreated i.p. with AM1241 (0.33 mg/kg) that were blocked by AM630 (1 mg/kg) but not by AM251 (1 mg/kg). In spite of CB₂R being present in the CNS also under normal, non-pathological conditions (Gong et al. 2006; Van Sickle et al. 2005), above DD data suggest minimal, if any contribution of this cannabinoid receptor subtype for behaviors primarily related to CB₁R activation/inactivation. Although we do not have a ready explanation for the U-shaped dose-response curve in tests with AM630, the results again indicate that a cannabinoid ligand administered in pure DMSO do not necessarily lead to reduced drinking.

When Δ^9 -THC (0.3 to 5.6 mg/kg) was tested together with rimonabant (3 mg/kg), there was a dose-related increase in fluid consumption among the EXP rats in Study 3; fluid intake for the CONT essentially was unchanged. Such an outcome is consistent with the idea that a cannabinoid CB₁R agonist blocks the effects of a cannabinoid CB₁R antagonist (see also McMahon 2006b) in much the same manner by which a CB₁R agonist-induced discriminative stimulus is blocked by rimonabant in previous DD studies using operant methodology (De Vry and Jentsch 2003; Järbe et al. 2001, 2006a, b; Mansbach et al. 1996; McMahon 2006a; Pério et al. 1996; Wiley et al. 1995, 2004) as well as DTA (Järbe et al. 2004). Such antagonism, however, was not clearly extended to test conditions involving combinations of mAEA and (1) rimonabant (3 mg/kg) or (2) AM251 (5.6 mg/kg). These tests were associated with unconditioned effects as reflected by reduced drinking in the CONT group. This was particularly evident in testing with 10 mg/kg mAEA combined with 5.6 mg/kg AM251. Tests with mAEA alone resulted in no change (CONT) or an increase (EXP) in drinking. In several instances, EXP rats tended to consume more fluid during vehicle sessions compared to the CONT group. This may reflect a compensatory behavior in

regard to the small amounts taken during rimonabant conditioning (“danger”) sessions (see also Järbe et al. 2004).

The outcomes of the antagonism tests are reminiscent of previous studies assessing surmountable antagonism of rimonabant by mAEA (Järbe et al. 2001); animals ceased responding before full generalization (80% drug lever responding) might have been achieved (see also Solinas et al. 2007). When agonist dose was held constant, i.e., the mAEA training dose, both rimonabant and AM251 (1 to 3 mg/kg) reduced mAEA (10 mg/kg) appropriate responding to around 25% or lower, suggesting CB₁R mediation for the DD effect; this was accompanied by a downward trend in response rate (Järbe et al. 2006a). Although sharing many effects, it has become increasingly clear that there are also differences in the mode of action between endogenous cannabinoids, such as anandamide and its more stable analog mAEA, and exogenous cannabinoids, such as Δ⁹-THC. Whereas rimonabant attenuated Δ⁹-THC induced open-field effects including circling, behaviors such as ambulation and rearing were dose-dependently augmented by the CB₁R antagonist in mAEA treated rats (Järbe et al. 2002, 2003a). Circling did not occur after mAEA administration (Järbe et al. 1998, 2003a). Similarly, mAEA rate decreases of lever pressing were also augmented by rimonabant, whereas such rate changes induced by Δ⁹-THC essentially were unaltered (Järbe et al. 2003b; see also Baskfield et al. 2004). To account for such findings, it has been suggested that cannabinoid ligands interact with CB₁R/CB₂R through different binding motifs and/or through non-CB₁R/CB₂R cellular targets including additional cannabinoid subtypes or cannabinoid-like receptors and involvement of vanilloid receptor (VR₁) mechanisms (Howlett 2004; Pacher et al. 2006; Oz 2006 for reviews). Of these, vanilloid mechanism(s) do not appear important for cannabinoid DD, as the VR₁ antagonist capsazepine did not antagonize the Δ⁹-THC-like discriminative stimulus effects of anandamide (Solinas et al. 2007), nor did the VR₁ agonist O-1839 substitute for DDs maintained by the CB₁R agonists Δ⁹-THC and O-1812 (ACEA), a CB₁R selective anandamide analog (Wiley et al. 2004). All of the above postulated mechanisms may, however, affect response rate.

In conclusion, coupled with previous data (Järbe et al. 2004), our results suggest that rimonabant induces a discriminative stimulus complex in DTA that has a potential for further examination of cannabinoid receptor antagonism. The reason(s) why our DTA approach was successful in establishing a reliable rimonabant DD whereas the operant approaches did not awaits explanation.

Acknowledgments

We thank NIDA, Bethesda, MD, for supplies of Δ⁹-THC, rimonabant, and SR142845. We thank Ms. M. Harris for technical assistance and Dr. J. Troisi, Ms. S. Tai, and Mr. B. LeMay as well as three anonymous reviewers for comments on the manuscript. Supported by NIDA Grants DA 09064, 00253, and 13429 (Philadelphia, PA), 03801, 9158, 7215, and 00493 (Storrs, CT and Boston, MA).

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Fig. 1.

Acquisition of 5.6 mg/kg rimonabant DTA in experimentally naive rats. **a** Average ($\pm$ SEM) fluid consumption (grams) during drug (rimonabant) and nondrug (vehicle) sessions for the experimental (*EXP*) rats. **b** Average ($\pm$ SEM) fluid consumption (grams) during drug (rimonabant, 5.6 mg/kg) and nondrug (vehicle) sessions for the controls (*CONT*). *EXP* rats discriminated between 5.6 mg/kg rimonabant paired with LiCl (120 mg/kg) vs. vehicle (2 ml/kg) followed by NaCl (10 ml/kg). *CONT* were treated similarly except that NaCl replaced LiCl after rimonabant sessions. Training conditions alternated on a daily basis. Pre-session injections were i.p. 20 min before saccharin fluid presentation, and post-session injections occurred immediately after the 30-min drinking opportunity. Data points represent the average ($\pm$ SEM) of one determination in each rat ($N=32$; $n=16$). *Cross mark* significant difference in fluid intake between drug and nondrug sessions in the *EXP* group; *asterisk* significant difference in fluid intake between drug sessions of *EXP* and *CONT* rats; *plus symbol* significant difference in fluid intake between nondrug (vehicle) sessions of *EXP* and *CONT* rats; *number symbol* significant difference in fluid intake between drug and nondrug sessions in the *CONT* group ($p<0.05$)

Fig. 2.

Average ($\pm$ SEM) fluid intake (grams) in experimental (*EXP*) and control (*CONT*) rats in Study 1 (training drug: rimonabant, 5.6 mg/kg) after treatment with different doses of the CB₁R antagonists/inverse agonists rimonabant (**a** and **b**) and AM281 (**c**), as well as the CB₂R antagonists/inverse agonists SR144528 and AM630 (**d**). Data points above D and V represent the average ($\pm$ SEM) fluid intake during the two (drug and vehicle) training, maintenance sessions immediately preceding testing. In tests with the DMSO vehicle alone (1 ml/kg), *EXP* and *CONT* rats drank an average ($\pm$ SEM) of 14.0 ($\pm$ 3.06) g and 14.5 ($\pm$ 2.15) g of fluid, respectively (*EXP*: *n*=7; *CONT*: *n*=8; not shown graphically). *Asterisk* significant pair-wise difference between *EXP* and the corresponding data point in the *CONT* group; *plus symbol* significantly different from *EXP* drug baseline (D) in the *EXP* group; *cross mark* significantly different from *CONT* drug baseline (D) in the *CONT* group; *number symbol* significantly different from *EXP* vehicle baseline (V) in the *EXP* group; *section sign* significantly different from *CONT* vehicle baseline (V) in the *CONT* group; (*p*<0.05)

Fig. 3.

Reacquisition of 3 mg/kg rimonabant DTA in rats previously trained with 5.6 mg/kg rimonabant. **a** Average ($\pm$ SEM) saccharin fluid consumption (grams) during drug (rimonabant) and nondrug (vehicle) sessions for the experimental (*EXP*) rats. **b** Average ($\pm$ SEM) saccharin fluid consumption (grams) during drug (rimonabant, 3 mg/kg) and nondrug (vehicle) sessions for the controls (*CONT*). Experimental rats discriminated between 3 mg/kg rimonabant paired with LiCl (120 mg/kg) vs. vehicle (2 ml/kg) followed by NaCl (10 ml/kg). *CONT* were treated similarly except that NaCl replaced LiCl after rimonabant sessions. Training conditions alternated on a daily basis. Pre-session injections were i.p. 20 min before fluid presentation, and post-session injections occurred immediately after the 30-min drinking opportunity. Data points represent the average ($\pm$ SEM) of one determination in each rat ($N=16$; $n=8$). *Cross mark* significant difference in fluid intake between drug and nondrug sessions in the *EXP* group; *asterisk* significant difference in fluid intake between drug sessions of *EXP* and *CONT* rats; *plus symbol* significant difference in fluid intake between nondrug (vehicle) sessions of *EXP* and *CONT* rats ($p<0.05$)

Fig. 4.

Average ($\pm$ SEM) saccharin fluid intake (grams) in experimental (EXP) and control (CONT) rats in Study 2 (training drug: rimonabant, 3 mg/kg) after pretreatments with different doses of the CB₁R antagonist rimonabant. Data points above D and V represent the average ($\pm$ SEM) fluid intake during two (one drug and one vehicle) training, maintenance sessions immediately preceding testing. *Asterisk* significant pair-wise difference between EXP and the corresponding data point in the CONT group; *plus symbol* significantly different from EXP drug base-line (D) in the EXP group; *number symbol* significantly different from EXP vehicle base-line (V) in the EXP group; ($p < 0.05$)

Fig. 5.

Acquisition of 3 mg/kg rimonabant DTA in experimentally naive animals. **a** Average ($\pm$ SEM) saccharin fluid consumption (grams) during drug (rimonabant) and nondrug (vehicle) sessions for the experimental (*EXP*) rats. **b** Average ($\pm$ SEM) saccharin fluid consumption (grams) during drug (rimonabant, 3 mg/kg) and nondrug (vehicle) sessions for the controls (*CONT*). Experimental rats discriminated between 3 mg/kg rimonabant paired with LiCl (120 mg/kg) vs. vehicle (2 ml/kg) followed by NaCl (10 ml/kg). *CONT* were treated similarly except that NaCl replaced LiCl after rimonabant sessions. Training conditions alternated on a daily basis. Pre-session injections were i.p. 20 min before fluid presentation, and post-session injections occurred immediately after the 30-min drinking opportunity. Data points represent the average ($\pm$ SEM) of one determination in each rat ($N=16$; $n=8$). *Cross mark* significant difference in fluid intake between drug and nondrug sessions in the *EXP* group; *asterisk* significant difference in fluid intake between drug sessions of *EXP* and *CONT* rats; *plus symbol* significant difference in fluid intake between nondrug (vehicle) sessions of *EXP* and *CONT* rats; *number symbol* significant difference in fluid intake between drug and nondrug sessions in the *CONT* group ($p<0.05$)

Fig. 6.

Average ($\pm$ SEM) saccharin fluid intake (grams) in experimental (*EXP*) and control (*CONT*) rats in Study 3 (training drug: rimonabant, 3 mg/kg) after treatment with different doses of the CB₁R antagonists/inverse agonists rimonabant (**a**), AM251 (**b**), and AM281 (**c**), as well as the CB₁R agonist mAEA (AM356; **d**). Data points above D and V represent the average ($\pm$ SEM) fluid intake during two (drug and vehicle) training, maintenance sessions immediately preceding testing. Asterisk significant pair-wise difference between *EXP* and the corresponding data point in the *CONT* group; plus symbol significantly different from *EXP* drug base-line (D) in the *EXP* group; cross mark significantly different from *CONT* drug baseline (D) in the *CONT* group; number symbol significantly different from *EXP* vehicle baseline (V) in the *EXP* group; section sign significantly different from *CONT* vehicle baseline (V) in the *CONT* group ($p < 0.05$)

Fig. 7.

Average ($\pm$ SEM) saccharin fluid intake (grams) in experimental (*EXP*) and control (*CONT*) rats in Study 3 (training drug: 3 mg/kg rimonabant) after treatment with different doses of the CB₁R agonist Δ^9 -THC combined with 3 mg/kg rimonabant (**a**) as well as the CB₁R agonist mAEA in combination with (1) 3 mg/kg rimonabant (**b**) and (2) 5.6 mg/kg AM251 (**c**). Data points above D and V represent the average ($\pm$ SEM) fluid intake during two (drug and vehicle) training, maintenance sessions immediately preceding testing. Asterisk (*) significant pair-wise difference between *EXP* and the corresponding data point in the *CONT* group; plus symbol (+) significantly different from *EXP* drug baseline (D) in the *EXP* group; cross mark (x) significantly different from *CONT* drug baseline (D) in the *CONT* group; number symbol (#) significantly different from *EXP* vehicle baseline (V) in the *EXP* group; section sign (§) significantly different from *CONT* vehicle baseline (V) in the *CONT* group ($p < 0.05$).