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Pharmacology, Biochemistry and Behavior 83 (2006) 396–402
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Suppression of food intake and food-reinforced behavior produced by the novel CB1 receptor antagonist/inverse agonist AM 1387

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Received 8 December 2005; received in revised form 14 February 2006; accepted 18 February 2006
Available online 6 March 2006

Abstract

Cannabinoid CB1 receptor antagonist/inverse agonists are becoming increasingly recognized for their potential therapeutic utility as appetite suppressants. In the current paper we characterize the biochemical and behavioral effects of AM 1387, which is a novel CB1 antagonist. AM 1387 exhibited binding affinity and selectivity for the CB1 over the CB2 receptor. Moreover, AM 1387 decreased GTPγS (EC_{50}: 22.82 nM) and increased forskolin-stimulated cAMP (EC_{50}: 274.6 nM), as did the CB1 inverse agonist AM 251 (GTPγS EC_{50}: 25.82 nM; cAMP EC_{50}: 363.8 nM), indicating that AM1387 also has inverse agonist properties in vitro. In the behavioral characterization in rats, AM 1387 suppressed lever pressing for food on two operant schedules (fixed-ratio 1 and 5). Timecourse of the effect on fixed-ratio 5 responding was then determined, and the half-life ($t_{1/2}$=4.87 h) was found to be threefold shorter than what has been shown for SR 141716A, and fourfold shorter than AM 251. Finally, AM 1387 was found to suppress food intake using three diets of differing macronutrient composition and palatability. It was concluded that AM 1387 may be a useful tool for examining the effects of CB1 receptor antagonism or inverse agonism on food intake.

Keywords: Cannabinoid; CB1; Inverse agonism; Antagonism; Appetite; Operant; Timecourse; Feeding; Rat

1. Introduction

Cannabinoid antagonists/inverse agonists such as SR 141716A (Rinaldi-Carmona et al., 1994) and AM 251 (Gatley et al., 1996) have been shown repeatedly to suppress food intake in rodents (Arnone et al., 1997; Hildebrandt et al., 2003; McLaughlin et al., 2003). SR 141716A dose-dependently reduced intake of high-sucrose pellets, but not lab chow, in subjects given a choice between these two diets (Arnone et al., 1997). In contrast, Colombo et al. (1998) reported that SR 141716A did reduce lab chow intake among free-fed rats. Anorectic effects were demonstrated following administration of the CB1-selective antagonist, AM 281, but not the CB2-selective antagonist AM 630 (Werner and Koch, 2003). The CB1-selective antagonist AM 251 has been shown to reduce food intake and in both mice (Hildebrandt et al., 2003) and rats (McLaughlin et al., 2003). McLaughlin et al. (2003) compared the effects of SR 141716A and AM 251 on several tasks related to food intake. SR 141716A and AM 251 both reduced food-reinforced operant responding on two different schedules of reinforcement (fixed-ratio 1 and fixed-ratio 5). The fixed-ratio 5 task was also used to determine the duration of the behavioral effects of SR 141716A ($t_{1/2}$=15.1 h) and of AM 251 ($t_{1/2}$=22.0 h). Moreover, both compounds were found to have similar, dose-dependent effects on intake of high-fat and high-carbohydrate diets, as well as standard laboratory chow.

It is important to develop novel CB1 antagonists with various pharmacological characteristics in order to assess their effects on food intake. The present studies investigated the neurochemical and behavioral effects of a novel CB1 antagonist, AM 1387. AM1387 is a pyrazole analog structurally

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0091-3057/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2006.02.022

related to SR141716A. Experiment 1a examined binding of [^{3}H]-CP 55,940 to rat brain and mouse spleen to determine CB1 and CB2 receptor binding. In order to determine if AM 1387 also acts as an inverse agonist at the cellular level, signal transduction effects of this drug were investigated using GTPγS and cAMP assays (Experiments 1b and 1c). Experiments 2–4 assessed the behavioral effects of AM 1387 employing the same food-related tasks previously used to characterize the actions of AM 251 and SR 141716A (McLaughlin et al., 2003). Experiment 2 examined the effects of different doses of AM 1387 on suppression of fixed-ratio 1 (FR1) and fixed-ratio 5 (FR5), respectively. SR 141716A has been shown to affect performance on both ratio schedules similarly when food (McLaughlin et al., 2003) but not heroin (Solinas et al., 2003) was used as the reinforcer. Because duration of action is an important feature for drugs with potential therapeutic utility, Experiment 3 tested the time course of the effect of AM 1387 on reductions of FR5 lever pressing. Based upon the chemical properties of the drug (i.e., the lower lipophilicity relative to SR 141716A, which would alter accumulation in depot fat), it was hypothesized that AM 1387 should have a shorter duration of action than SR 141716A and AM 251. Finally, effects of AM 1387 on intake of diets differing in macronutrient composition were investigated (Experiment 4). We hypothesized that AM 1387 would suppress food intake with all three food types, as has been shown with SR 141716A and AM 251 (McLaughlin et al., 2003).

2. Materials and methods

2.1. Animals

For the behavioral experiments (Experiments 2, 3, and 4), adult male Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing approximately 280–330 g on arrival were used. Rats were housed in pairs, and all studies were conducted in the light portion of the 12 h light–dark cycle (lights on 0800–2000 h). Approximately a week after arrival, rats were food-restricted to 85% of initial body weight, and received all of their daily food allotment during daily experimental sessions, except on weekends and where otherwise noted. Water was freely available in the home cage throughout the experiments. Animal protocols were approved by the University of Connecticut's institutional animal care and use committee, and the methods were in accord with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996).

2.2. Drugs

For Experiment 1 (i.e., in vitro studies), AM 1387 and AM 251 were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 10 mM and stored at −20 °C. For behavioral studies (Experiments 2, 3, and 4), AM 1387 was dissolved in DMSO and Tween-80 (both Fisher, St. Louis, MO), and then the solution was brought to volume with artificial cerebrospinal fluid (aCSF), in a final ratio of 1:2:7. This solution also was used as the vehicle control treatment (1.0 mL/kg) for the behavioral studies. In Experiments 2 and 4, subjects were administered vehicle and various doses of AM 1387 via i.p. injection (see Procedures section for specific doses). In experiment 3, AM 1387 (4.0 mg/kg, i.p.) was injected every other week with varying pretreatment times. Doses were selected based on pilot studies (data not shown).

2.3. Procedures

2.3.1. Experiment 1a—rat brain CB1 and mouse spleen CB2 binding assay

Competitive binding assays for cannabinoid receptors were performed using rat brain (CB1) and mouse spleen (CB2) membranes, which are membrane preparations that have been previously described (Lan et al., 1999). The concentrated stocks (10 mM) were diluted into TME buffer (50 mM Tris–HCl, 3 mM $MgCl_2$, 100 mM NaCl, 0.2 mM EDTA, pH 7.4) with 0.1% BSA, and transferred to 96 well plates containing [^{3}H]-CP 55,940 (specific activity 128 Ci/mmol; NIDA) at a final concentration of 0.76 nM. Non-specific binding was assessed in the presence of 100 nM CP 55,940. Binding was initiated with the addition of the respective membrane suspension (~50 μg membrane protein) followed by incubation at 30 °C with gentle agitation in a shaking water bath for 60 min. Binding was terminated by rapid filtration of the membrane suspension over Unifilter GF/B-96 Well Filter Plates (Packard Instruments) using a Packard Filtermate-196 Cell Harvester. The filter plates were washed four times with ice-cold wash buffer (50 mM Tris–base, 5 mM $MgCl_2$ with 0.5% BSA) and bound radioactivity was determined using a Packard TopCount Scintillation Counter. The results were analyzed using nonlinear regression to determine the actual IC_{50} of the ligand (Prism by GraphPad Software, Inc.) and the K_i values were calculated from the IC_{50} (Cheng and Prusoff, 1973). All data were in duplicate with IC_{50} and K_i values determined from at least three independent experiments.

2.3.2. Experiment 1b—GTPγS binding assay

HEK293 cells expressing hCB1 or hCB2 were harvested, lysed in a cell disruption bomb, centrifuged at 1000×*g* for 10 min and again at 175,000×*g*, and then resuspended in TME buffer with 0.1% BSA to a protein concentration of 0.6 mg/mL. Various concentrations of compound were incubated in 96 well plates containing 24–40 μg protein of the cell membrane preparation, 30 μM GDP, and 0.05 nM GTPγ^{35}S to a final volume of 250 μL. Non-specific binding was assessed in the presence of 10 μM non-radiolabeled GTPγS. After incubating the plate for 2 h at 30 °C, the wells were filtered using GF/B filters and washed with wash buffer. Bound GTPγ^{35}S was determined using a Packard Topcount scintillation counter and results were analyzed using Prism software (GraphPad Software, Inc., San Diego, CA).

2.3.3. Experiment 1c—cAMP assay

Intact HEK293 cells expressing hCB1 or hCB2 were harvested and resuspended in 20 mM HEPES buffer, pH 7.3, containing 0.1 mM RO-20-1724 (4-[(3-butoxy-4-methoxyphenyl)-methyl]-

2-imidazolidinone) and 1 mM IBMX (isobutylmethylxanthine) in DME media with 0.1% BSA to a final concentration of 1×10^6 cells/mL. Cells were incubated for 5 min at 37 °C with forskolin, the HEPES/DME buffer, and various concentrations of compound. The reaction was stopped and the cells lysed by boiling water followed by cooling on ice. Cell debris was removed by centrifugation and the amount of cAMP in the supernatant was determined using the cAMP assay kit from Diagnostic Products Corporation (Los Angeles, CA). EC_{50} was determined by nonlinear regression using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA).

2.3.4. Experiments 2a and 2b—effects of AM 1387 on food-reinforced operant behavior

Operant testing was conducted using Med Associates (St. Albans, VT) operant chambers and interface equipment controlled by custom software written in QBASIC. In Experiment 2a, food-deprived rats ($n=8$) were trained on a FR1 schedule in which each response on a lever was reinforced with a single 45 mg Bioserv pellet (Research Diets, New Brunswick, NJ). Rats were tested in 30-min sessions 5 days per week. When performance stabilized (i.e., consistent performance greater than 250 lever presses per day), drug testing began. A repeated-measures design was used, in which each rat received all treatments, in a counterbalanced order, once per week (i.e., baseline testing 4 days each week, with the 5th day being the drug treatment day). AM 1387 in doses of 1.0, 2.0, 4.0, and 8.0 mg/kg, as well as the vehicle control solution, were administered i.p. 30 min before testing. In Experiment 2b, a separate group of rats ($n=11$) was initially trained on FR1 as described above, and then shifted to a FR5 schedule (i.e., 5 lever presses required for each reinforcer to be received). These rats were trained in 30-min sessions 5 days per week until performance was stable (i.e., 2 weeks with >1000 responses per 30 min). After training, the drug testing phase began. During this phase each rat ($n=11$) received baseline testing 4 days each week, and on the 5th day received a drug treatment. Drug treatment days were conducted once per week, and over the course of the experiment each rat received all drug treatments in a counterbalanced order. Drug treatments consisted of i.p. injections of either vehicle or AM 1387 at doses of 0.25, 0.5, 1.0, 2.0, and 4.0 mg/kg 30 min prior to testing.

2.3.5. Experiment 3—timecourse of behavioral effect of AM 1387

A new group of rats ($n=7$) was trained to achieve stable FR5 performance as described above. Based on the results of the second experiment, a dose of 4.0 mg/kg was selected for assessment of the time course of the drug effect on FR5 performance. Rats were administered AM 1387 either 10, 120, 480, or 1440 min (0.167, 2.0, 8.0, and 24.0 h; McLaughlin et al., 2003) prior to Friday operant sessions in a counterbalanced repeated-measures design, once every 2 weeks. In the intervening weeks, vehicle was given in an identical time regimen. Furthermore, subjects were randomly assigned to begin the 8-week injection schedule either on a drug-injected ($n=4$) or a vehicle-injected ($n=3$) week. Two-way ANOVA revealed no main effect of treatment order (i.e., whether drug or vehicle was given first), and there was no treatment order × preinjection time interaction.

2.3.6. Experiment 4—effects of AM 1387 on intake of diets of differing macronutrients

Rats ($n=30$) were assigned to one of three diet types: high fat (HF; diet # D12451, Research Diets, New Brunswick, NJ; 45% kcal from fat), high carbohydrate (HC; diet # D12450B, Research Diets; 67% kcal from carbohydrate), or lab chow (LC, Prolab 3000 LabDiet, Purina Mills, St. Louis, MO), which they received during 30 min sessions in test chambers (i.e., stainless steel rat cages, hung in cage racks). Rats were food-restricted to 85% of free-feeding body weight and were given 5 habituation sessions in the test chamber with the experimental diet. Following the habituation week, rats were shifted to the feeding procedure that was used throughout the drug test phase (McLaughlin et al., 2003). For this procedure, rats were fed on a weekly schedule in which lab chow was available ad lib in the home cage for 4 consecutive days, after which they were food restricted and then tested for 3 consecutive days in the feeding test chamber with the test diet (i.e., either high fat, high carbohydrate or lab chow) available for 30 min. Each week rats had drug-free days for the first 2 feeding sessions, and the third test day was used as the drug treatment day. Rats were administered vehicle or AM 1387 (doses: 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0 mg/kg) 30 min prior to the third test session each week in a counterbalanced, repeated-measures design (i.e., each rat received all treatments in a counterbalanced order once per week). Food was weighed before and after each session, and sufficient food was provided to allow for ad-lib feeding during the session. Intake was defined as the difference between pre- and post-session food weight, including spillage, which was collected on paper sheets below the wire-mesh floor of the test chamber.

2.4. Data analysis

Binding, GTPγS, and cAMP analyses (Experiment 1) were conducted using curve-fitting programs in GraphPad Prism. Behavioral data were analyzed using SYSTAT 7.0. In Experiment 2, a one-way analysis of variance was used to test for dose effects on number of responses per session. Non-orthogonal planned comparisons were performed comparing each drug dose to vehicle performance (Keppel, 1982). A two-way ANOVA was performed in which treatment (drug vs. vehicle) and pretreatment time were repeated-measures factors. Curve-fitting measures (GraphPad Prism) were also used to determine half-life of behavioral effect of the drug. In experiment 4, effect of drug on each diet type was examined with a diet × dose factorial ANOVA, in which dose was a repeated measure. To account for drastic differences in baseline intake between diets, additional analyses were performed in which data were transformed by expressing each injection session performance as a percentage of each subject's mean performance over the two preceding feeding sessions. A diet × dose factorial ANOVA was then performed on the

percent-transformed data. Binding, GTPγS, and cAMP analyses (Experiment 1) were conducted using curve-fitting programs in GraphPad Prism.

3. Results

3.1. Experiment 1: rat brain CB1 and mouse spleen CB2 binding assays and signal transduction assays

As shown in Table 1, AM 1387 bound with relatively high affinity to CB1 receptors. AM 1387 exhibited over 48-fold selectivity for CB1 versus CB2 receptors. In Experiment 1b, AM 1387 decreased GTP binding. In addition, AM 1387 enhanced forskolin-stimulated cAMP accumulation in Experiment 1c. These effects were similar to those found with the CB1-selective inverse agonist AM 251. For direct comparison of both compounds see Table 1.

3.2. Experiment 2: effects of AM 1387 on food-reinforced operant behavior

Analysis of FR1 lever pressing data in Experiment 2a revealed a significant suppressive effect of AM 1387 treatment on total number of responses (Fig. 1A; $F(4, 28)=5.87$, $p<.001$). In Experiment 2b (FR5 lever pressing) AM 1387 also produced a significant suppression of overall number of lever presses (Fig. 1B; $F(5, 50)=10.67$, $p<.001$). With the FR5 schedule, doses of 1.0 mg/kg and higher produced significant suppression of responding compared to vehicle, while only the 4.0 and 8.0 mg/kg doses were significantly different from vehicle on the FR1 schedule.

3.3. Experiment 3: timecourse of behavioral effect of AM 1387

In the timecourse experiment (Fig. 2), ANOVA revealed an effect of treatment ($F(1, 6)=12.53$, $p<.05$) but not of pretreatment time. There was a significant interaction between pretreatment time and treatment condition ($F(3, 18)=3.89$, $p<.005$), indicating that the suppressive effects of AM 1387 on FR5 lever pressing were different depending upon the pretreatment time. Half-life of the effect of AM 1387 was determined to be 4.87 h using the curve-fitting analysis in GraphPad Prism.

Table 1
Comparison of AM 1387 to AM 251. Values for K_i and EC_{50} are in nM (shown with 95% confidence intervals)

Assay		AM 1387	AM251
CB1 binding	K_i (nM)	30.78 (25.29, 37.46)	4.785 (3.922, 5.838)
	R^2	0.9826	0.9799
CB2 binding	K_i (nM)	1481 (887.3, 2473)	2058 (1689, 2508)
	R^2	0.9213	0.9802
GTPγ^{35}S	EC_{50} (nM)	22.82 (1.791, 290.8)	25.82 (6.527, 102.1)
	R^2	0.3563	0.6338
	% decrease	14.39	44.25
cAMP	EC_{50} (nM)	274.6 (53.55, 1409)	363.8 (58.43, 2265)
	R^2	0.6305	0.7612
	% increase	96.5	77.5

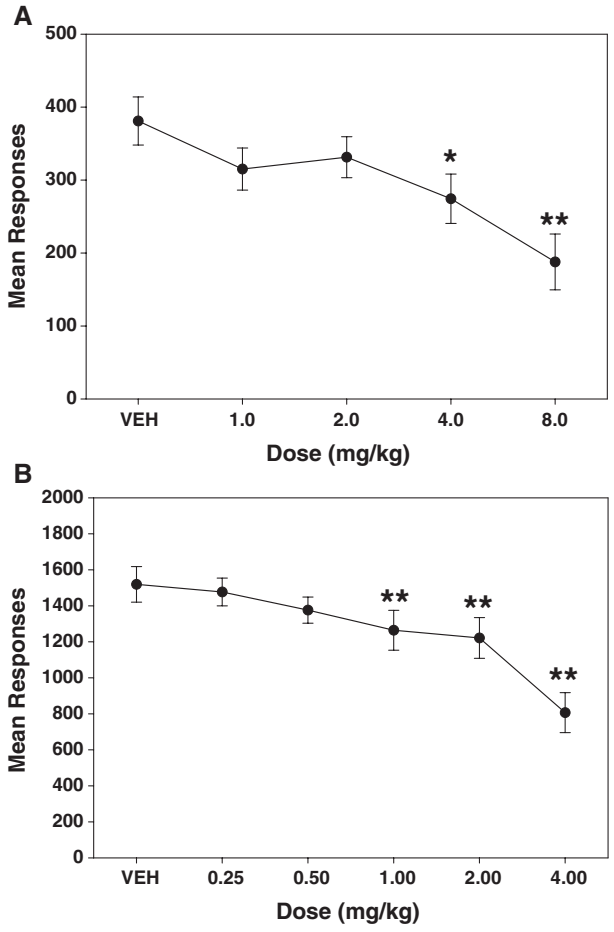

Fig. 1. Mean (±S.E.M.) number of lever presses for rats that received injections of vehicle (VEH) or various doses of AM 1387. (A) FR1 Schedule. (B) FR5 Schedule. *$p<.05$; **$p<.01$ different from vehicle.

3.4. Experiment 4: effects of AM 1387 on intake of diets of differing macronutrients

As seen in Fig. 3, there was an overall significant effect of AM 1387 on food intake ($F(6, 162)=26.49$, $p<.001$). A

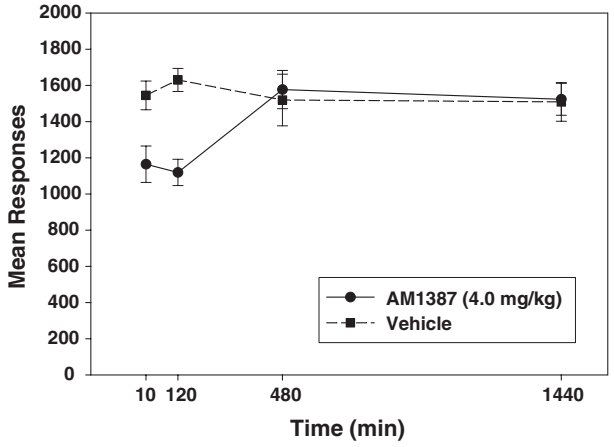

Fig. 2. Mean (±S.E.M.) number of lever presses (FR5 schedule) after treatment with vehicle or 4.0 mg/kg AM 1387 using various pretreatment times (10 min, 2 h, 8 h, and 24 h). $t_{1/2}=4.87$ h.

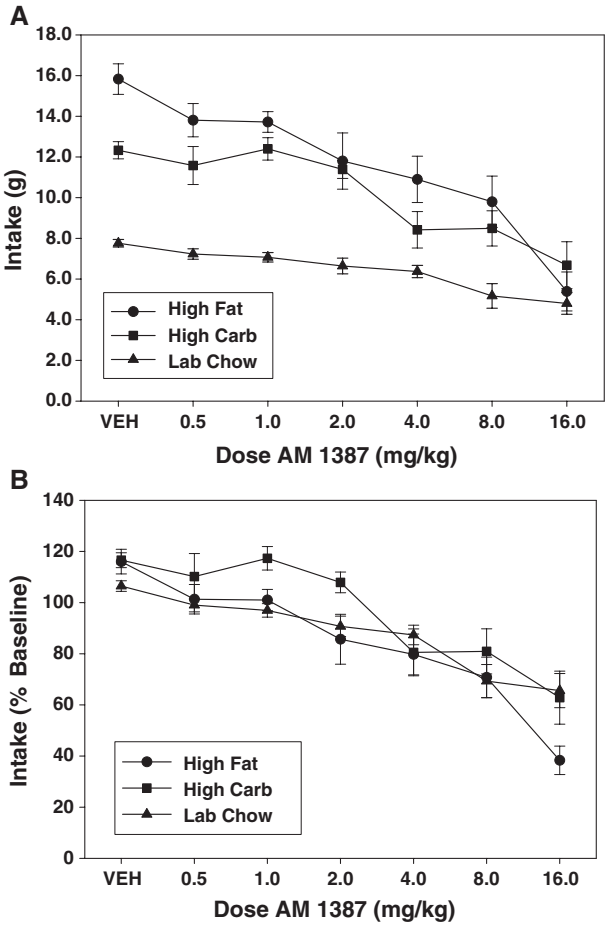

Fig. 3. Intake of three different types of foods in 30 min sessions after injections of vehicle (VEH) or various doses of AM 1387. (A) Mean (±S.E.M.) raw intake (expressed as gram quantity) of three different types of food. (B) Mean (±S.E.M.) food intake (expressed as percent of baseline) of three different types of food.

significant difference in the diet factor also was found ($F(2, 27) = 55.11$, $p < .001$). As the diet × dose interaction was significant ($F(12, 162) = 3.01$, $p < .005$), separate one-way ANOVAs were performed on each diet type. It was determined that AM 1387 dose-dependently decreased intake in all groups (LC: ($F(6, 54) = 11.25$, $p < .001$; HC: $F(6, 54) = 7.57$, $p < .001$; HF: $F(6, 54) = 13.36$, $p < .001$). As indicated by the significant main effect of diet (see also the vehicle injection data in Fig. 3), a sizeable difference in baseline intake existed across the three foods. Thus, it is possible that the dose × diet interaction reported above was largely due to the fact that baseline chow intake is much lower than consumption of the other two foods. To account for these differences in baseline, intake data were transformed into a percentage of each subject's baseline performance. Dose × diet factorial ANOVA on the transformed data revealed a main effect of dose ($F(6, 162) = 1.12$, $p < .001$) and of diet ($F(2, 27) = 7.38$, $p < .005$), but no significant interaction ($F(12, 162) = 1.43$, $p > .15$).

4. Discussion

AM 1387 is a novel CB1 receptor antagonist with the biochemical characteristics of an inverse agonist. Receptor binding studies involving displacement of radiolabeled CP 55,940 in rat brain and mouse spleen revealed that AM 1387 showed over a 48-fold binding selectivity for CB1 over CB2 receptors. These results indicate that AM 1387 is a selective CB1 ligand. In the present studies, AM 1387 also decreased GTPγS binding and increased cAMP production. These results demonstrate that AM 1387 is not a CB1 agonist, and in fact this drug has the biochemical characteristics of a CB1 inverse agonist. In this regard, AM 1387 appears to have biochemical properties similar to other CB1 antagonists/inverse agonists such as SR 141716A and AM 251 (Landsman et al., 1997; Mato et al., 2002; Pertwee, 2005).

In addition to these biochemical results, AM 1387 also was found to suppress food intake and food-reinforced behavior in rats. AM 1387 decreased operant responding for food reinforcement using two schedules of reinforcement, FR1 and FR5, which is similar to the pattern of effects previously reported for SR 141716A and AM 251 (McLaughlin et al., 2003). The half-life of the effect of AM 1387 on FR5 responding was found to be 4.87 h, which is considerably shorter than the half-life previous shown for SR 141716A (15.1 h) and AM 251 (22.0 h; McLaughlin et al., 2003). Thus, in comparison to SR 141716A and AM 251, AM 1387 produced a much shorter-lasting effect. Time of onset was similar for all three drugs (i.e., approximately 10 min after injection; see also McLaughlin et al., 2003). In view of the possible therapeutic utility of these drugs for appetite suppression, it is worth noting that duration of action is an important dimension of drug activity. Although there may be circumstances in which a longer-acting drug is preferred, it may also be desirable under some therapeutic circumstances to employ drugs with limited duration of action.

In addition to suppressing food-reinforced operant responding, AM 1387 also suppressed intake of three types of food with varying macronutrient composition and palatability. At first glance, AM 1387 appeared to produce one effect on food intake that was notably different from SR 141716A or AM 251. In previous studies using similar methods (McLaughlin et al., 2003), it was shown that SR 141716A and AM 251 did not produce a significant interaction between drug treatment and type of diet, in terms of gram quantity of food consumed. However, in the present study AM 1387 did produce a significant interaction drug treatment × diet type interaction (Experiment 4; Fig. 3A) when data were analyzed in terms of raw intake (i.e., in grams). The issue of whether or not the effects of CB1 antagonism on food intake interact with the palatability or nutritional content of the food is a subject of great interest (e.g., Ward and Dykstra, 2005). In some studies, SR 141716A was shown to have a greater effect on intake of more palatable versus less palatable foods (Arnone et al., 1997; Simiand et al., 1998). Thus, it could be argued that the effects of AM 1387 on gram quantity of food consumed that were seen in the present study may reflect a greater sensitivity of this drug for suppressing intake of high palatability or high-fat foods. On the other hand, CB1 antagonists may suppress intake of more palatable foods to a

larger extent than less palatable foods in some studies because of a possible limit in feeding suppression efficacy that may produce a "floor effect" when measuring intake (McLaughlin et al., 2003). This would mean that, as dose of the CB1 antagonist gets higher, intake of all three foods would converge towards a common level, but the absolute effect on chow intake would be smaller because baseline intake of this food is closer to the floor level. Such an observation is consistent with the data obtained in the present studies (Fig. 3A). In considering the significance of the present effects, it is important to emphasize that, despite the significant interaction between drug treatment and food type that was observed, there was still a significant overall suppression of lab chow, which was the least palatable food used in the present study. This finding is consistent with several other studies that have reported suppressive effects of CB1 antagonists on lab chow (Rowland et al., 2001; Colombo et al., 1998; McLaughlin et al., 2003; Verty et al., 2004). Moreover, no drug treatment × food type interaction was found when the data were expressed as percent change from baseline (Fig. 3B). Taken together, these analyses suggest that reported interactions with palatability may be due to differences in baseline intake, or scaling differences, rather than being a specific property of CB1 receptor antagonism. Along with the results reported in a recent study (McLaughlin et al., 2003), in which AM251 and SR141716A produced suppressive effects on feeding that did not interact with the palatability of the food being consumed, the present results indicate that CB1 receptor antagonists/inverse agonists do not suppress feeding in a manner that is highly selective for the intake of more palatable diets.

Future studies should investigate the site of action for the anorectic effects of CB1 antagonists. While some researchers have found a peripheral, but not central site of action for SR 141716A (Gómez et al., 2002), others have found decreased intake with intracranial injections of the CB1 antagonist/inverse agonist AM 281 (Werner and Koch, 2003). In fact, there may be more than one food-related effect being produced by CB1 antagonism, which could suggest multiple sites of action for CB1 antagonists such as AM 1387. Furthermore, while AM 1387 has been shown to decrease food consumption and food-maintained responding, a selective effect on hunger or satiety mechanisms has not been demonstrated, and an increase in food aversion and avoidance has been found recently at anorectic doses of AM 251 (McLaughlin et al., 2005). Additional studies should investigate food handling, taste aversion, and satiety-related behavior (i.e., the Behavioral Satiety Sequence; Halford et al., 1998) that may appear following AM 1387 administration. The development of tolerance with repeated administration should also be assessed. Tolerance develops rapidly to the suppressive effect of SR 141716A (Colombo et al., 1998) and AM 251 (Hildebrandt et al., 2003). As these compounds suppress food intake 24 h following administration, carryover effects of the drug or chronic blockade of CB1 receptors may contribute to the formation of tolerance. It is possible that with a shorter duration of effect, chronic daily administration of AM 1387 would produce effects more resistant to tolerance than treatment with longer acting compounds.

Acknowledgements

This work was supported by grants to J.S. and A.M. from NIH/NIDA.

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