Pharmacology, Biochemistry and Behavior 95 (2010) 443–448

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

AM 251 differentially effects food-maintained responding depending on food palatability

Suzanne M. Droste [a], Samantha K. Saland [a,b], Emily K. Schlitter [a], Joshua S. Rodefer [a,b,*]

[a] Department of Psychology, Florida State University, USA
[b] Program in Neuroscience, Florida State University, USA

ARTICLE INFO

Article history:
Received 15 January 2010
Received in revised form 1 March 2010
Accepted 13 March 2010
Available online 21 March 2010

Keywords:
AM 251
Cannabinoid
Food intake
Food reinforcement
Motivation
Obesity
Progressive-ratio schedule
Rat

ABSTRACT

Ligands functioning as antagonists and inverse agonists at the cannabinoid CB_1-receptor (e.g., AM 251, AM 281, and rimonabant (previously identified as SR141716)) have been demonstrated to have effects on satiety, consumption of, and the motivation to work for, or obtain food. These represent behavioral effects that may also be linked to characteristics such as food palatability or motivation to obtain food. Given the recent removal of rimonabant from clinical trials, a thorough characterization of ingestive behaviors that are associated with other likely candidate drugs is warranted. In the present study, normal weight male Long Evans rats were trained to respond for grain or chocolate-flavored food pellets under progressive-ratio schedules of reinforcement. Rats received acute injections of the CB_1 receptor antagonist AM 251 (0.3–3.0 mg/kg) or vehicle prior to daily testing sessions. Administration of AM 251 produced significant dose-dependent reductions in responding for, deliveries of, and break points (BP) associated with chocolate-flavored but not grain pellets. These data add to the literature demonstrating the ability of CB_1 antagonists to selectively reduce motivation to obtain highly palatable reinforcers.

1. Introduction

The association of marijuana (*Cannabis sativa*) with altered levels of food intake and hunger has existed for hundreds of years (Gaoni & Mechoulam, 1964), but only recently have these behaviors been attributed to Δ^9-THC (Gaetani et al., 2008). More recent investigations have confirmed the complex role that endogenous cannabinoids (e.g., anandamide) and other exogenous and synthetic cannabinoid compounds (e.g., WIN 55,212-2) have on these feeding-related behaviors. Systematic clinical (Greenberg et al., 1976; Halikas et al., 1985) and preclinical studies (cf. Di Marzo and Matias, 2005) have demonstrated the role of cannabinoids in food intake and energy balance. The ability of CB_1 agonists and antagonists to modulate food intake has made them attractive targets for drug development both for conditions in which food intake is increased (e.g., anorexia nervosa, wasting syndrome) (Elamin et al., 2006; Gaetani et al., 2008; Haney et al., 2007) or decreased (e.g., obesity, binge eating disorder, bulimia nervosa) (Cota et al., 2003; Gaetani et al., 2008; Huang and Glass, 2008; Pertwee, 2006). However, the exact mechanisms involved for these effects are unclear. Cannabinoid compounds seem to produce changes in multiple behaviors that implicate different pathways and circuitry relevant for feeding and food-related behaviors. Specifically, food intake changes following administration of CB_1 agonists in a dose-dependent manner, but higher doses do not produce hyperphagia (Salamone et al., 2007; Sofia and Knobloch, 1976). Cannabinoid administration alters both body weight (Tallet et al., 2008) and macronutrient intake (Escartín-Pérez et al., 2009; Mathes et al., 2008) of rodents. In addition, variations in meal size, frequency or duration (Hao et al., 2000; Tallet et al., 2008), as well as satiety (Escartín-Pérez et al., 2009; Hodge et al., 2008) and the reinforcing and motivational properties of food (Maccioni et al., 2008; Mathes et al., 2008; Rasmussen and Huskinson, 2008) have all been associated with altered levels of cannabinoid-mediated activity.

Rimonabant (SR 141716) had been a leading drug candidate for investigation until its recent removal from clinical trials. However, there are other cannabinoid compounds that may be effective in altering reinforcing and motivational properties without the same undesirable behavioral effects. AM 251 is a recently developed synthetic CB_1 antagonist that produces effects similar to those of rimonabant on feeding-related and food-reinforced behaviors (Hodge et al., 2008; McLaughlin et al., 2003, 2005; Tallet et al., 2008). Reports have demonstrated that administration of AM 251 and other CB_1 antagonists result in mostly consistent behavioral effects: decreased food-maintained responding under fixed-ratio (FR) schedules of reinforcement (Maccioni et al., 2008) and suppressed intake of foods with differing macronutrient compositions (Arnone et al., 1997; Colombo et al., 1998; McLaughlin et al., 2003), including those highly palatable foods and natural rewards that are sweet and/or fatty. Yet there is some suggestion that some drugs,

* Corresponding author. Department of Psychology, Florida State University, 1107 W. Call Street, Tallahassee FL 32306 USA. Tel.: +1 850 645 9142; fax: +1 850 644 7739.
E-mail address: rodefer@neuro.fsu.edu (J.S. Rodefer).

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.03.005

like AM 251 and rimonabant, may have more complex activity within the cannabinoid system, functioning both as CB_1 receptor antagonists but also having inverse agonist effects on CB_1 receptors (Salamone et al., 2007; Sink et al., 2009). These different activities might suggest that compounds acting as CB_1 neutral antagonists might lack some specific behavioral effects (e.g., food aversion) associated with CB_1 inverse agonists. In addition there has been recent work suggesting that CB_1 antagonists like AM 251 may also impact measures of drug-reinforcement and drug-related rewards and cues (Budzynska et al., 2009; Di Chiara et al., 2003; Xi et al., 2008). These data suggest that CB_1 antagonists like AM 251 may produce some behavioral effects that are consistent with altered dopaminergic reward circuitry and activity (O'Neill et al., 2009), thus, AM 251 may impact highly palatable food reinforcement through similar means. However, recent reports found that AM 251 was effective in attenuating reinforcing effects of cocaine whereas SR 141716 was not, thus suggesting AM 251 may have more robust behavioral activity in procedures dealing with motivation and potent reinforcers (Xi et al., 2008).

The present experiments were designed to study the effects of the CB_1 antagonist AM 251 on food intake under progressive-ratio schedules of reinforcement using foods that differed in initial levels of palatability. Progressive-ratio (PR) schedules (Hodos, 1961) require subjects to emit systematically increasing numbers of responses to receive successive reinforcers. At some point, subjects cease responding due to the increased requirements to obtain reinforcement. The last completed ratio is termed the break point (BP). These operant schedules of reinforcement can generate large amounts of behavior with a limited amount of food deliveries, thereby minimizing any satiating effect of the food. It has been suggested that PR schedules may provide a measure of relative reinforcing efficacy or assess motivation to obtain reinforcers (Rasmussen and Huskinson, 2008; Rodefer and Carroll, 1997). Progressive-ratio schedules have been documented to be robust in their sensitivity to detect motivational differences in both drug- and food-maintained responding (Hodos, 1961; Rasmussen and Huskinson, 2008; Rodefer and Carroll, 1996, 1997; Xi et al., 2008). We hypothesized that rats would respond more for chocolate-flavored food pellets versus grain pellets, indicating that chocolate-flavored pellets were more palatable and reinforcing than grain based. Moreover, we hypothesized that AM 251 would selectively impact motivation to respond for the highly palatable chocolate-flavored pellets more than grain pellets, and that this would subsequently result in fewer food deliveries and lower BP values.

2. Methods

2.1. Subjects

Fifteen male Long Evans rats were purchased from Harlan (Dublin VA) and arrived in the lab at approximately 60 days of age. Body weights of rats ranged from about 225 g at the beginning of the experiments to about 325–350 g at the conclusion. Rats were housed individually and maintained in polycarbonate shoebox-style cages. Sentinel animals were monitored for pathogens and none were detected during the course of this study. The colony room was temperature and humidity controlled with a 12 h light:dark cycle (lights on 07.00–19.00); all experiments were done in the light part of the cycle. All rats were initially trained to lever press for food pellets in operant chambers. During initial operant training, animals were slightly food restricted (~90–95% of free feeding weight) by manipulating post-session daily food allotments (Purina Chow #5001). This was to ensure consistent levels of behavioral performance while learning the food-reinforced task. Subsequently, animals were maintained at about 100% of their free feeding weight. In addition, the normal animal chow diet was supplemented with both varieties of the 45 mg pellets that were dispensed in the operant chambers so that the animals could experience and obtain those foods outside of the daily testing environment. All animals had free access to water when not in testing chambers. All procedures described followed the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee and the facilities were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

2.2. Apparatus

Rats were trained and tested in standard operant chambers (Med Associates, Georgia, VT; internal dimensions of $20 \times 21 \times 28$ cm) 7 days a week using a progressive-ratio (PR) schedule (1, 2, 4, 6, 9, 12, 15, 20, 25, 32, etc.) of food reinforcement (Richardson and Roberts, 1996). One wall housed two retractable response levers and animals were randomly assigned to learn to lever press the right or left lever for food. Responses on the inactive lever had no programmed consequences and responses that occurred during time out periods were not counted. Standard 45 mg grain- or chocolate-flavored pellets (Dustless Precision Pellets; Bioserv; Frenchtown NJ) served as food reinforcement in the experiments and were delivered into a pellet tray recessed into the same wall as the levers. The pellets were comparable, except for taste, and did not differ markedly in their macronutrient content (Grain: 21% protein, 55% carbohydrates, 4% fat; chocolate: 19% protein, 62% carbohydrate, 5% fat) or caloric density (grain: 3.4 kcal/g; chocolate: 3.6 kcal/g). The chambers were controlled through Med State software programming that automatically recorded the number of lever responses, the number of food deliveries received, the last PR value achieved (BP), and the time to complete the session. Sessions were conducted 7 days a week.

2.3. Procedure

To train lever pressing, each subject was placed in the operant chamber, and responses on the right or left lever (randomly assigned and counterbalanced across subjects) were reinforced under a FR-1 schedule of food reinforcement. Reinforcement was then gradually increased over successive days to a FR-5 schedule of reinforcement. After lever pressing was reliable and stable (no increasing or decreasing trends over a 3-day period) under FR-5 schedules of food reinforcement, each rat was then trained under a PR schedule (Richardson and Roberts, 1996) of food reinforcement that increased the response requirement following each reinforcement delivery (1, 2, 4, 6, 9, 12, 15, 20, 25, 32, etc.). When a specific ratio requirement of the PR schedule was not completed within a 5 min period, the daily session ended. Sessions generally lasted approximately 30 min. After the daily PR sessions were complete, rats were returned to their cages, and fed their daily food allotment and allowed free access to water. Daily feedings included normal rat chow as well as an extra-experimental allocation of both grain and chocolate-flavored pellets (~3–5 g each) to ensure that animals had access to both foods outside of the experimental session. Animals had experience with both grain and chocolate-flavored pellets prior the beginning of the experimental sessions.

Once stable responding behavior under the PR schedule had been established, rats were tested by administering injections of AM 251 (0.3, 1.0, 3.0 mg/kg, ip) or vehicle, or saline, 30 min before experimental sessions where normal grain or chocolate-flavored pellets (counterbalanced across rats) were available. Following a test session, subjects were required to demonstrate a return to stable responding (as described above) and where number of food deliveries did not deviate by more than 2 steps from previous levels during daily training sessions before the next test session. Each rat received all doses of AM 251 in a nonsystematic order in both food conditions, with the exception that the highest dose was administered last in the sequence within each food condition.

2.4. Drug

AM-251 (Sigma-Aldrich, St. Louis, MO, USA) was initially dissolved in 100% DMSO and subsequently dissolved in a final vehicle solution that consisted of 100% dimethylsulfoxide (DMSO), 95% ethanol, and saline in a 1:1:8 ratio. This DMSO vehicle served as the control conditions for all experimental comparisons. Since DMSO has pharmacological effects, we also obtained data following saline administration for comparison purposes only. Operant behavior following DSMO injections was not statistically significantly different from behavior following saline injections (all $ps>0.05$). Vehicle (0.0), 0.3, 1, and 3 mg/kg doses were administered by intraperitoneal (ip) injection 30 min before the start of PR sessions. The doses were selected to include a range previously reported to decrease feeding in deprived and non-deprived animals without affecting other behaviors (Tallet et al., 2008). Pilot experiments also revealed that higher doses up to 10 mg/kg AM 251 significantly affected locomotion and operant responding and therefore were not used in these experiments. Lastly, the recurring testing paradigm (active drug tests occurring twice per week) was selected because tolerance to the anorectic effects resulting from repeated administration of AM 251 (e.g., 1–5 mg/kg, for 5 days) has not been observed (Chambers et al., 2006).

2.5. Data analysis

Duplicative data points were obtained for all animals and were averaged across all animals to create the group means. Initial baseline performance data (grain vs. chocolate) comparisons were assessed with planned paired *t* tests to examine the initial hypotheses that chocolate-flavored food pellets would produce greater food-reinforced lever pressing and result in greater numbers of food deliveries in baseline conditions following vehicle administration. The saline baseline data were not used for any other analyses. All other data comparisons were analyzed with a repeated measures analysis of variance (ANOVA) and assessed for homogeneity using Bartlett's test for equal variances (Snedecor and Cochran, 1989). When a significant *F* was obtained, Dunnett's Multiple Comparison *post hoc* tests (*q* tests) were employed to discern significant treatment differences when compared to the vehicle control group data. Dependent variables included responding, food deliveries received, and BP values resulting from the PR schedule of reinforcement. Responding that occurred during food presentation or during the time out following food delivery was not counted in the overall responses. All *p*-values were set *a priori* at alpha = 0.05 and all statistical calculations were completed using Prism version 5.0b (GraphPad Software, San Diego CA).

3. Results

Our initial validation check revealed a significant difference in food deliveries (two-tailed $t=4.43$, $df=1$, $p<0.001$) and BP (two-tailed $t=2.79$, $df=1$, $p=0.01$) between normal and chocolate-flavored food pellets following saline administration (see Fig. 1, top frame), but the greater amount of responding maintained by chocolate-flavored pellets (versus grain) did not reach statistical significance (two-tailed $t=1.16$, $df=1$, $p=0.26$). These data partially supported the original hypothesis that chocolate-flavored pellets would engender greater amounts of behavior versus normal grain pellets under the PR schedule of food reinforcement and result in higher levels of chocolate pellet deliveries versus grain pellets.

AM 251 decreased responding (see Fig. 1, row 2 frames) maintained by chocolate pellets in a dose-dependent manner ($F(3,42)=8.85$, $p<0.001$), with significant reductions in responding observed at 0.3 mg/kg ($q=2.86$, $p<0.05$), 1.0 mg/kg ($q=2.53$, $p<0.05$) and 3.0 mg/kg ($q=5.14$, $p<0.001$) doses of AM 251 when compared to vehicle treatment. In contrast, AM 251 did not significantly attenuate responding maintained by grain pellets ($F(3,42)=0.29$, $p=0.82$).

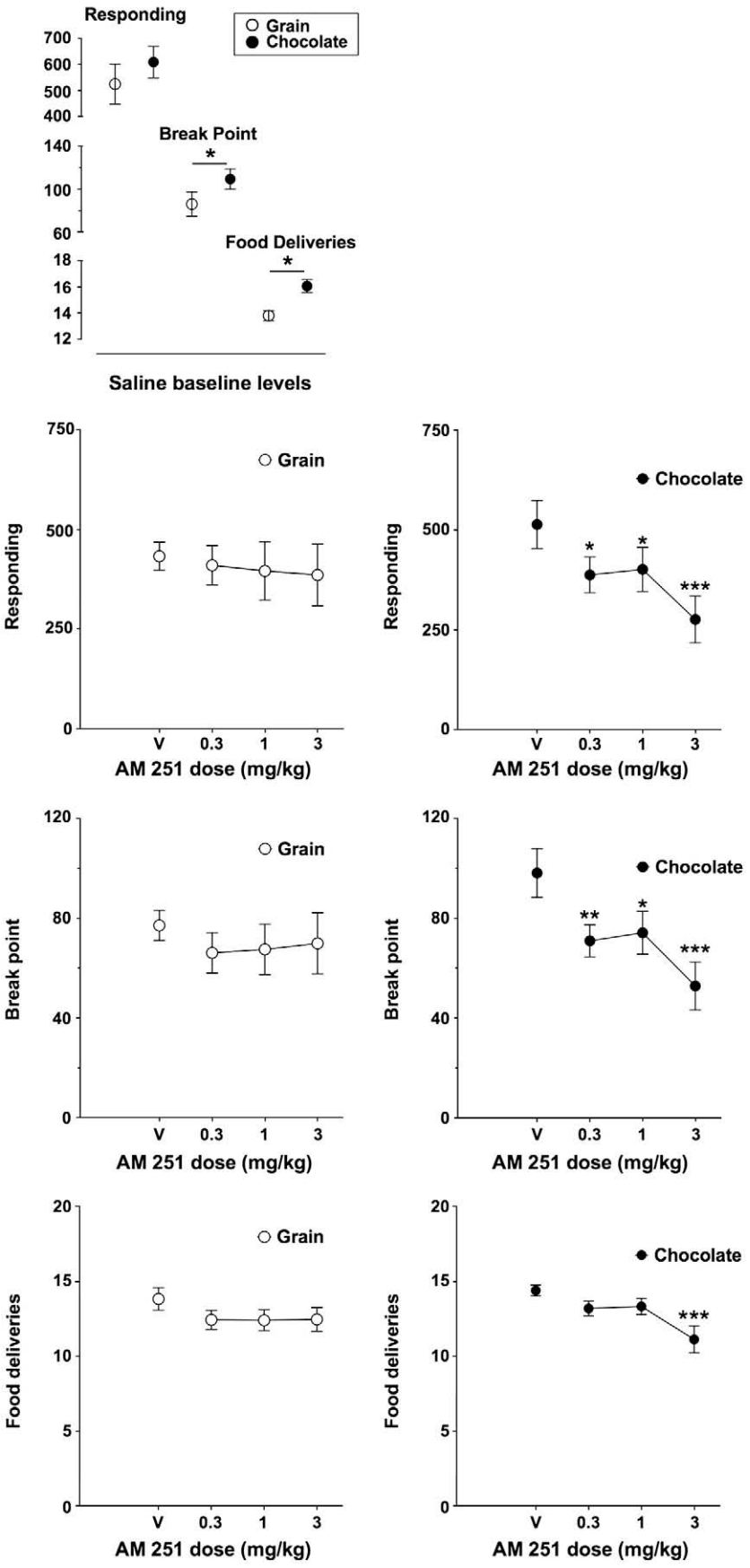
A similar pattern of results was observed when examining BP data (see Fig. 1, row 3 frames). AM 251 produced a robust reduction in BP values maintained by chocolate-flavored pellets ($F(3,42)=9.37$, $p<0.001$), with significant reductions observed at all three AM 251 doses examined: 0.3 mg/kg ($q=3.16$, $p<0.01$); 1.0 mg/kg ($q=2.79$, $p<0.05$); and 3.0 mg/kg ($q=5.27$, $p<0.001$) when compared to treatment with vehicle. However, AM 251 did not have a comparable effect on BP maintained by grain pellets, and no significant effects were observed ($F(3,42)=0.54$, $p=0.65$).

Lastly, deliveries of chocolate pellets (see Fig. 1, bottom frames) were significantly impacted by treatment with AM 251 ($F(3,42)=9.21$, $p<0.001$), with maximal reduction observed following treatment with 3.0 mg/kg AM 251 ($q=5.13$, $p<0.001$). The two lower doses of AM 251 did not result in a significant attenuation of chocolate pellet deliveries (0.3 mg/kg: $q=1.88$, $p>0.05$; 1.0 mg/kg: $q=1.68$, $p>0.05$). Inspection of delivery data of grain pellets revealed no significant effect ($F(3,42)=2.21$, $p=0.11$) of AM 251 at any dose (all $qs<2.25$, all $ps>0.05$).

4. Discussion

In this study, AM 251 significantly decreased BP, responding for, and food deliveries of chocolate pellets in normal weight, adult male, Long Evans rats under a PR schedule of reinforcement. In contrast, administration of AM 251 did not significantly decrease responding, BP or food deliveries when grain pellets were made available. The results were fairly consistent across the three separate dependent variables, as expected, since all three are measures of the same behavioral output. These data supported our hypothesis that AM 251 would have different effects depending upon the specific food available. This selective effect of AM 251 is consistent with previous research that has demonstrated decreased motivation for food consumption in laboratory studies following administration of CB_1 antagonists. These data compare favorably with findings using other CB_1 antagonists (Escartín-Pérez et al., 2009; Maccioni et al., 2008; Mathes et al., 2008; Pertwee, 2005; Rasmussen and Huskinson, 2008; Salamone et al., 2007; Ward and Dykstra, 2005) as well as from studies with AM 251 (Hodge et al., 2008; Mathes et al., 2008; McLaughlin et al., 2003, 2005; Tallet et al., 2008). Our results, while not providing a direct measure of food intake, did provide measures of motivation (BP) and the reinforcing effectiveness of grain and more palatable food in rats.

One key aspect to our study that has been consistent with the work of others is that CB_1 antagonists like AM 251 sometimes appear more effective with those foods that rate higher in level of hedonics or palatability. In the present experiments the nutritional value of chocolate-flavored food did have slightly higher levels of carbohydrates and fat, as well as being a slightly more caloric dense food, but in general both foods were relatively comparable in macronutrient composition but differed in terms of palatability. A number of studies have shown that cannabinoid antagonists can vary in effectiveness depending upon the palatability of food. For instance, Arnone et al. (1997) and Simiand et al. (1998) both found that high versus low palatable diets produced different results in feeding behavior following AM 251 administration. Mathes et al. (2008) demonstrated that AM 251 decreased 24 h food intake in rats, but that this decrease was specific to food high in sugar and fat contents. In a similar manner, Escartín-Pérez et al. (2009) reported that AM 251 reversed a CB_1 agonist-induced increase in carbohydrate consumption suggesting that CB_1 activation may stimulate hunger and inhibit mechanisms of satiety. Finally, two recent studies (Maccioni et al., 2008; Rasmussen and Huskinson, 2008) reported rimonabant administration decreased consumption of a chocolate beverage (Maccioni et al., 2008) and of sucrose pellets (Rasmussen and Huskinson, 2008) under different schedules of reinforcement. Thus, our data seems to complement a number of recent


Responding
Grain
Chocolate
700
600
500
400
Break Point
140
100
60
Food Deliveries
18
16
14
12
Saline baseline levels
Responding
750
500
250
0
Grain
V
0.3
1
3
AM 251 dose (mg/kg)
Chocolate
*
*
***
Break point
120
80
40
**
Food deliveries
20
15
10
5

studies that suggest a selective nature of CB_1 antagonist effects on grain and highly palatable food.

One non-motivational mechanism that might produce decreased feeding and intake in animals treated with CB_1 antagonists is some type of aversion. Tallet et al. (2008) reported that 1.0 mg/kg AM 251 did not alter feeding behaviors, but it did decrease overall food consumption, suggesting that alternative mechanisms, like aversion, might be involved with CB_1 antagonism. There have been some conflicting reports that suggest higher doses of AM 251 do (McLaughlin et al., 2005), and do not (Vickers et al., 2003), impact non-motivational factors (e.g., nausea and conditioned taste aversion), and thus might be responsible for some of the CB_1 antagonist-related effects. Thus, noxious direct effects that produce aversion (McLaughlin et al., 2005) might emerge at higher doses of AM 251, similar to that observed with other drug classes (Platt et al., 2003). This would be consistent with our data gathered from pilot studies for these experiments that suggested 10 mg/kg AM 251 produced locomotor suppression and short-term weight loss. Others (Chambers et al., 2006; Xi et al., 2008) have reported that higher doses (>5 mg/kg) of AM 251 produced altered or inconsistent behavioral effects, including long lasting (4 days) reductions in food intake. Thus, taken together, it would appear that the hypothesis of lower AM 251 doses producing a more selective effect on foods with increased palatability is the most parsimonious explanation for the behavioral effects observed.

One alternative interpretation of our data is that the increased palatability of the chocolate pellets would lead to motivational differences to obtain an uncommonly available preferred food. We attempted to control for this possibility by providing both chocolate and grain food pellets to the animals in their home cage environment as a food supplement. The rationale for this was to ensure that animals had prior experience with the food pellets before encountering them in the operant chambers during session and to keep both foods in an open (versus closed) economy situation. Previous work has demonstrated that goods available only in a closed economy situation can be resistant to pharmacological treatments (Carroll et al., 2000; Rodefer et al., 1999).

A second possible interpretation of our data is that of experience or behavioral momentum. Specifically, that because animals may have demonstrated a preference for chocolate-flavored pellets, the increased numbers of chocolate reinforcements received might subsequently increase the reinforcing value of subsequent reinforcers. Although we did not limit the maximal number of reinforcers available to animals, it is worth noting that maximum food deliveries obtained by any animal were similar across food and dose conditions (*data not plotted*; Vehicle: grain = 18, chocolate = 18; 0.3 mg/kg: grain = 16, chocolate = 17; 1.0 mg/kg: grain = 17, chocolate = 17; 3.0 mg/kg: grain = 16, chocolate = 17). Thus, any greater palatability of chocolate food did not manifest itself as robust differences in total intake of chocolate food. Moreover, it should be noted that total food intake during daily experimental sessions was less than 1 g, and thus constituted a small portion (~5%) of each animals daily food (~20 g).

The use of PR schedules of reinforcement to assess motivation is not new (Hodos, 1961), but it does offer behavioral advantages compared to traditional FR schedules of reinforcement when dealing with reinforcing substances (e.g., foods, beverages, or drugs) where satiety might play a critical factor in modulating intake. Our results are consistent with prior studies that have demonstrated that PR schedules of reinforcement can effectively measure reinforcing qualities of food (Maccioni et al., 2008; Rasmussen and Huskinson, 2008) and drug (Xi et al., 2008) following administration of CB_1 ligands. Second, although PR schedules carry a limitation of an unequal response requirement per unit of reinforcer compared to a standard FR schedule of reinforcement, previous work (Rodefer and Carroll, 1996, 1997) has demonstrated that the PR measure of BP is analogous to other traditional response measurements (Hursh and Winger, 1995; Rodefer et al., 1996). One other important value of PR schedules is the different behavioral measures they produce. Although the three dependent variables used in this study seemed to reveal similar patterns of behavior resulting from AM 251, it should be noted that BP and food deliveries were differentially sensitive measures (i.e., only the highest dose of AM 251 significantly reduced food deliveries, whereas BP was sensitive to lower doses of AM 251) and the magnitude of change differed across measures. The large change in response output required as subjects advanced through the various steps of the PR schedule resulted in both larger differences in responding and BP, and larger variability across animals.

When considering the experiment in total, there were a number of strengths. First, we utilized a well-documented behavioral methodology to assess reinforcing effectiveness across three different behavioral measures. Second, we examined multiple doses of AM 251 across a range demonstrated effective in altering feeding-related behaviors but that which did not alter motor behavior or body weight. Lastly, the repeated measures, within-subjects design allowed us to examine a fewer number of animals and to have each animal serve as its own control. The consistent significant effect of AM 251 on behavior suggests that our experimental design was robust and powerful enough to detect behavioral differences across groups resulting from some of our experimental manipulations. Although we did limit the time duration allowed for completion of the PR response requirement, the BP values achieved in our study were greater than (Rasmussen and Huskinson, 2008) or comparable to those (Maccioni et al., 2008) reported by other groups using similar procedures. One other limitation may have been that both foods engendered similar behavior across vehicle and lower doses of drug. Chocolate appeared to be a more palatable, but not a more clearly preferred, food over grain pellets. A direct comparison using concurrent reinforcers, or using a range of palatable foods (e.g., cake icing) was beyond the scope of these experiments, but is a possible future direction.

In summary, the cannabinoid CB_1 antagonist AM 251 significantly reduced behavior emitted towards obtaining chocolate-flavored food in a dose-dependent manner, but did not produce corresponding reductions in behavior when animals were reinforced with grain pellets. Moreover, these corresponding decreases in BP levels suggest that AM 251 also significantly decreased the reinforcing effectiveness of the chocolate food pellets. These data suggest that CB_1 antagonists like AM 251 may be more effective when the foods are more palatable, such as those high in sugar or fat, and contribute to the growing arena of research demonstrating how CB_1 ligands can selectively modulate food-reinforced and feeding-related behaviors.

Acknowledgments

This project partially fulfilled requirements for a B.S. degree with *Honors* (SMD). The authors thank Jennifer Hafling for her technical assistance. This study was supported in part by seed funds to JSR. EKS was partially supported by an IREU grant (to JSR). The authors have no conflicts of interests to declare.

Fig. 1. Baseline levels of performance (top frame) when reinforced by chocolate (filled circles) or grain (open circles) pellets following saline administration. Effects of AM 251 on responding for (panels in row 2), break point (frames in row 3), and deliveries of (bottom row frames) grain (open circles; left frames) or chocolate-flavored pellets (filled circles; right frames) under PR schedules of food reinforcement. Group means during daily test sessions are plotted following acute administration of saline (S), vehicle (V) or drug (0.3–3.0 mg/kg). Analyses revealed significant baseline differences in BP and food deliveries, but not responding. Significant effects of AM 251 were observed on behavior maintained by chocolate-flavored, but not grain, food pellets. Asterisks indicate significant group differences ($^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$) when individual doses of AM 251 were compared to corresponding vehicle administration. All error bars represent 1 SEM.

References

Arnone M, Maruani J, Chaperon F, Thiebot MH, Poncelet M, Soubrie P, et al. Selective inhibition of sucrose and ethanol intake by SR 141716A, an antagonist of central cannabinoid (CB1) receptors. Psychopharmacology (Berl) 1997;132:104–6.

Budzynska B, Kruk M, Biala G. Effects of the cannabinoid CB1 receptor antagonist AM 251 on the reinstatement of nicotine-conditioned place preference by drug priming in rats. Pharmacol Rep 2009;61:304–10.

Carroll ME, Cosgrove KP, Campbell UC, Morgan AD, Mickelberg JL. Reductions in ethanol, phencyclidine, and food-maintained behavior by naltrexone pretreatment in monkeys is enhanced by open economic conditions. Psychopharmacology 2000;148:412–22.

Chambers AP, Koopmans HS, Pittman QJ, Sharkey KA. AM 251 produces sustained reductions in food intake and body weight that are resistant to tolerance and conditioned taste aversion. Br J Pharmacol 2006;147:109–16.

Colombo G, Agabio R, Diaz G, Lobina C, Reali R, Gessa GL. Appetite suppression and weight loss after the cannabinoid antagonist SR 141716. Life Sci 1998;63(8):PL113–7.

Cota D, Marsicano G, Lutz B, Vicennati V, Stalla GK, Pasquali R, et al. Endogenous cannabinoid system as a modulator of food intake. Int J Obes Relat Metab Disord 2003;27:289–301.

Di Chiara G, Acquas E, Tanda G, Cadoni C. Drugs of abuse: biochemical surrogates of specific aspects of natural reward? Biochem Soc Symp 2003;59:65–81.

Di Marzo V, Matias I. Endocannabinoids control of food intake and energy balance. Nat Neurosci 2005;8:585–9.

Elamin EM, Glass M, Camporesi E. Pharmacological approaches to ameliorating catabolic conditions. Curr Opin Clin Nutr Metab Care 2006;9:449–54.

Escartín-Pérez EE, Cendejas-Trejo NM, Cruz-Martínez AM, González-Hernández B, Mancilla-Díaz JM, Florán-Garduño B. Role of cannabinoid CB1 receptors on macronutrient selection and satiety in rats. Physiol Behav 2009;96:646–50.

Gaetani S, Kaye WH, Cuomo V, Piomelli D. Role of endocannabinoids and their analogues in obesity and eating disorders. Eat Weight Disord 2008;13:e42–8.

Gaoni Y, Mechoulam R. Isolation, structure, and partial synthesis of an active constituent of hashish. J Am Chem Soc 1964;86:1646–7.

Greenberg I, Kuehnle J, Mendelson JH, Bernstein JG. Effects of marijuana use on body weight and caloric intake in humans. Psychopharmacology (Berl) 1976;49:79–84.

Halikas JA, Weller RA, Morse CL, Hoffman RG. A longitudinal study of marijuana effects. Int J Addiction 1985;20:701–11.

Haney M, Gunderson EW, Rabkin J, Hart CL, Vosburg SK, Comer SD, et al. Dronabinol and marijuana in HIV-positive marijuana-smokers. Caloric intake, mood, and sleep. J Acquir Immune Defic Syndr 2007;45:545–54.

Hao S, Avraham Y, Mechoulam R, Berry EM. Low dose anandamine affects food intake, cognitive function, neurotransmitter and corticosterone levels in diet-restricted mice. Eur J Pharmacol 2000;392:147–56.

Hodge J, Bow JP, Plyler KS, Vemuri KV, Wisniecki A, Salamone JD, et al. The cannabinoid CB1 receptor inverse agonist AM 251 and antagonist AM 4113 produce similar effects on the behavioral satiety sequence in rats. Behav Brain Res 2008;193:298–305.

Hodos W. Progressive ratio as a measure of reward strength. Science 1961;269:943–4.

Huang TTK, Glass TA. Transforming research strategies for understanding and preventing obesity. J Amer Med Assoc 2008;300:1811–3.

Hursh SR, Winger G. Normalized demand for drugs and other reinforcers. J Exp Anal Behav 1995;64:373–84.

Maccioni P, Pes D, Carai MAM, Gessa GL, Colombo G. Suppression by the cannabinoid CB1 receptor antagonist, rimonabant, of the reinforcing and motivational properties of a chocolate-flavoured beverage in rats. Behav Pharmacol 2008;19:197–209.

Mathes CM, Ferrara M, Rowland NE. Cannabinoid-1 receptor antagonists reduce caloric intake by decreasing palatable diet selection in novel dessert protocol in female rats. Am J Physiol Regul Integr Comp Physiol 2008;295:R67–75.

McLaughlin PJ, Winston K, Swezey L, Wisniecki A, Aberman J, Tardif DJ, et al. The cannabinoid CB1 antagonists SR 141716A and AM 251 suppress food intake and food-reinforced behavior in a variety of tasks in rats. Behav Pharmacol 2003;14:583–8.

McLaughlin PJ, Winston KM, Limebeer CL, Parker LA, Makriyannis A, Salamone JD. The cannabinoid antagonist AM 251 produces food avoidance and behaviors associated with nausea but does not impair feeding efficiency in rats. Psychopharmacology 2005;180:286–93.

O'Neill C, Evers-Donnelly A, Nicoholson D, O'Boyle KM, O'Conner JJ. D2 receptor-mediated inhibition of dopamine release in the rat striatum in vitro is modulated by CB1 receptors: studies using fast cyclic voltammetry. J Neurochem 2009;108:545–51.

Pertwee RG. Inverse agonism and neutral antagonism at cannabinoid CB1 receptors. Life Sci 2005;76:1307–24.

Pertwee RG. The pharmacology of cannabinoid receptors and their ligands: an overview. Int J Obes 2006;30:S13–8.

Platt DM, Rodefer JS, Rowlett JK, Spealman RD. Suppression of cocaine- and food-maintained responding by the D2-like receptor partial agonist terguride in squirrel monkeys. Psychopharmacology 2003;166:298–305.

Rasmussen EB, Huskinson SL. Effects of rimonabant on behavior maintained by progressive ratio schedules of sucrose reinforcement in obese Zucker (fa/fa) rats. Behav Pharmacol 2008;19:735–42.

Richardson NR, Roberts DC. Progressive ratio schedules in drugs self-administration studies in rats: a method to evaluate reinforcing efficacy. J Neurosci Methods 1996;66:1-11.

Rodefer JS, Carroll ME. Evaluation of the reinforcing efficacy of phencyclidine and ethanol under varied feeding conditions using progressive ratio schedules in rhesus monkeys. Psychopharmacology 1996;128:265–73.

Rodefer JS, Carroll ME. A comparison of progressive ratio schedules versus behavioral economic measures: effect of an alternative reinforcer on the reinforcing efficacy of phencyclidine. Psychopharmacology 1997;132:95-103.

Rodefer JS, DeRoche KK, Lynch WA, Carroll ME. A behavioral economic analysis of the effects of food deprivation and satiation on self-administration of phencyclidine and ethanol self-administration. Exp Clin Psychopharmacol 1996;4:61–7.

Rodefer JS, Campbell UC, Cosgrove KP, Carroll ME. Naltrexone pretreatment decreases the reinforcing effectiveness of ethanol and saccharin but not PCP or food in rhesus monkeys under concurrent progressive-ratio schedules in rhesus monkeys. Psychopharmacology 1999;141:436–46.

Salamone JD, McLaughlin PJ, Sink K, Makriyannis A, Parker LA. Cannabinoid CB1 receptor inverse agonists and neutral antagonists: effects on food intake, food-reinforced behavior and food aversions. Physiol Behav 2007;91:383–8.

Simiand J, Keane M, Keane PE, Soubrie P. SR 141716, a CB1 cannabinoid receptor antagonist, selectively reduces sweet food intake in marmoset. Behav Pharmacol 1998;9:179–81.

Sink KS, Segovia KN, Nunes EJ, Collins LE, Vemuri VK, Thakur G, et al. Intracerbroventricular administration of cannabinoid CB11 receptor antagonists AM251 and AM4113 fails to alter food-reinforced behavior in rats. Psychopharmacology 2009;206:223–32.

Snedecor GW, Cochran WG. Statistical methods. Eighth Edition. Iowa State University Press; 1989.

Sofia RD, Knobloch LC. Comparative effects of various naturally occurring cannabinoids on food, sucrose and water consumption by rats. Pharmacol Biochem Behav 1976;4:591–9.

Tallet AJ, Blundell JE, Rodgers RJ. Effects of acute low dose combined treatment with naloxone and AM 251 on food intake, feeding behavior and weight gain in rats. Pharmacol Biochem Behav 2008;91:358–66.

Vickers SP, Webster LJ, Wyatt A, Dourish CT, Kennett GA. Preferential effects of the cannabinoid CB-sub-1 receptor antagonist, SR 141716, on food intake and body weight gain of obese (fa/fa) compared to lean Zucker rats. Psychopharmacology 2003;167:103–11.

Ward SJ, Dykstra LA. The role of CB1 receptors in sweet versus fat reinforcement: effect of CB1 receptor deletion, CB1 receptor antagonism (SR141716A) and CB1 receptor agonism (CP-55940). Behav Pharmacol 2005;16:381–8.

Xi ZX, Spiller K, Pak AC, Gilbert J, Dillon C, Li X, et al. Cannabinoid CB1 receptor antagonists attenuate cocaine's rewarding effects: experiments with self-administration and brain-stimulation reward in rats. Neuropsychopharmacology 2008;33:1735–45.