

Effects of rimonabant on behavior maintained by progressive ratio schedules of sucrose reinforcement in obese Zucker (*fa/fa*) rats

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This experiment reports on the ability of rimonabant to alter the reinforcing properties of food in the genetically obese Zucker (*fa/fa*) rat, a strain that exhibits higher levels of endocannabinoids in brain regions that correspond to heightened food intake. We characterized food reinforcement in obese and lean Zucker rats by placing behavior under progressive ratio schedules of sucrose reinforcement. Then, doses of rimonabant (1–10 mg/kg), a CB1 receptor antagonist, were administered. Obese Zuckers had slightly higher breakpoints for sucrose under baseline conditions compared with leans, and also demonstrated significantly higher response rates than leans. Rimonabant dose-dependently decreased breakpoints and response rates for both groups, though only obese Zuckers demonstrated suppressed behavior under the 1 mg/kg dose. The 10 mg/kg dose of rimonabant reduced breakpoints equally for both groups (by about 60%). This dose of rimonabant also reduced food intake by 20% in lean Zuckers, and by 30% in obese Zuckers. These

findings extend the literature that rimonabant reduces food reinforcer efficacy, and suggest that obese Zuckers may exhibit a heightened sensitivity to rimonabant. The findings also suggest that the effort required to obtain food reinforcement may also play a role in the efficacy of rimonabant. *Behavioural Pharmacology* 19:735–742 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

The endocannabinoid neurotransmitter system plays a role in feeding and hyperphagia. Activation of the CB1 receptor by endogenous cannabinoids, such as 2-arachidonyl glycerol (2-AG) or anandamide, or exogenous compounds, like Δ -9 tetrahydrocannabinol, increases food intake (Drewnowski and Grinker, 1978; Williams *et al.*, 1998; Williams and Kirkham, 1999; Hao *et al.*, 2000; DiMarzo *et al.*, 2001; Kirkham and Williams, 2001; Berry and Mechoulam, 2002; Jarrett *et al.*, 2005). The aforementioned studies also demonstrate that the induction of eating occurs even when the organism is not food deprived, suggesting that one behavioral mechanism affected by cannabinoid activity is enhancement of food reward.

SR141716, or rimonabant, is a cannabinoid antagonist that blocks the CB1 receptor, thereby reducing food intake (Colombo *et al.*, 1998; Simiand *et al.*, 1998; McLaughlin *et al.*, 2003; Vickers *et al.*, 2003; Thornton-Jones *et al.*, 2005; Carai *et al.*, 2006; Gardner and Mallet, 2006; Herling *et al.*, 2008; Serrano *et al.*, 2008). This effect seems to be especially relevant to palatable foods, such as those that are fatty or sweet (Verty *et al.*, 2004; Gessa *et al.*, 2006; Melis *et al.*, 2007; Thornton-Jones *et al.*, 2007). On a neural level, rimonabant reduces extracellular dopamine release in the reward areas of the brain of rodents when

palatable food is ingested (Melis *et al.*, 2007), so rimonabant may reduce motivation for food through this mechanism.

As is gleaned from the above studies, food intake is the most frequently used dependent variable for behavior assessment of food reward. Food intake usually occurs in an environment where food is readily available, that is, available at a low response cost to the organism. For example, a rat may simply move toward a food aperture located within its home cage and eat from a large amount of freely available food. Although food intake is an informative dependent variable, it does little to assess the value of food at higher response costs, when the value of food may decrease (Hursh, 1984; Hursh *et al.*, 1988).

The progressive ratio (PR) schedule of reinforcement is a procedure that shows the relation between response effort and the value of a particular reinforcer, for example, food or drugs of abuse (Hodos, 1961; see also Markou *et al.*, 1993; Stafford *et al.*, 1998 for reviews). Under this schedule, the initial response requirement for a reinforcer is low, then the ratio requirement increases systematically within a single session. The 'breakpoint' or strain, in which the animal no longer responds, is the referent for the value of the reinforcer. Indeed, the PR schedule is used extensively in determining reinforcing efficacy

for drugs of abuse (see Spealman and Goldberg, 1978; Stafford *et al.*, 1998 for reviews). However, its application to the conditions under which food functions as a reinforcer, especially with food-related problems such as obesity, has been limited in recent times.

Although many studies have shown how cannabinoids influence food intake, fewer studies have examined how cannabinoids affect food reinforcement using operants that require larger response requirements. Two reports that used PR schedules, for example, confirmed that tetrahydrocannabinol increased breakpoints for food under PR schedules in standard rat strains such as Sprague-Dawley and Wistar (Higgs *et al.*, 2005; Solinas and Goldberg, 2005). In addition, rimonabant has been shown to reduce breakpoints for food reinforcement in rats (Solinas and Goldberg, 2005) and mice (Ward and Dykstra, 2005). These studies expand the food intake literature by providing a clearer characterization that food reinforcement may be a behavioral mechanism altered by cannabinoid drugs.

Genetics may also influence the reinforcing properties of food by way of the cannabinoid system. The obese Zucker rat, for example, exhibits leptin insensitivity. Leptin is a peptide secreted by adipose tissue that, in a normal lean animal, blocks hunger signals, such as neuropeptide Y (Figlewicz, 2003; Sahu, 2004a, b). One manner by which leptin lowers the reinforcing properties of food (Harrold and Williams, 2003; Fulton *et al.*, 2004; Jo *et al.*, 2005) is by blocking receptors in reward areas of the brain (Figlewicz *et al.*, 2003) and by reducing endocannabinoid activity in the brain (DiMarzo *et al.*, 2001). The Zucker rat, in contrast, is missing the leptin receptor. As a result, this strain eats approximately three times more food than control wild types (e.g. Zucker and Zucker, 1962; DiMarzo *et al.*, 2001) and exhibits an obese phenotype. Moreover, the obese Zucker has been shown to have higher 2-AG (an endocannabinoid) levels in areas of the brain that are relevant to food intake (Wenger and Moldrich, 2002; Harrold and Williams, 2003; Jo *et al.*, 2005), and these higher levels seem to be mediated by an insensitivity to leptin (DiMarzo *et al.*, 2001). Few studies, have examined how these physiological changes manifest in behavioral choice that go beyond food intake as a dependent variable. One exception, however, is found in a study by Glass *et al.* (1999), who showed that naloxone, a drug that blocks the opioid receptor (which is also implicated in food reinforcement), decreases the reinforcing properties of food under PR schedules in lean and obese Zuckers.

This study examined the degree to which the cannabinoid antagonist rimonabant affected food reinforcer efficacy in obese Zucker rats. As the obese Zucker strain exhibits higher endocannabinoid levels, it was

hypothesized that this rat strain may exhibit altered behavioral sensitivity to rimonabant compared with lean controls. We also compared the effects of rimonabant in Zuckers using the standard dependent variable of food intake to determine whether this drug would reduce food intake to the same extent as the effect on behavior maintained by PR schedules of reinforcement.

Method

Subjects

Nine male genetically obese Zucker (*fa/fa*) and nine male lean Zucker rats were purchased from Harlan at 4–5 weeks of age, and singly housed in home cages with free access to standard food and water. The home cages were located in a temperature-controlled and humidity-controlled room with a 12:12 h light/dark cycle (08.00 h). After 7–8 weeks of free feeding, lean controls weighed a mean (\pm SEM) of 295.23 (\pm 13.23) g and obese (*fa/fa*) rats weighed a mean of 474.43 (\pm 10.34) g; these weights were significantly different [$t(18) = -10.68$, $P < 0.01$]. At 12–13 weeks, subjects began a food restriction protocol in which they were allowed 2 h of free access to food at the same time period in the early afternoon, followed by 22 h without access to food (before an experimental session) to ensure that food would function as a reinforcer. After 2 weeks under this deprivation protocol, obese rats ate an average of 11.0 (\pm 0.64) g, or about 2.3% of their body weights, during the 2-h free-feed sessions; leans ate an average of 7.2 (\pm 0.49) g, or about 2.4% of their body weights. After this 2-week period, deprivation continued and all rats were trained to lever press for sucrose food pellets. Over the course of the experiment, two rats (one lean and one obese) died, so eight rats from each group completed the study. All rats had previous exposure to acute doses of 2-AG (0.03–3 mg/kg), an endocannabinoid, though these data will not be presented here.

Apparatus

Seven Coulbourn Habitest standard (Coulbourn Instruments, Whitehall, PA, USA) rat experimental chambers were used for data collection. Each chamber was equipped with two levers on one panel situated from the bottom of a grid floor. When response criteria were met, a cue light above the lever, and one in the collection area (magazine) illuminated for 5 s as a 45-mg sucrose pellet was delivered. During this reinforcer interval, lever presses has no scheduled consequences. A 28-v house-light was situated 13 cm above the food dispenser. A speaker placed in the upper left corner of the left wall of the chamber generated white noise. A 2" \times 2" fan was situated in the upper right corner of the left wall. A sound-attenuating cubicle surrounded each chamber. Graphic State software (Coulbourn Instruments, Whitehall, PA, USA) on an IBM compatible computer, with 0.01" resolution, controlled all reinforcement contingencies and data collection. Computers and software were

stationed in a room separate from the chambers. Sessions were conducted in the mornings at the same time (± 15 min) from Monday to Thursday.

Procedure

To train lever pressing, each subject was placed in an experimental chamber for a 3-h session, in which responses on the right lever were reinforced under a fixed ratio 1 (FR1) schedule of reinforcement. Lever pressing was considered trained when 90 reinforcers were earned in a session. If this criterion was not met within six sessions, the rat was hand shaped, or reinforced for successive approximations until the lever press was sufficiently trained. Of the 18 rats, three lean Zuckers and one obese Zucker were hand shaped.

Lever pressing was placed under a PR schedule of sucrose reinforcement 2 days/week, for example, Tuesdays and Thursdays. Under the PR schedule, a single lever press produced a food pellet, and the response requirement increased within the experimental session by an exponent of 0.2 multiplied by the number of reinforcers earned in the session to that point and rounded to the nearest integer (Roberts and Bennett, 1993). This resulted in the following ratio steps: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, and 95. When a ratio was not completed within 20 min, the session ended. The breakpoint was the main dependent variable of interest. A breakpoint baseline was determined for each rat before the administration of each drug by averaging the behavior across the last three stable sessions. Stability was defined as at least three sessions in which the breakpoint did not deviate by more than two steps (response requirements) and there were no trends.

An FR1 schedule was placed in effect on days before PR schedules (e.g. Monday and Wednesday) and operated as a maintenance schedule. Here, every response resulted in sucrose pellet. The session terminated after 25 reinforcers were earned.

Once baseline data were collected, rats received vehicle (saline) injections 1 h before PR sessions until behavioral stability was reached. Next, acute doses of rimonabant (dosing range: 1–10 mg/kg) were administered to each rat 1 h before the beginning of a PR session. Doses were given in ascending order in half-log unit increases. If a particular dose reduced behavior by greater than 70% for a particular animal, the next higher dose was not attempted, to reduce the chances of overdose. No fewer than 2 days separated injections. No injections were administered before FR1 sessions.

No-food delivery condition

To ensure the food pellet maintained behavior, each subject was exposed to a no-food delivery, or extinction condition, following the completed rimonabant

dose–response determination. Here, lever presses produced environmental conditions that were identical to the PR sessions, except that no food delivery occurred. After behavior stabilized across three sessions of extinction, the 10 mg/kg dose of rimonabant for each rat was administered 1 h before a subsequent extinction session. This was done to ensure that changes in responding observed in the food-delivery condition during the drug phase were a result of motivation for food rather than another mechanism, for example, motor effect. All experimental procedures were approved and in compliance with the Idaho State University's Institution for Animal Care and Use Committee.

Food intake

Food intake in the home cage during the 2-h free-feed sessions was monitored throughout the experiment. The amount of food placed in the home cage was measured before a 2-h session and then compared with the amount of food that was left after the 2-h session.

Drug

Rimonabant (National Institute of Mental Health Chemical Synthesis and Drug Supply Program) was dissolved in a 1:1:18 ethanol (Sigma, Sigma-Aldrich Co., St Louis, MO, USA), Cremaphor (Sigma), and saline solution (1 ml/kg) and was administered by intraperitoneal injection 1 h before the start of PR sessions. A saline vehicle (1 ml/kg) was administered intraperitoneally before the beginning of some PR sessions.

Data analysis

Dependent variables included mean breakpoints and response rates per session, as well as mean food intake in grams during the 2-h free-feed session. Response rates were determined by examining only the number of responses per session that counted toward the response ratios, that is, responses made during the reinforcement interval were subtracted from the total number of responses across the session. The amount of time during the delivery of reinforcers was subtracted from the total session time. For example, if 12 pellets were earned, and the reinforcement interval was 5 s, then 60 s was subtracted from the session duration (e.g. 45 min), such that the net session time was 44 min. The number of responses made during the session was then divided by the session time in minutes.

A two-way analysis of variance (ANOVA) with repeated measures (SPSS, version 14.0, SPSS, Inc., Chicago, IL, USA) was used to analyze the data, with obese versus lean Zuckers as a between-subjects variable and dose of drug as a within-subjects variable. For each rat, the last three baseline sessions (no vehicle administrations) were averaged into a single mean; the same process was applied to the vehicle (0 mg/kg) data. In other words, for each rat,

a single datum represented each within-subject condition (control, vehicle, and each dose of rimonabant). In addition, the food-delivery versus no-food delivery condition was analyzed using a repeated measures ANOVA, with food-delivery versus no-food delivery as a between-subjects variable and drug condition (baseline vs. 10 mg/kg rimonabant) as a within-subjects variable.

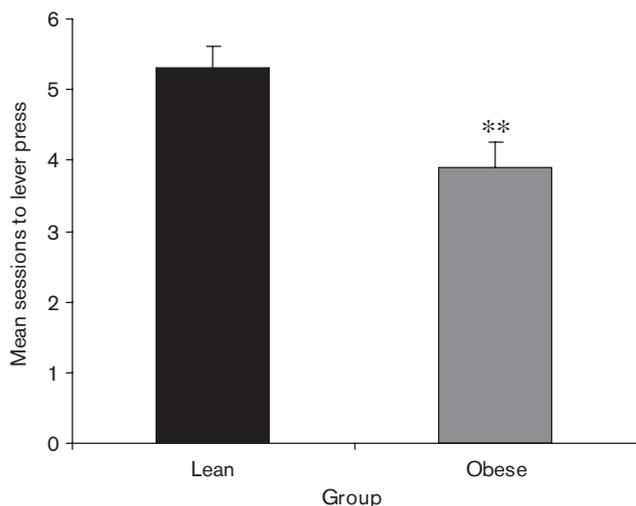
Effect sizes, as reported by partial eta squared (η_p^2), are included. The traditional P value is used to reflect statistical significance levels, though they are often limited in that they do not describe effect sizes. Partial η^2 values refer to the proportion of variance caused by the effect, or independent variable, which can extend information provided by a P value (Neter *et al.*, 1996). Partial η^2 provides similar information to an r^2 value in a regression analysis.

Results

Figure 1 shows the mean number of sessions to acquire the lever press, including data for the four animals that required hand shaping (these four animals were treated as though they completed six sessions). The lean Zuckers acquired the lever press in an average of 5.3 (± 0.95) sessions and the obese Zuckers acquired it in 3.9 (± 1.1) sessions [$t(18) = 3.05$, $P < 0.01$]. When the four animals that required hand shaping were removed from the analysis, the result did not change [lean: 5.0 ± 0.38 ; obese: 3.67 ± 0.29 ; $t(14) = 2.86$, $P < 0.01$].

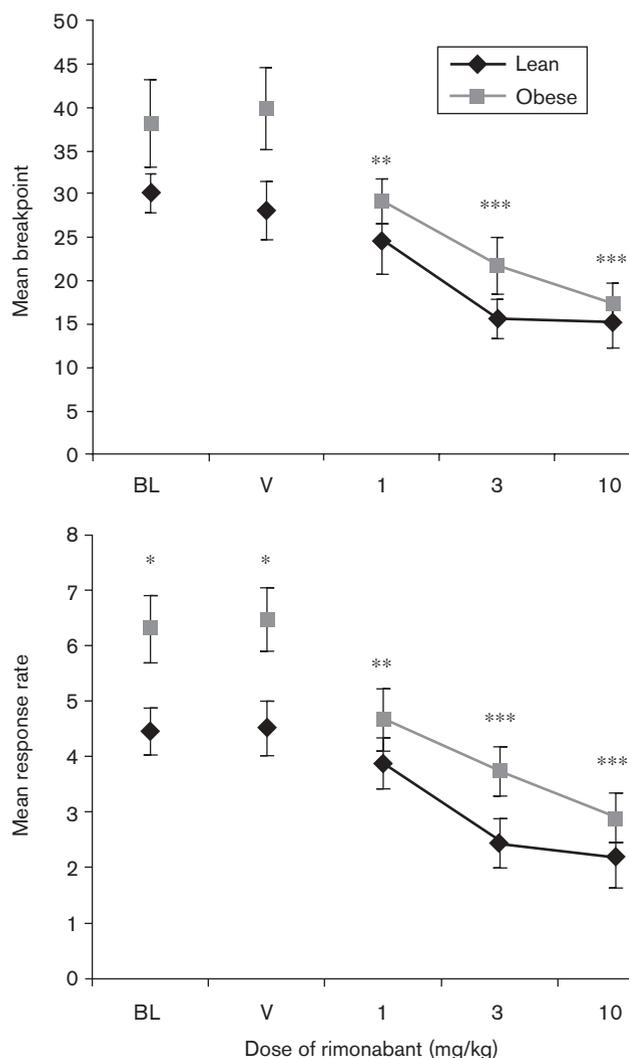
The upper panel of Fig. 2 shows mean breakpoints as a function of dose of rimonabant. The baseline and vehicle (0 mg/kg) means were compared using a two-way ANOVA with repeated measures, which yielded no differences

Fig. 1



Mean (\pm SEM) number of sessions to acquire the lever-press response in lean (black) and obese (grey) Zucker rats. ** $P < 0.01$.

Fig. 2



Mean (\pm SEM) breakpoint (upper panel) and response rate per session (lower panel) as a function of dose of rimonabant. * $P < 0.05$ difference between lean and obese Zucker rats; ** $P < 0.05$ difference between vehicle and dose of rimonabant for obese Zuckers only; *** $P < 0.05$ difference between vehicle and dose of rimonabant for lean and obese Zuckers.

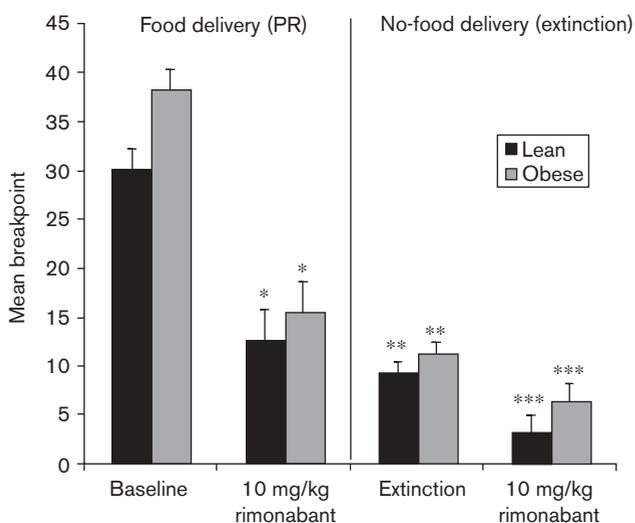
between baseline and vehicle conditions ($P = 0.52$). However, there was a marginal group difference in these data [$F(1,17) = 3.83$, $P = 0.07$, $\eta_p^2 = 0.19$], but no significant interaction ($P = 0.52$).

Rimonabant dose-dependently reduced breakpoints for lean and obese Zuckers [$F(3,42) = 25.98$, $P < 0.01$, $\eta_p^2 = 0.65$]. A significant main effect of group [$F(1,14) = 4.9$, $P < 0.05$, $\eta_p^2 = 0.26$] was observed, but no significant group \times dose interaction ($P = 0.21$). Within-subject contrasts were conducted on vehicle versus each dose of rimonabant for each group. The 1 mg/kg dose of rimonabant reduced mean breakpoints by 28% for the

obese Zuckers, and this was statistically significant [$F(1,7) = 15.51, P < 0.01$]. This same dose reduced breakpoints for the leans by only 12%, and this was not statistically significant ($P = 0.24$). The 3 and 10 mg/kg doses, however, reduced breakpoints significantly for the lean rats [3 mg/kg $F(1,7) = 20.79, P < 0.01$; 10 mg/kg: $F(1,7) = 15.04, P < 0.01$] and obese rats [$F(1,7) = 12.76, P < 0.01$; $F(1,7) = 27.73, P < 0.01$]. Lean and obese rats' breakpoints did not differ significantly at any dose (1–10 mg/kg) of rimonabant.

The lower panel of Fig. 2 shows mean response rates as a function of dose of rimonabant. Baseline and vehicle conditions did not differ significantly ($P = 0.76$), though there was a significant group difference [$F(1,14) = 8.1, P < 0.1, \eta_p^2 = 0.32$]. Rimonabant dose-dependently decreased response rate [$F(4,36) = 17.96, P < 0.01, \eta_p^2 = 0.66$]. In addition, there was a significant main effect of group [$F(1,9) = 7.13, P < 0.02, \eta_p^2 = 0.44$] and an interaction [$F(4,36) = 3.04, P < 0.05, \eta_p^2 = 0.25$]. Vehicle-dose contrasts revealed that the 1 mg/kg of rimonabant reduced response rates for obese Zuckers significantly, by about 28% [$F(1,7) = 159.37, P < 0.01$], but only reduced lean Zucker rates by about 14.5% ($P = 0.32$). The 3 mg/kg dose significantly reduced rates in lean rats [$F(1,7) = 7.3, P < 0.03$] and obese rats [$F(1,7) = 41.02, P < 0.01$]. The 10 mg/kg dose reduced both groups' rates significantly [lean $F(1,7) = 15.79, P < 0.01$; obese $F(1,7) = 5.83, P < 0.01$]. Lean and obese rats' rates did not differ at any dose of rimonabant ($P < 0.32$).

Fig. 3



Mean (\pm SEM) breakpoint in food-delivery (left) and no-food delivery (right) conditions comparing behavior under baseline and 10 mg/kg of rimonabant. * $P < 0.01$ for progressive ratio (PR) baseline vs. 10 mg/kg, ** $P < 0.01$ for PR vs. extinction, *** $P < 0.01$ for extinction vs. 10 mg/kg under extinction.

Figure 3 summarizes effects on breakpoint under food-delivery (PR) and no-food delivery (extinction) conditions. The left half of Fig. 3 shows baseline and 10 mg/kg data from Fig. 2. The 10 mg/kg dose of rimonabant reduced breakpoints equally (about 58% for lean controls and obese Zuckers. The right half of Fig. 3 shows mean breakpoint under the no-food delivery condition. Extinction reduced the mean breakpoint (from PR) to 9.33 (± 0.78) and 11.25 (± 1.1) for the lean and obese Zuckers, respectively, and this reduction was significant. [$F(1,15) = 76.54, P < 0.01, \eta_p^2 = 0.84$]. The 10 mg/kg dose of rimonabant significantly reduced behavior under extinction for the lean (3.11 ± 0.94) and obese Zuckers (6.38 ± 1.81) [$F(1,15) = 22.88, P < 0.01, \eta_p^2 = 0.60$]. In addition, there was a significant main effect of group [$F(1,15) = 4.56, P < 0.05, \eta_p^2 = 0.23$], but no significant interaction ($P = 0.57$). Similar effects were found with response rate, and these data are summarized in Table 1.

Figure 4 shows food intake (g) in the 2-h free-feed session after rimonabant was administered. Rimonabant

Table 1 Response rates (responses/minute) for lean and obese Zucker rats under food-delivery (PR) and no-food delivery (extinction) conditions

	Food delivery (PR) (from Fig. 2)		No-food delivery (extinction)	
	Baseline (progressive ratio)	10 mg/kg rimonabant	Extinction	10 mg/kg rimonabant
Lean controls	4.47 (0.42)	1.7 (0.35) ^a	1.24 (0.17) ^b	0.3 (0.11) ^c
Obese (<i>fa/fa</i>)	6.32 (0.6)	2.74 (0.41) ^a	1.76 (0.22) ^b	0.41 (0.16) ^c

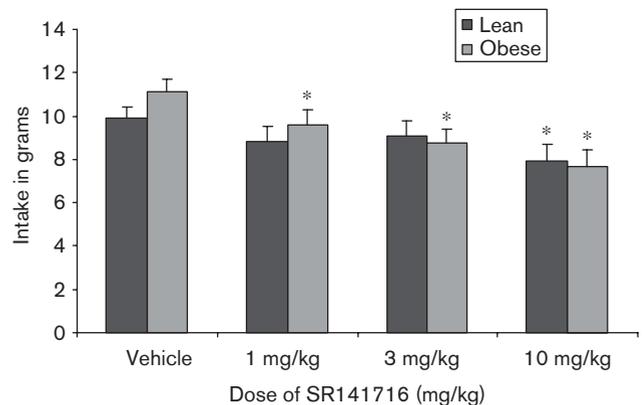
Mean response rates (SEM) under baseline and the 10 mg/kg of rimonabant are shown.

^a10 mg/kg reduced breakpoints significantly for both groups (see text for details).

^bResponse rate under PR vs. extinction: $P < 0.01$ [$F(1,14) = 79.93, \eta_p^2 = 0.85$; no main effect of group or interaction ($P > 0.16$).

^cResponse rate under extinction vs. 10 mg/kg dose of rimonabant under extinction: $P < 0.01$ [$F(1,14) = 48.35, \eta_p^2 = 0.76$]; no main effects of group or interaction ($P > 0.08$).

Fig. 4



Mean (\pm SEM) food intake (g) as a function of dose of rimonabant. * $P \leq 0.01$.

dose-dependently decreased food intake for lean and obese Zuckers [$F(3,39) = 9.29, P < 0.01, \eta_p^2 = 0.42$]. The 10 mg/kg dose reduced food intake by about 20% (from a mean of 9.9 under vehicle to 7.94) for the lean rats and by over 30% (from 11.1 to 7.65) for the obese Zuckers. No significant main effect of group or a group \times dose interaction ($P > 0.15$) was observed. Vehicle-dose contrasts revealed a significant reduction from baseline in the 1 mg/kg [$F(1,7) = 11.66, P < 0.01$], 3 mg/kg [$F(1,7) = 9.19, P < 0.01$], and 10 mg/kg doses [$F(1,7) = 128.48, P < 0.01$] for the obese rats. Only the 10 mg/kg dose reduced food intake significantly for the lean rats [$F(1,7) = 11.82, P < 0.01$].

Discussion

In this study, food-deprived lean and obese Zucker rats responded under a PR schedule for sucrose pellets. During training, obese Zucker rats acquired lever pressing for sucrose pellets in fewer sessions compared with lean controls and more leans required hand shaping compared with obese Zuckers (3 vs. 1). This finding, to our knowledge, has not been reported in the literature with Zucker rats. The mechanism for this finding is unclear; it may reflect genetic differences in learning, for example. We believe, however, in light of other effects to be discussed, the mechanism may likely be due to differences in sensitivity to food reinforcement, with obese Zucker rats showing a higher sensitivity than lean controls.

The baseline data under PR schedules suggest that obese Zuckers had higher, though not significantly higher, breakpoints for sucrose reinforcement. This finding supports an observation reported by Glass *et al.* (1999), who also found that lean and obese Zucker rats did not differ significantly with regard to motivation for sucrose under PR schedules. It is noteworthy to mention, however, that response rates under PRs in this study differed between groups – obese Zuckers had significantly higher response rates compared to leans. It may be the case that response rate is a more sensitive measure than breakpoints. It is noteworthy, for example, that in this study, the mean breakpoint averaged between 25 and 40 across both groups. The relevant steps of the PR sequence progressed from 25 to 32 to 40. Consider two animals that exhibited ratio strain between 32 and 40. Both would be assigned a breakpoint of 32. However, if the first of those rats stopped responding immediately after the reinforcer was delivered, its response rate would be lower than the second rat, who may have ceased responding just before the next pellet delivery (just shy of the 40-response requirement). As there is a potential for more variability within each ratio step, response rate, as opposed to breakpoint, may be a more sensitive measure, and thus better able to reveal a group difference between Zuckers.

Rimonabant dose-dependently reduced breakpoints and response rates for all rats, and the effect sizes were substantial (0.65 or greater). This is consistent with what others have found in standard rat strains (Solinas and Goldberg, 2005). Despite the food reinforcement-suppressing effects of rimonabant, obese Zuckers had higher breakpoints and response rates for sucrose pellets than lean controls across all doses, and this difference was significant for rate. Moreover, between 25 and 66% of the variance in the data was accounted for by group. This study is the first to show that rimonabant reduced the reinforcing properties of food in the obese Zucker rat, which is noteworthy because Zuckers have altered endocannabinoid systems compared with standard laboratory rat strains (DiMarzo *et al.*, 2001). In addition, the 1 mg/kg dose of rimonabant reduced breakpoints and response rates by almost 30% for the obese Zuckers, and did not reduce behavior significantly for the leans. Indeed, a three-fold increase in the drug dose was necessary to cause a significant decrease in the lean rats. The significant reduction for Zuckers at the 1 mg/kg dose may be due to an endocannabinoid-related sensitivity to rimonabant. It is, however, important to note that obese Zuckers did exhibit higher response rates and breakpoints under baseline conditions, so the 1 mg/kg effect may be due to a rate-dependent effect (Dews, 1955) or may be confounded with the number of contacts made with the reinforcer (Nevin *et al.*, 1983). A study that holds response rate and the number of reinforcers constant between the two groups may be able to elucidate the mechanism involved in the strain difference at the 1 mg/kg dose.

The no-food delivery condition (extinction) confirmed that the lever press was maintained by the sucrose pellet. Extinction reduced breakpoints for sucrose pellets in the lean and obese Zucker rats by about 70% for both groups, and accounted for 84% of the variance between conditions. A group difference was found in which obese Zuckers responded more under extinction than controls. This may have resulted from the degree of conditioning to cues that accompanied pellet delivery during PR sessions. Although there was no pellet delivery during the 5 s reinforcer interval throughout the extinction condition, the cues associated with pellet delivery (e.g. the cue light) were presented. As a food pellet was a more potent reinforcer for the obese Zuckers, it may have conditioned more strongly to these cues (Rescorla and Wagner, 1972). This may create a situation in which the cues were stronger conditioned reinforcers for the obese Zuckers, thereby resulting in higher breakpoints and response rates under extinction.

Rimonabant also significantly reduced behavior in the no-food delivery condition. This suggests that another mechanism unrelated to food directly may also be

possible, for example, a motor effect. A more likely reason may have to do with the properties of food that were conditioned to the cues associated with food delivery. In this instance, rimonabant may have suppressed the reinforcing efficacy of these conditioned reinforcers during extinction. Wickelgreen (1997), for example, showed that after repeated presentations, stimuli paired with food can activate the mesolimbic dopamine system more than consumption of food itself. If that is the case here, rimonabant may act to reduce sensitivity to stimuli associated with food. Regardless of the mechanism of action of rimonabant during extinction, it is clear the majority of the drug-induced reduction in breakpoint occurred in the food-delivery condition which suggests that the drug's primary mechanism (in this context) is reduction of the reinforcing properties of food.

Rimonabant also dose-dependently reduced food intake in both lean and obese Zuckers, and this finding replicates what others have found in standard rat strains (e.g. Colombo *et al.*, 1998; Simiand *et al.*, 1998; McLaughlin *et al.*, 2003; Vickers *et al.*, 2003; Carai *et al.*, 2006; Gardner and Mallet, 2006; Herling *et al.*, 2008; Serrano *et al.*, 2008). The 1 and 3 mg/kg doses of rimonabant did not significantly reduce food intake in the lean rats, but those doses did reduce food intake in the obese Zuckers. Moreover, the 10 mg/kg dose of rimonabant only reduced food intake by 20% for leans and just over 30% for obese, suggesting that the obese Zuckers showed stronger effects to the 10 mg/kg dose. Vickers *et al.* (2003) also showed that a 10 mg/kg dose of rimonabant reduced food intake in free-feeding Zuckers by 45% in obese Zuckers, but only 25% in the leans. Hence, our data are consistent with this study in showing differences in Zuckers in terms of the effects of rimonabant on food intake.

The effects of rimonabant at the 10 mg/kg dose were smaller for food intake (20–30% reduction; effects size 0.42) compared with the PR task (~60% reduction; 0.65 effect size). Although it may be unfair to compare food intake with PR performance because they are qualitatively different types of tasks, a brief discussion seems necessary. One reason for the discrepancy may have to do with the observation that food intake was measured after operant sessions were conducted, so motivation for food may have been weaker during food intake assessment. However, the typical number of food pellets earned by lean and obese Zucker rats was 10–12 per session. Ten 45-mg food pellets would equal 450 mg. The Zuckers ate a range of 8–15 g of food during 2 h free-feed sessions, so the 450 mg of food probably did not subtract much from the reinforcing properties of food during these 2 h free-feed sessions.

A second reason why rimonabant may be more effective for food gained under PR schedules versus free food

intake may be the nature of the food. Some studies suggest that palatable food is more sensitive to effects of rimonabant compared with less palatable food (e.g. Arnone *et al.*, 1997; Ward and Dykstra, 2005). This assertion, however, is confounded by the number of times an animal makes contact with palatable versus less palatable food during baseline conditions (Thornton-Jones *et al.*, 2007 for a discussion on this topic). Moreover, some studies have reported little difference in the ability of rimonabant to reduce food intake in foods that differ in palatability (Verty *et al.*, 2004). Therefore, this issue is still unclear. We offer a third reason: the type of response output that is required to gain access to food in each task differs. With food intake, the rat only needed to move toward the food aperture once and was able to eat as much as possible. Under the PR schedule, the rat was required to move toward the lever and emit a series of lever presses to gain access to a single 45 mg pellet. Indeed, the rat had to repeat this process for each pellet, and the requirement increased with each reinforcer. Hence, the response to reinforcer ratio in the PR task was greater (and increased throughout the session) compared with the food intake task, in which the response cost was small and the potential reinforcer was large. It would seem then, that effects of rimonabant may be greatest when the response to reinforcer ratio is larger. Future research may focus on better characterizing this relationship by examining food access across a range of costs (small to large) that vary from session to session and hold the amount of food in each 'eating bout' constant. The behavioral economic approach, which characterizes reinforcer efficacy by examining the number of reinforcers earned at different unit prices (e.g. 1–100 lever presses for access to 1–5 food pellets) between sessions, may be a good place to begin. This would allow for careful control of response requirement, reinforcer amount, as well as palatability of the reinforcer.

In summary, rimonabant dose-dependently reduced breakpoints for obese and lean Zucker rats. Obese Zucker rats exhibited effects of rimonabant at doses lower than lean Zuckers with regard to food reinforcer efficacy under a PR schedule, as well as food intake. Effects of rimonabant were also revealed in the extinction condition, by possible disruption of conditioned reinforcement associated with cues paired with food. These data have implications for rimonabant as an antiobesity treatment. Some individuals who may exhibit genetically induced or environmentally induced leptin insensitivity (Sahu, 2004a, b), a common observation with obese individuals, may exhibit a sensitivity to this drug, though the mechanism for this sensitivity (e.g. leptin-mediated endocannabinoid sensitivities, higher contacts with food, etc.) is unclear at this point. Further research on interactions of rimonabant with behavior is needed.

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