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Voluntary exercise augments acute effects of CB1-receptor inverse agonist on body weight loss in obese and lean mice

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Abstract

Cannabinoid CB1 receptor (CB1R) inverse agonists reduce appetite and body weight (BW) gain in various species. Exercise is thought to be a natural reward process and the cannabinoid system is also believed to influence reward. We tested the hypothesis that voluntary exercise would augment the effects of AM251, a CB1R inverse agonist, on food intake (FI) and BW loss in murine genetic models of obesity. *ob/ob*, agouti yellow (A^y), and lean C57BL/6J mice were treated via oral gavage with vehicle or AM251 (1, 3, or 10 mg/kg) 1 h before the dark cycle. The suppressive effects of 3 and 10 mg/kg AM251 on overnight FI, BW gain, and water intake (WI) were significant in *ob/ob* mice. In contrast, in A^y mice, 10 mg/kg AM251 decreased FI and BW gain while it did not influence WI. Food consumption of *ob/ob* and A^y mice, as evidenced by feeding frequency (FF) and feeding duration (FD), was reduced by AM251 for 4–6 h. AM251 at these doses had no impact on the appetitive behavior or BW gain of lean mice. After a 1-week wash-out period, mice were given running wheels in their home cages. With running wheel exercise, lean and obese mice exhibited increased sensitivity to AM251. Low voluntary wheel running activity of *ob/ob* mice precluded detection of combined effects of AM251 and exercise in this genetic model of obesity. Lean and agouti mice given AM251 combined with exercise lost a greater amount of BW than with AM251 alone. Our data suggest that voluntary exercise can enhance CB1R inverse agonist effects on appetite and BW loss in both lean and agouti obese mice.

Keywords: Food intake; Obesity; Cannabinoid; Running wheels; *ob/ob* mice; Agouti mice

1. Introduction

The central cannabinoid system has been implicated in the control of appetite in humans and rodents following the discovery of cannabinoid receptors and the identification of endogenous ligands for these receptors (Mattes et al., 1994; Kirkham and Williams, 2001). The function of endocannabinoids in the regulation of energy homeostasis is mediated by G-protein coupled cannabinoid CB1 receptors (CB1R), located primarily in the central nervous system (Berrendero et al., 1998). CB1R are densely expressed in the cerebral cortex, basal ganglia, hippocampus, amygdala, and classical pain pathways, including brain areas implicated in motivation, mood, and appetite regulation (Herkenham et al., 1991; Matsuda et al., 1993). There is evidence that CB1R density in the hippocampus, cortex, nucleus accumbens, and entopeduncular nucleus is inversely correlated with palatable food intake (FI) in diet-induced obese (DIO) rats (Harrold et al., 2002). Delta^9-tetrahydrocannabinol (delta^9-THC), an active component of marijuana and exogenous cannabinoid receptor agonist, increases feeding in humans (Foltin et al., 1988) and rodents (Koch, 2001; Williams et al., 1998; Williams and Kirkham, 2002b).

Conversely, acute blockade of CB1R with the selective inverse agonist, SR141716A, suppresses FI in rats (Rinaldi-Carmona et al., 1994; Arnone et al., 1997), marmosets (Simiand et al., 1998), and mice (Ravinet-Trillou et al., 2003) and attenuates the orexigenic effect of anandamide and delta^9-THC in rats (Williams and Kirkham, 1999, 2002a). Longer term studies with SR141716 have shown desensitization of its effects on appetite with a sustained reduction in body weight (BW) gain, indicating possible effects on energy expenditure (Colombo et al., 1998; Ravinet-Trillou et al., 2003). Moreover, chronic treatment with

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CB1R inverse agonists SR141716A or AM251 prevents diet-induced obesity in mice (Hildebrandt et al., 2003; Ravinet-Trillou et al., 2003). Deletion of a gene coding for the CB1R is associated with a reduction in FI, as *Cb1r*-deficient mice (Zimmer et al., 1999) consume less food than wild-type mice following a period of food deprivation (Di Marzo et al., 2001).

There is increasing evidence implicating the involvement of endocannabinoids in the regulation of FI and BW. Endogenous cannabinoids such as anandamide and 2-arachidonoyl ethanolamide (2-AG) increase FI in rats (Kirkham and Williams, 2001). Central injection of anandamide into the ventromedial hypothalamus or of 2-AG into the nucleus accumbens shell stimulates feeding in rats (Jamshidi and Taylor, 2001; Williams and Kirkham, 1999; Kirkham et al., 2002). Levels of endocannabinoids vary depending on metabolic state. Fasting increases anandamide and 2-AG levels in the brain while refeeding decreases those levels (Kirkham et al., 2002). In addition, endogenous cannabinoids may be controlled by the adipocyte hormone leptin (Zhang et al., 1994; Friedman and Halaas, 1998). Endocannabinoid levels are increased in the hypothalamus of mice lacking leptin signaling (*ob/ob*) and exogenous leptin treatment decreases their levels (Di Marzo et al., 2001). The hyperphagia observed in *ob/ob* and *db/db* mice may be attributed, in part, to high levels of endocannabinoids in the brain. SR141716A reduces FI of leptin-deficient *ob/ob* and *db/db* mice (Di Marzo et al., 2001), indicating that its effects may result from disruption of endocannabinoid tone.

Leptin has been shown to induce anorectic effects by acting through central melanocortin-4 receptors (MC4R) (Seeley et al., 1997). The hyperphagia and adult-onset obesity seen in agouti yellow obese (A^y) mice results from ectopic expression of agouti protein in the brain where it acts as an inverse agonist at MC4R (Fan et al., 1997). Although agouti mice have high levels of leptin in the plasma, they are in a leptin-resistant state such that animals do not respond to endogenous or exogenous leptin (Correia et al., 2002; Halaas et al., 1997, 2002; Rahmouni et al., 2002). It is possible that the normal inhibition of leptin on endocannabinoids is lifted in agouti mice, which leads to high levels of endogenous cannabinoids that in turn cause hyperphagia and obesity. The role of CB1R in the control of FI in agouti obese mice has not been reported.

Obesity has become a dramatic public health problem in the United States and other developed countries. Increased fat and high caloric intake, impaired fat mobilization from adipose tissue, and oxidation are believed to be responsible for obesity (Raben et al., 1994; Ranneries et al., 1998; Ravussin and Smith, 2002). Exercise is one method for reducing BW and adiposity. Exercise has been shown to increase lipolysis by activating the sympathetic nervous system (SNS), increasing adipose tissue blood flow, and stimulating fatty acid oxidation (Bulow and Madsen, 1976; Arner, 1995; Horowitz, 2001; Stich et al., 2000). We used a voluntary running wheel model to study exercise in mice; it has been proposed as a natural reward model (Sherwin, 1998).

Here we determined effects of CB1R inverse agonist treatment alone and in combination with voluntary running wheel exercise on ingestive behavior and BW gain in lean and obese *ob/ob* and A^y mice.

2. Materials and methods

2.1. Animals

All testing protocols used in this study were reviewed and approved by the Merck Research Laboratories Institutional Animals Care and Use Committee in Rahway, NJ. Mice were housed in either standard shoebox Nalgene mouse cages or in cages equipped with running wheels according to experimental requirements. Mice housed in running wheel cages were allowed access to the wheel at all times during the study period. The room was maintained at an ambient temperature of 22 ± 2 °C with a 12:12-h light/dark cycle (lights off at 1700 h). Mice were fed standard low fat mouse chow (Harlan Teklad Diet #7012, 5% kcal from fat, 3.75 kcal/gm). Mice were purchased from Jackson Laboratories (Bar Harbor, ME). Female (noncycling) *ob/ob* mice were studied at 20–22 weeks of age. Male A^y obese (C57BL/6J) and age-matched lean C57BL/6J mice were evaluated at 15–17 weeks of age. Animals were randomized and re-evaluated in experiments with or without access to running wheels. A minimum 7-day washout period was implemented between experiments.

2.2. Drug administration

AM251 was purchased from Tocris (Ellisville, MO) and dissolved in 5% Tween 80 (Sigma) in 0.5% methylcellulose. AM251 and vehicle were administered via oral gavage in a volume of 1 ml/100 g BW. Mice were treated with vehicle, 1, 3, or 10 mg/kg AM251 1 h prior to the dark cycle.

2.3. Feeding behavior and water intake (WI)

Mice were separated into individual microisolator cages for a minimum of 7 days before measuring FI. Milled chow was provided in a food hopper attached to the side of the cage. Food consumption was determined by measuring the amount of food remaining in the food cup overnight (18 h) and 24 h following vehicle or AM251 administration ($n = 6$ mice/dose). The difference between these measurements represented daily FI. BW changes over the 24-h period were also determined. Animal and food weights were measured using an electronic balance (Mettler Toledo). An automated FI system with infrared-feeding monitors (Mini-Mitter, Sunriver, OR) provided information about feeding frequency (FF) and feeding duration (FD). FF was reported as the number of times an animal broke the infrared photo

beam to approach the food hopper (bouts of feeding). FD was defined as the length of time the photo beam was interrupted. The cumulative pattern of FD typically matched the pattern of actual FI (in grams or kilocalories). Data were collected at 5-min intervals by using the VitalView data acquisition system (Mini-Mitter). Cumulative FF and FD (counts per 5-min interval) were determined for each animal. WI was determined by weighing the water bottle before and 18 or 24 h after drug treatment.

2.4. Running wheel activity

Mice were placed into their home cages with unlimited access to the running wheels (Mini-Mitter). Wheel revolutions were recorded using VitalView software (Mini-Mitter), and the distance traveled (in meters) by each animal was calculated. Drug treatments began after 5–7 days of acclimation to the running wheel cages.

2.5. Data analysis

Results are shown as the mean ± S.E.M. All data were analyzed by one-way ANOVA using StatView software (Abacus, CA). Fisher's post hoc analysis was performed to identify differences between treatment groups. Statistical significance was set at $P < .05$.

3. Results

3.1. Effects of AM251 on FI, BW, and WI in ob/ob, A^y obese, and lean mice without access to running wheels

AM251 dose-dependently reduced ad libitum FI of *ob/ob* mice without access to running wheels. AM251 at 10 mg/kg decreased overnight FI significantly [$F(1,8) = 32.7$, $P < .001$ vs. vehicle] (Fig. 1A). Cumulative 24-h FI was decreased following 3 and 10 mg/kg AM251 administration compared to vehicle-treated mice [$F(1,8) = 12.4$, $P < .001$; $F(1,8) = 39.1$, for 3 and 10 mg/kg, respectively] (Fig. 1A). These doses of AM251 evoked a reduction in overnight BW gain of *ob/ob* mice [$F(1,8) = 6.9$, $P < .05$, 3 mg/kg vs. vehicle; $F(1,8) = 20.5$, $P < .001$, 10 mg/kg vs. vehicle] and continued to have an effect at 24 h (Fig. 1B). Furthermore, 3 and 10 mg/kg AM251 reduced overnight WI of *ob/ob* mice [$F(1,8) = 12.8$; $F(1,8) = 33.8$, $P < .01$, for 3 and 10 mg/kg, respective-

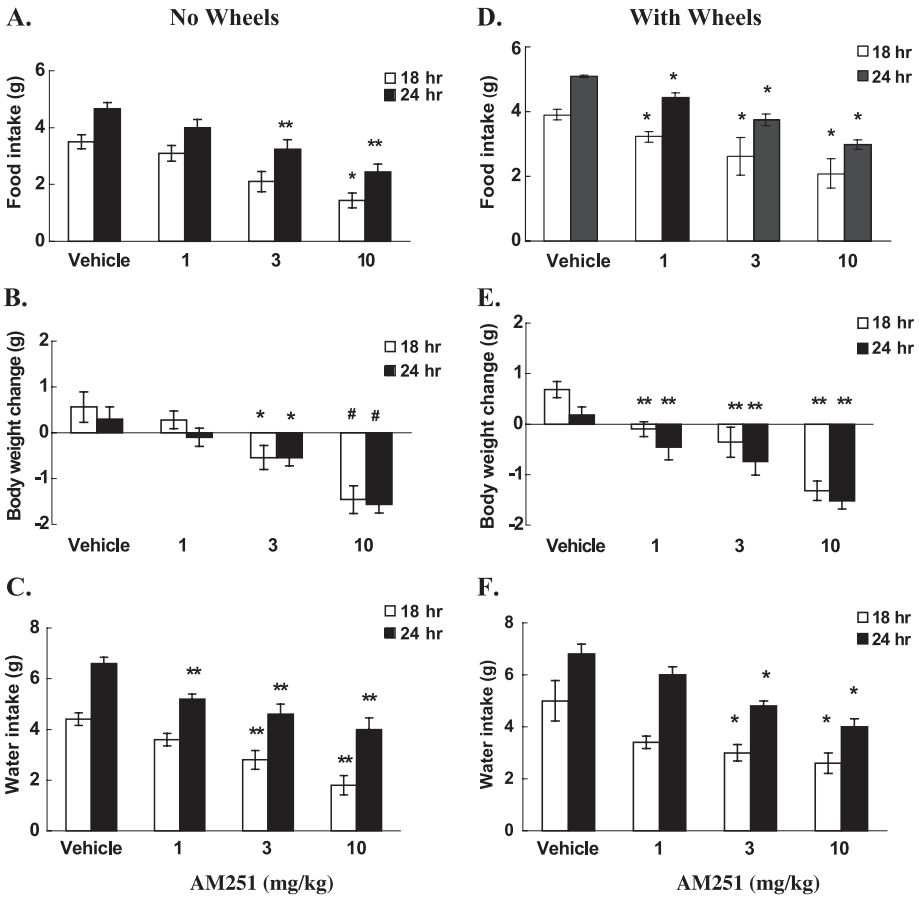

Fig. 1. Effects of vehicle or AM251 (1, 3, or 10 mg/kg) treatment on FI, BW gain, and WI of *ob/ob* mice with and without access to running wheels. (A) AM251 reduced FI, (B) BW change, and (C) WI of *ob/ob* mice without wheel access. (D) AM251 coupled with running wheel access reduced FI, (E) BW gain, and (F) WI of *ob/ob* mice. $*P < .05$, $**P < .01$, $^{\#}P < .001$ versus vehicle; $n = 6$/group. Data are presented as mean ± S.E.M.

ly]. All doses of AM251 decreased daily (24 h) WI of *ob/ob* mice significantly [$F(1,8)=19.6$; $F(1,8)=18.2$; $F(1,8)=26$, $P<.01$, for 1, 3, and 10 mg/kg, respectively] (Fig. 1C).

Male agouti obese mice were given the same doses of AM251. Significant reductions in FI and BW gain were evident only at the high dose (10 mg/kg) of AM251 [$F(1,8)=41.7$, $P<.01$] (Fig. 2A). FI following 10 mg/kg AM251 treatment remained decreased at 24 h [$F(1,8)=22.4$, $P<.01$] (Fig. 2A). Although all animals lost some BW overnight, mice given 10 mg/kg AM251 lost the most BW [$F(1,8)=17.3$, $P<.01$]. The effects of 10 mg/kg AM251 on BW were evident at 24 h [$F(1,8)=30.3$, $P<05$] (Fig. 2B). AM251 treatment had no effect on WI in agouti obese mice at the doses examined (data not shown).

Lean mice received the same drug treatments as the obese mice. Blockade of CB1R using AM251 did not exert obvious effects on feeding or drinking behavior of lean mice at any doses tested (Fig. 3A). Lean mice were less sensitive to the anorectic effects of AM251 than agouti obese mice.

We measured FF and FD to further characterize feeding in response to AM251 treatment. Reductions in FF and FD reflected the decreased FI in *ob/ob* mice (Table 1). AM251 reduced FF in a dose-dependent manner [$F(1,288)=20$, 22, 46.1, $P<.001$]. The reduction in FD after AM251 treatment was similar to that of FF [$F(1,288)=17.4$, 31.5, 47.5, $P<.001$ vs. vehicle].

Agouti obese mice exhibited different feeding patterns following AM251 treatment. The low dose of AM251, 1 mg/kg, which did not reduce FI, suppressed FF measured overnight [$F(1,288)=12$, $P<.01$]. However, FD was not affected by 1 mg/kg AM251 [$F(1,288)=0.2$, $P>.05$]. AM251 at 3 mg/kg had no effect on FF or FD. In agouti obese mice, 10 mg/kg AM251 reduced FF [$F(1,288)=16.3$, $P<.001$] without an effect on FD (Table 1).

AM251 treatment did not change FF of lean mice, consistent with its lack of effect on the amount of food consumed. However, FD was altered significantly following 1 or 3 mg/kg AM251 treatment [$F(1,288)=12.7$, $P<.001$ for 1 mg/kg; $F(1,288)=8.3$, $P<.001$ for 3 mg/kg]. Mice given 10 mg/kg AM251 did not show changes in FD or FF. The increased FD observed in lean mice given lower doses of AM251 may reflect alterations in ambulatory behavior or other behaviors (e.g., grooming) that were not monitored.

3.2. Effects of AM251 on FI, BW, and WI in ob/ob, A^y obese, and C57BL/6 lean mice with access to running wheels

Voluntary wheel running was found to augment the effects of AM251 on feeding behavior and BW loss in both obese and lean mouse models. The 1 mg/kg dose of AM251, which had no effect on FI or BW gain in *ob/ob* mice without access to running wheels (Fig. 1A), decreased overnight FI [$F(1,8)=9.6$, $P<.05$] (Fig. 1D) and resulted in a significant BW loss [$F(1,8)=12.8$, $P<.01$] (Fig. 1E). AM251, at 3 and 10 mg/kg, decreased FI, BW, and WI (Fig. 1F), similar to the findings in mice without exercise. Decreases in FI correlated with reductions in FF and FD in *ob/ob* mice (Table 1). The baseline running wheel

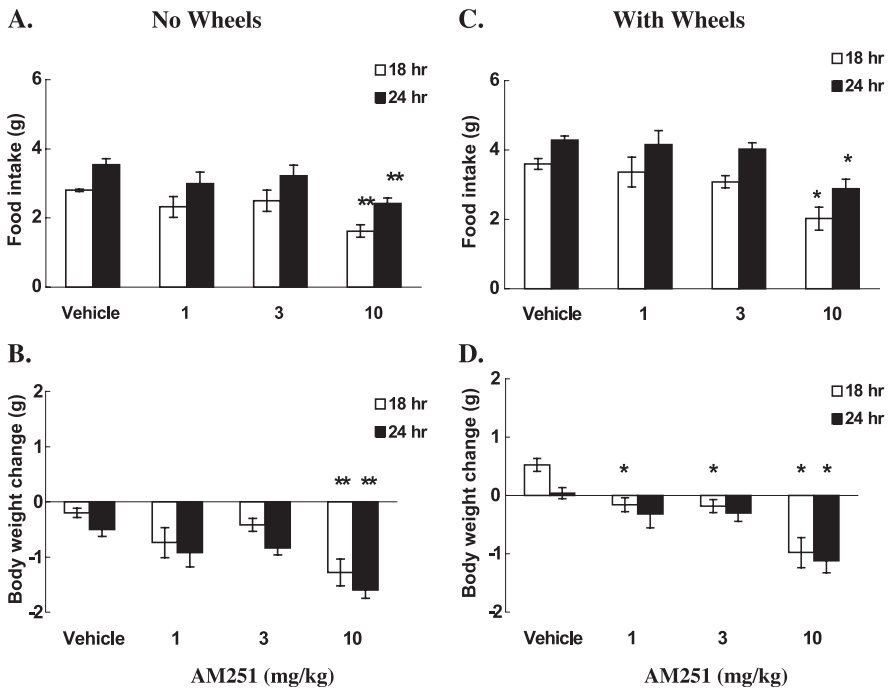

Fig. 2. Effects of vehicle or AM251 (1, 3, or 10 mg/kg) treatment on FI and BW gain of agouti mice with and without access to running wheels. (A) AM251 reduced FI and (B) BW gain of agouti mice without running wheels. (C) AM251 reduced FI and (D) BW gain of agouti mice with running wheel access. * $P<.05$, ** $P<.01$ versus vehicle; $n=6$/group. Data are presented as mean ± S.E.M.

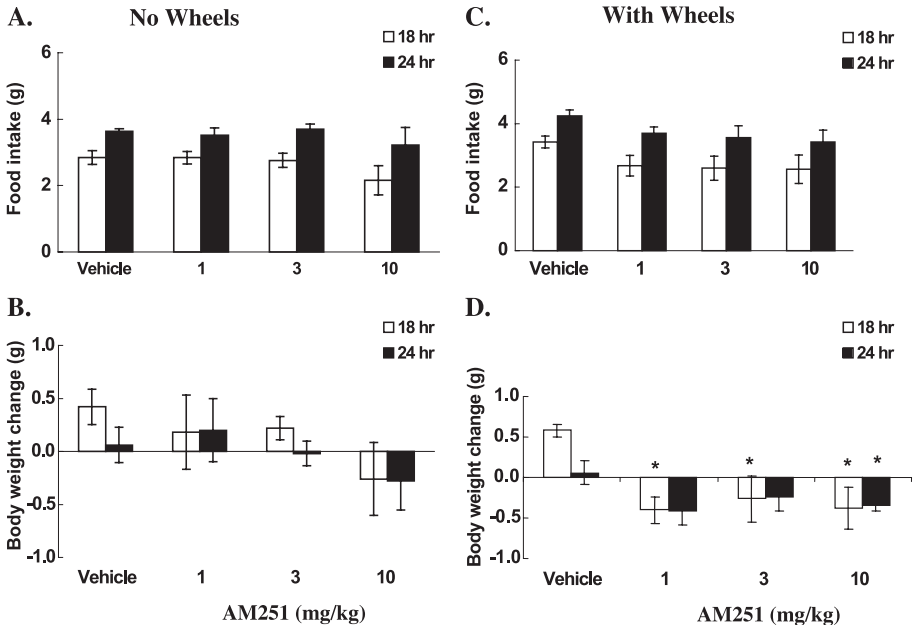

Fig. 3. Effects of vehicle or AM251 (1, 3, or 10 mg/kg) treatment on FI and BW gain of lean mice with and without running wheel access. (A) AM251 did not significantly alter FI or (B) BW gain of lean mice without running wheels. (C) AM251 coupled with running wheel access did not significantly alter FI of lean mice. (D) AM251 coupled with running wheel access significantly reduced BW gain of lean mice. Data are presented as mean ± S.E.M. * $P < .05$ versus vehicle; $n = 6$/group.

activities of *ob/ob* mice were very low (1–2 turns/day) even after AM251 administration (data not shown). The low voluntary running wheel activity of *ob/ob* mice precluded us from detecting combined effects of exercise and CB1R inverse agonist treatment in this genetic model of obesity (Fig. 5A).

Overnight and cumulative 24-h FIs were not different between vehicle- and AM251-treated (1 and 3 mg/kg) agouti obese mice with running wheels (Fig. 2C). However, mice given 1 or 3 mg/kg AM251 lost BW overnight relative to vehicle-treated controls [$F(1,8) = 17.1$ or 19.7, $P < .01$, respectively]. Vehicle-treated mice maintained their BW over the 24-h period, while 1 and 3 mg/kg AM251 treatment

Table 1
Overnight FF and FD of *ob/ob*, A^y, and lean mice given vehicle or AM251 with or without (bold) access to running wheels

Vehicle			AM251 1 mg/kg	AM251 3 mg/kg	AM251 10 mg/kg
ob/ob	FF	**11.1 ± 1.4**	**4.4 ± 0.3** *	**4.1 ± 0.6** *	**1.5 ± 0.3** *
		13.6 ± 1.4	4.6 ± 0.5 *	3.6 ± 0.5 *	2.2 ± 0.3 *
	FD	**28.5 ± 2.9**	**14.2 ± 1.8** *	**10.1 ± 1.4** *	**6.8 ± 1.1** *
		26.6 ± 2.3	17.1 ± 1.7 *	13.3 ± 1.7 *	12.2 ± 1.6 *
A^y	FF	**6.0 ± 0.7**	**3.4 ± 0.4** *	**5.6 ± 0.5**	**2.9 ± 0.4** *
		6.0 ± 0.6	5.7 ± 0.5	5.4 ± 0.5	2.7 ± 0.4 *
	FD	**9.5 ± 1.0**	**10.3 ± 1.3**	**12.1 ± 1.1**	**7.5 ± 1.1**
		13.8 ± 1.4	20.7 ± 1.6 *	8.8 ± 0.9 *	7.3 ± 1.1 *
C57BL/6	FF	**4.2 ± 0.6**	**4.5 ± 0.5**	**4.4 ± 0.6**	**2.9 ± 0.4**
		6.0 ± 0.7	3.2 ± 0.4 *	4.1 ± 0.4 *	3.6 ± 0.4 *
	FD	**7.1 ± 1.1**	**13.2 ± 1.3** *	**11.8 ± 1.2** *	**6.7 ± 1.1**
		11.6 ± 1.5	9.3 ± 1.1	9.3 ± 1.0	9.6 ± 1.0

Values shown are the mean ± S.E.M. ($n = 6$/group). Bold values indicate mice without running wheels. Other values represent mice with running wheels. Units for FF and FD are represented as counts/5 min interval.

* Indicates a significant difference between vehicle and AM251-treated group ($P < .05$).

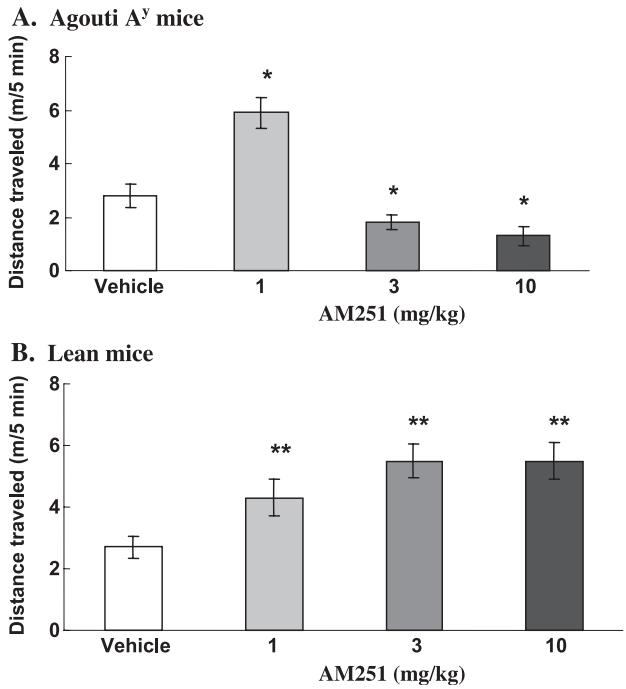

Fig. 4. Distance traveled overnight by agouti and lean mice following vehicle or AM251 treatment (1, 3, and 10 mg/kg). (A) Low-dose AM251 increased the distance traveled by agouti obese mice and higher doses decreased their running wheel activity. (B) AM251 significantly increased the distance traveled by lean mice in a running wheel. Data are presented as mean ± S.E.M. * $P < .05$, ** $P < .01$ versus vehicle; $n = 6$/group.

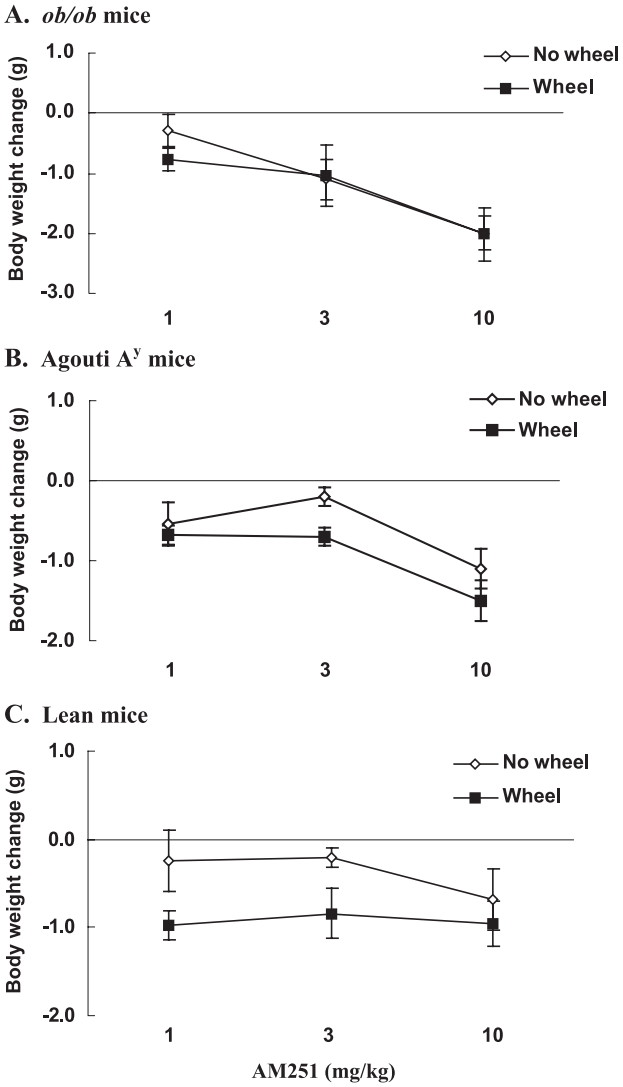

Fig. 5. AM251 effects on BW change relative to vehicle when administered alone or combined with voluntary running wheel exercise in (A) *ob/ob* mice, (B) agouti obese mice, or (C) lean mice.

evoked slight BW losses [$F(1,8)=2.1$, 4.0, $P>.05$ for both] (Fig. 2D). When coupled with running wheel exercise, 10 mg/kg AM251 decreased overnight FI [$F(1,8)=18.3$, $P<.005$] and BW gain of agouti obese mice significantly [$F(1,8)=28.6$, $P<.005$] and had effects at 24 h.

FI reductions in agouti mice following 10 mg/kg AM251 treatment with exercise correlated with decreased FF [$F(1,290)=22.8$, $P<.001$] and FD [$F(1,290)=14.9$, $P<.01$] (Table 1). Effects of lower AM251 doses on FD were variable in agouti obese mice. AM251 at 1 mg/kg increased FD [$F(1,290)=10.4$, $P<.005$] while 3 mg/kg AM251 decreased FD overnight [$F(1,290)=9.6$, $P<.01$]. Importantly, 1 mg/kg AM251 increased running wheel activity of agouti obese significantly, as measured by distance traveled overnight [$F(1,290)=34.7$, $P<.001$] (Fig. 4A). In contrast, 3 or 10 mg/kg AM251 reduced running wheel activity significantly. Vehicle-treated agouti obese mice traveled 2.8 ± 0.4 m during the night, while AM251-treated mice traveled 1.8 ± 0.3 and 1.3 ± 0.3 m [$F(1,290)=7.0$; $F(1,290)=12.7$, $P<.001$] for 3 and 10 mg/kg, respectively. The amount of BW loss following a single AM251 dose was augmented by exercise in agouti obese mice (Fig. 5B).

AM251 treatment did not modify the amount of food consumed by lean mice with running wheel access (Fig. 3C). However, overnight BW gain was reduced significantly when mice received AM251 [$F(1,8)=27.9$, 8.7, 13.2, $P<.05$ for 1, 3, or 10 mg/kg AM251, respectively] (Fig. 6D). BW gain at 24 h post-treatment was reduced significantly by 10 mg/kg AM251 [$F(1,8)=6.1$, $P<.05$]. AM251 treatment did not influence WI of lean mice (data not shown). All doses of AM251 suppressed FF overnight (Table 1). Although AM251-treated mice had less frequent feeding bouts than vehicle-treated mice [$F(1,288)=12.5$, 5.6, 9.6, $P<.01$ for 1, 3, and 10 mg/kg AM251, respectively], their FD was not affected. Thus, the absolute

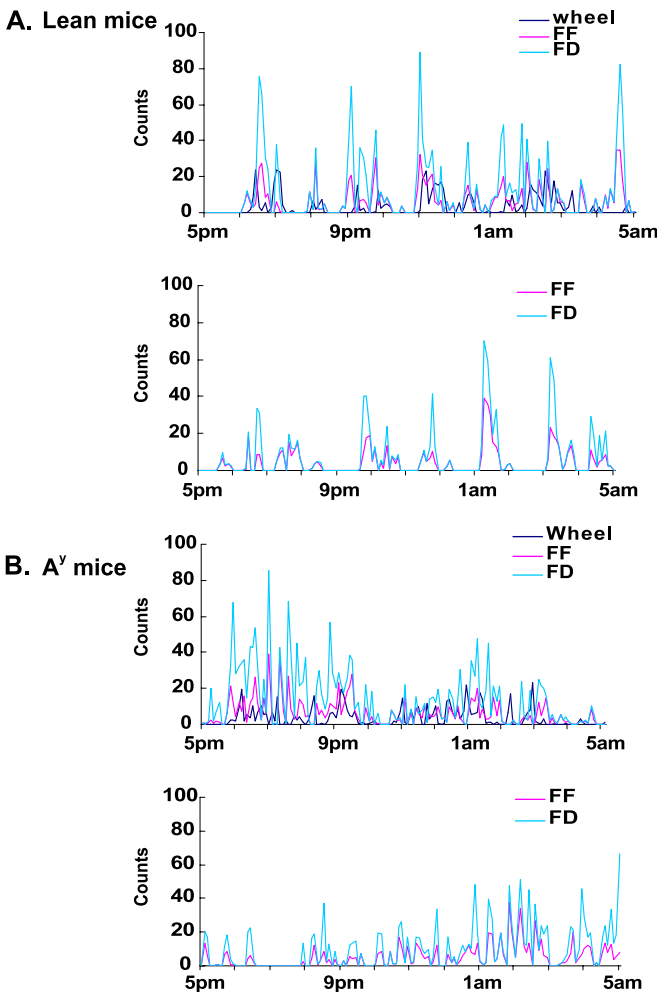

Fig. 6. Overnight FD and FF of vehicle-treated (A) lean or (B) agouti obese mice with and without running wheel access. Measures of FD and FF are shown as units/5 min interval. Animals housed with running wheels are indicated by "wheel."

amount of food consumed by lean mice was not decreased by AM251.

Moreover, AM251 increased overnight running wheel activity (distance traveled) of lean mice in a dose-dependent manner (Fig. 4B). Lean mice treated with AM251 traveled 4.3 ± 0.6, 5.5 ± 0.5, and 5.5 ± 0.6 m/5 min overnight for 1, 3, and 10 mg/kg AM251, respectively, while vehicle-treated mice ran less (2.7 ± 0.4 m/5 min) [$F(1,288) = 5.4$, 18.1, 15.1, $P < .05$, respectively]. The increased running wheel activity of lean mice likely reduced FF because animals spent more time running in wheels rather than visiting the food cup. The magnitude of BW loss following a single AM251 dose was augmented by exercise in lean mice (Fig. 5C).

We evaluated the feeding patterns of vehicle-treated *ob/ob*, A^y obese, and lean mice during the 12-h dark cycle. The running wheel was without effect on the feeding behavior of *ob/ob* mice as they did not run appreciably (data not shown). In contrast, the presence of a running wheel in the cage modified the feeding behavior (frequency and duration of feeding bouts) of both lean and agouti mice (Fig. 6). Lean mice increased their FD and FF when allowed to run in a wheel. The temporal characteristics of feeding behavior of agouti obese mice were changed when a wheel was present. Agouti obese mice exhibited more frequent feeding bouts and greater duration of meals during the early portion of the dark cycle. Representative plots of overnight feeding and wheel running recordings are shown (Fig. 6).

4. Discussion

This work demonstrated that voluntary running wheel exercise can augment the effects of the CB1R inverse agonist AM251 on BW gain in agouti A^y obese and lean mice. When using AM251 alone, a high dose was required to decrease FI and BW gain in agouti obese mice. However, when combined with exercise, a lower dose of AM251 could reduce BW gain in both agouti obese and lean mice. This might result, in part, from the ability of AM251 to increase locomotor activity in lean and agouti obese mice. AM251 increased the distance traveled overnight by lean mice in a dose-dependent manner. Intraperitoneal administration of the CB1R inverse agonist, SR141716A, increases c-Fos expression in the nucleus accumbens via reduced dopamine D_2 function (Alonso et al., 1999), which indicates that CB1R inverse agonists may activate the dopamine reward system in the brain to stimulate locomotor activity. Voluntary wheel running regulates brain monoamine metabolites (Elam et al., 1987), increases CSF β-endorphin levels (Hoffmann et al., 1990), and up-regulates brain dynorphin mRNA (Werme et al., 2000). Notably, AM251 increased the locomotor activity of agouti obese mice at a low dose but suppressed activity at higher doses, suggesting that interactions between the cannabinoid and dopamine systems might be altered by overexpression of agouti protein. Alternatively, differences in the pharmacokinetics of AM251 in different mouse models might explain the variability in locomotor stimulation observed. Direct comparisons between the groups of mice cannot be made as they were not studied contemporaneously and female *ob/ob* mice were used while male lean and agouti mice were examined.

Locomotor stimulatory effects of AM251 were not seen in *ob/ob* mice due to their low baseline running wheel activities. Obese *ob/ob* mice are known to exhibit reduced locomotor activity, metabolism, and body temperature (Pelleymounter et al., 1995). In addition, *ob/ob* mice exhibit increased immobility in a model of depression (Collin et al., 2000). Long-term CB1R inverse agonist treatment may be needed to stimulate wheel running activity of *ob/ob* mice. The suppressive effects of AM251 on the feeding we observed in female *ob/ob* mice are in agreement with previous findings with SR141716A on fasting-induced feeding in *ob/ob* and *db/db* mice (Di Marzo et al., 2001). We demonstrated that AM251 decreased overnight FI, WI, and BW gain of *ob/ob* mice lacking circulating leptin. Furthermore, AM251 reduced the FF and FD of *ob/ob* mice with and without running wheel exercise. FF and FD in the presence of a running wheel were likely to be influenced by other behaviors evoked in response to its presence in the cage. The availability of an alternative activity, like wheel running, can alter daily feeding patterns and other behaviors such as climbing, grooming, and sleeping (Stewart et al., 1985; Harri et al., 1999).

Here we show that a CB1R inverse agonist can suppress FI in agouti A^y obese mice with defective hypothalamic melanocortin-4 receptor (MC4-R) signaling. It is unknown whether agouti obese mice have alterations in brain endocannabinoid levels. Agouti obese mice are in a leptin-resistant state (Halaas et al., 1997; Correia et al., 2002; Harris et al., 2002; Rahmouni et al., 2002), therefore the suppressive effects of leptin on endogenous cannabinoids may be disinhibited, leading to hyperphagia. Although 1 and 10 mg/kg AM251 decreased FF in agouti mice without running wheel access, FD was not affected. We found that 1 mg/kg AM251 increased the distance that agouti obese mice traveled in a running wheel and that higher doses decreased their running wheel activity. Similarly, low-dose AM251 increased FD and higher doses decreased FD. Spontaneous wheel running is a model for natural rewarding behavior (Sherwin, 1998). In the presence of a running wheel, vehicle-treated agouti obese mice exhibited increased FD and a shift in the temporal characteristics of their feeding behavior during the dark cycle with earlier and longer bouts of feeding. Agouti mice are reported to be stress sensitive when fasted or placed into social isolation; however, once they adapt to the housing conditions, their feeding normalizes (De Souza et al., 2000). The agouti obese mice used in our study were acclimated to their cages for 1 week prior to behavioral recordings. Together, these findings support a role for endogenous cannabinoids in the control of FI and BW gain in agouti obese mice. The mechanisms by which

cannabinoids are involved in appetite regulation of agouti obese mice and their interactions with the melanocortin system need further investigation.

We demonstrated that AM251 did not influence FI of lean mice fed ad libitum with low fat chow. Our finding is in agreement with the report that SR141716A did not affect regular chow intake in mice (Arnone et al., 1997). CB1R inverse agonists may preferentially affect the intake of more palatable foods. Chronic treatment with SR141716 was reported to have a greater effect on FI and BW gain in obese than in lean Zucker rats (Vickers et al., 2003). The endogenous cannabinoid system may be involved in modulating an animal's motivation to feed and the appetitive value of the diet by influencing brain reward systems. CB1R inverse agonists SR141716A and AM251 can block fasting-induced hyperphagia in lean mice (Di Marzo et al., 2001; Zhou and Shearman, unpublished). Further evidence for the role of reward systems is the finding that SR141716A decreases sucrose and ethanol intake in lean mice and the intake of a palatable cane sugar mixture in marmosets (Arnone et al., 1997; Simiand et al., 1998). Moreover, SR141716A has been shown to decrease the rewarding effects of intracranial electrical brain stimulation in rats (Deroche-Gamonet et al., 2001). This process may involve modulation of the mesencephalic dopamine or serotonin systems and release of catecholamines (Tzavara et al., 2003). Alternatively, the increased running wheel activity may be a reflection of increased arousal following AM251 treatment. SR141716A has been shown to enhance arousal in rats, suggesting that endogenous cannabinoid(s) may be involved in the sleep-waking cycle (Santucci et al., 1996). We have not observed changes in home cage locomotor or open field activity in response to AM251 treatment (with a 30 mg/kg dose) (data not shown).

Exercise increases lipolysis mainly by activating the SNS via beta-adrenergic receptors located on adipocytes (Bartness and Bamshad, 1998; Bartness et al., 2002). Lipolysis causes the breakdown of triglycerides to fatty acids and glycerol, which then enter the bloodstream. Cannabinoid receptor agonists WIN55212-2 and CP55940 were found to lower plasma adrenaline concentrations while SR141716A counteracted this effect (Niederhoffer et al., 2001). Moreover, SR171416A increased norepinephrine efflux in the anterior hypothalamus when measured in free moving rats (Tzavara et al., 2001). Therefore, it is possible that CB1R inverse agonist treatment increases sympathetic activity, which in turn stimulates lipolysis. Recent papers report direct CB1-mediated actions on fat metabolism (Bensaid et al., 2003; Cota et al., 2003). CB1 mRNA is present in adipocytes (Bensaid et al., 2003; Cota et al., 2003), and SR141716 treatment stimulated adiponectin (Acrp30) mRNA expression in adipocytes of obese Zucker (*fa/fa*) rats (Bensaid et al., 2003). Exercise may augment the lipolytic effects evoked by the CB1R inverse agonist AM251 alone. In summary, our findings demonstrate that voluntary running wheel exercise augments the acute effects of AM251 on BW loss in agouti A^y obese and lean mice. Long-term effects of combined exercise and CB1R inverse agonist treatment on BW and adiposity need further investigation.

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