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# AM 251 produces sustained reductions in food intake and body weight that are resistant to tolerance and conditioned taste aversion

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**1** The cannabinoid 1 ($CB_1$) receptor has been implicated in the regulation of food intake. Here, we examine the effect of the $CB_1$ receptor antagonist AM 251 on food intake and body weight over a prolonged period. Further, we examine whether AM 251 produces conditioned taste aversion (CTA) and if sustained antagonism at central receptors contributes to its anorectic effect.

**2** The effect of AM 251 of food intake and body weight was examined in daily (1 mg kg$^{-1}$) and 5-day (5 mg kg$^{-1}$) dosing schedules. Matching reductions in food intake and body weight were observed in both paradigms. A single administration of AM 251 (5 mg kg$^{-1}$) significantly reduced food intake for 4 days. Tolerance to the anorectic effects of AM 251 did not develop in either dosing strategy.

**3** Active avoidance of AM 251 (3; 5 mg kg$^{-1}$, i.p.) was examined using a CTA assay. Rats showed no evidence of CTA associated with AM 251.

**4** We investigated the sustained effect of AM 251 (5 mg kg$^{-1}$, i.p.) on $CB_1$ receptors in the hypothalamus using $\Delta^9$-tetrahydrocannabinol (8 mg kg$^{-1}$, i.p.) induced hypothermia. AM 251 initially blocked hypothermia, but this effect was not seen 2 or 4 days later.

**5** The results demonstrate that smaller, or infrequent, administrations of AM 251 can produce sustained reductions in food intake and body weight in rat. Reductions in food intake were sustained longer than AM 251 antagonized the effects of a $CB_1$ receptor agonist in the hypothalamus, and occurred independently of CTA.
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**Keywords:** AM 251; CB1 receptor antagonist; conditioned taste aversion; food intake; hypothermia; feed efficiency

**Abbreviations:** $CB_1$, cannabinoid 1 receptor; CTA, conditioned taste aversion; DMSO, dimethyl sulphoxide; i.p., intraperitoneal; LiCl, lithium chloride; THC, $\Delta^9$-tetrahydrocannabinol

## Introduction

The cannabinoid type 1 ($CB_1$) receptor has been strongly implicated in the regulation of food intake (Di Marzo & Matias, 2005). $CB_1$ receptors are present in the brain and periphery. In the brain, $CB_1$ receptors have been identified in pathways responsible for reward and energy balance (Cota *et al*., 2003; Robbe *et al*., 2003; Pickel *et al*., 2004). In the periphery, $CB_1$ receptors have been identified in the gut (Croci *et al*., 1998; Kulkarni-Narla & Brown, 2000; Coutts *et al*., 2002), as well as on hepatocytes (Osei-Hyiaman *et al*., 2005) and adipocytes (Bensaid *et al*., 2003; Cota *et al*., 2003). $CB_1$ receptor agonists such as $\Delta^9$-tetrahydrocannabinol (THC) and the endocannabinoids, anandamide and 2-arachidonoylglycerol, increase food intake in both humans (Hart *et al*., 2002) and animals (Kirkham *et al*., 2002). This action is blocked or reversed by the selective $CB_1$ receptor antagonist SR 141716A (Kirkham & Williams, 2004) (Rimonabant) and by the structurally and pharmacologically similar compound AM 251 (Hildebrandt *et al*., 2003; Chambers *et al*., 2004). In rodent hypothalamus, endogenous cannabinoid levels increase when animals are food deprived and decrease during feeding (Kirkham *et al*., 2002). Endogenous cannabinoid levels are also under negative control by the anorectic hormone leptin (Di Marzo *et al*., 2001). Currently, $CB_1$ receptor knockout mice are the only genetically engineered rodent to express a lean phenotype. Weight loss in these animals is associated with reductions in daily food intake as well as reduced fat synthesis and storage (Cota *et al*., 2003; Ravinet-Trillou *et al*., 2004; Jbilo *et al*., 2005; Osei-Hyiaman *et al*., 2005).

In a previous study, we showed that a single administration of the $CB_1$ antagonist AM 251 led to a dose-dependent reduction in food intake and body weight for up to 6 days (Chambers *et al*., 2004). Others have reported that chronic treatment with $CB_1$ antagonists leads to sustained weight loss, but only to a transient reduction in daily food intake (Colombo *et al*., 1998; Bensaid *et al*., 2003; Hildebrandt *et al*., 2003; Ravinet-Trillou *et al*., 2003; Vickers *et al*., 2003; Liu *et al*., 2005). We examined the effect of AM 251 on food intake and body weight over a prolonged period of treatment to see if the effects we previously observed waned over time. For comparison, we studied the same dose of AM 251 given repeatedly using a daily dosing regimen to assess whether the total amount of the drug or whether the frequency of administration altered the effects on body weight and food intake.

The sustained effect of AM 251 on food intake could be due to adverse effects of the $CB_1$ receptor antagonist. Speculation that reductions in food intake by $CB_1$ receptor antagonists, in

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part or in whole, result from nausea and/or malaise (De Vry *et al*., 2004b; McLaughlin *et al*., 2005) is supported anatomically and behaviourally. Endogenous cannabinoids (endocannabinoids) and $CB_1$ receptors are found in the brainstem in areas involved in emesis, including the area postrema (Van Sickle *et al*., 2001). In ferrets (Van Sickle *et al*., 2001) and shrews (Darmani, 2001), $CB_1$ receptor antagonists potentiate emesis, and in rats, $CB_1$ antagonists can induce active avoidance of a food item associated with treatment, also known as a conditioned taste aversion (CTA) (De Vry *et al*., 2004b; McLaughlin *et al*., 2005). In these studies, reductions in the consumption of flavoured water associated with the effects of SR 141716A or AM 251 were significant at doses often used in studies examining feeding behaviour ($\sim$8–10 mg kg$^{-1}$) (Colombo *et al*., 1998; Bensaid *et al*., 2003; Hildebrandt *et al*., 2003; Ravinet-Trillou *et al*., 2003; Vickers *et al*., 2003; Liu *et al*., 2005). The estimated $ED_{50}$ for AM 251 in the CTA assay was approximately 3.6 mg kg$^{-1}$ (McLaughlin *et al*., 2005), and 6.5 mg kg$^{-1}$ for SR 141716A (De Vry *et al*., 2004b). As this is within the dose range we previously employed, we investigated whether AM 251 caused CTA under our dosing regimen, using a previously described, and highly sensitive, paradigm for this assessment (Maggio & Koopmans, 1982).

The sustained action of AM 251 over 5 days (Chambers *et al*., 2004) could also be due to continued antagonism at the $CB_1$ receptor. The half-life of this drug injected intraperitoneally (i.p.) is approximately 22 h in rat (McLaughlin *et al*., 2003). Based on dose–response curves obtained in previous studies (Chambers *et al*., 2004; Rutkowska 2004) there could be enough AM 251 from a 5 mg kg$^{-1}$ dose of the receptor antagonist to sustain a significant anorectic effect over 5 days. We chose to examine the extent of $CB_1$ receptor antagonism in the central nervous system following a single administration of AM 251 over 5 days. We examined the effect of AM 251 on agonist-induced reductions in thermoregulation, because the effects of $CB_1$ agonists on thermoregulation are centrally mediated (Fitton & Pertwee, 1982; Rawls *et al*., 2002), and because $CB_1$ antagonists alone have no effect on body temperature (De Vry *et al*., 2004a).

## Methods

### *Animals*

In all experiments, male Lewis strain rats were individually housed in transparent plastic cages between 20 and 22°C under a 12 h light–dark cycle (lights off 19:30 h). Standard rat chow (Canadian Lab Diets, Inc., Leduc, AB, Canada) and water were freely available except where indicated. All rats were habituated to testing and handling procedures for at least 7 days prior to testing. All methods used in this study were approved by the University of Calgary Animal Care Committee and were carried out in accordance with the guidelines of the Canadian Council on Animal Care.

### *Drugs*

AM 251 (*N*-(piperidin-1-yl)-5-(4-iodophynyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; Tocris Cookson Inc., Ellisville, MO, U.S.A.) was dissolved in dimethyl sulphoxide (DMSO) using gentle sonication before being diluted with Tween 80 and saline (2% DMSO, 1% Tween 80, 97% saline) to final concentrations of 1, 3, and 5 mg ml$^{-1}$ delivered i.p. (Chambers *et al*., 2004). THC (Lipomed, Arlesheim, Switzerland) was supplied predissolved in anhydrous ethanol solution, spun into a viscous concentrate using a Speed Vac concentrator (Savant Instruments Inc., Farmingdale, NY, U.S.A.), and resuspended into vehicle consisting of DMSO (44%), Tween 80 (1%), and saline (55%) at a final concentration of 8 mg ml$^{-1}$ delivered i.p. (Pertwee & Tavendale, 1977). A 0.1 M solution of LiCl was made up in sterile distilled water. AM 251, THC, and vehicle treatments were delivered i.p. in a volume of 1 ml kg$^{-1}$, LiCl and saline were delivered i.p. in a volume of 10 ml kg$^{-1}$ (Benoit, Air, Wilmer *et al*., 2003). All chemicals were supplied by Fisher Scientific (Fairlawn, NJ, U.S.A.), except where indicated.

### *AM 251 and food intake*

To examine the effect of AM 251 given daily, or every 5 days, on food intake and body weight, 17 rats weighing between 440 and 540 g were fed vanilla flavoured Ensure Plus® liquid diet (Abbott Laboratories, Abbott Park, IL, U.S.A.). The Ensure is a highly palatable, nutrient dense, complete meal replacement composed of 53.3% carbohydrate, 29% fat, and 16.7% protein (1.41 kcal g$^{-1}$). Food was available for 17 h each day starting at 16:00 h. Food and water were presented in inverted glass bottles that were attached to the outside of the cage in order to minimize spillage. Rats were injected daily at 15:45 h with vehicle 1 ml kg$^{-1}$ i.p. (mean bodyweight $\pm$ standard error of the mean (s.e.m.); 461 $\pm$ 7.3 g, $n=5$), or AM 251 (1 mg kg$^{-1}$) (462 $\pm$ 8.5 g, $n=6$), or every 5 days with AM 251 (5 mg kg$^{-1}$) (465.3 $\pm$ 9.1 g, $n=6$). Food intake and body weight measurements were recorded daily.

### *AM 251 and CTA*

To determine whether two highly anorectic doses of AM 251 produced active avoidance of a food item associated with the effects of AM 251, 15 male Lewis rats (315–368 g) were assigned to either AM 251 (3 and 5 mg kg$^{-1}$) and vehicle treatment, or LiCl and saline ($n=5$) treatment, conditions (Hildebrandt *et al*., 2003; McLaughlin *et al*., 2003; Shearman *et al*., 2003; Chambers *et al*., 2004; Chen *et al*., 2004; Rutkowska, 2004; Zhou & Shearman, 2004). During training trials, rats learned to associate the effects of AM 251 with one flavour and the effects of vehicle with another flavour. During test trials, rats chose between AM 251 and vehicle-associated flavours. A similar protocol was used with LiCl and saline to demonstrate that this procedure results in CTA to flavours associated with the effects of a noxious substance (Garcia *et al*., 1985). Avoidance is shown by the selection of one flavour over another.

Rats were weighed each day between 10:00 and 11:00 h. Food intakes were adjusted on a daily basis to maintain each rat's original body weight and increase the incentive to eat food pellets. Each rat was habituated to training and handling procedures for at least 7 days prior to the beginning of the study. During habituation, rats had access to 20 unflavoured pellets. Rats were permitted to remain in the chamber for up to 20 min, or until they had eaten at least five of 20 pellets, which ever came first. All rats consumed at least 10 unflavoured

pellets during the last 2 days of habituation, and most rats ate most of their pellets.

*CTA assay*: The experimental design was adapted from a previous study (Maggio & Koopmans, 1982) and carried out in specially designed chambers. The outer wall of the chamber consisted of a transparent plastic cylinder 24 cm $h \times 11$ cm $r$. Rats were able to access and consume individual pellets placed on a circular shelf within the cylinder from a well located in the centre of the chamber (8 cm $h \times 12$ cm diameter).

During training trials, opposing flavours were paired with the effects of AM 251 or its vehicle *via* a counter balance design. Rats were placed in the chamber at approximately 13:00 h where they had access to 20 raspberry- or banana-flavoured food pellets (Research Diet, New Brunswick, NJ, U.S.A.). Injections were given 30 min after the last pellet was eaten. If no pellets were eaten, no treatment was given. The number of pellets eaten and the length of time in minutes before the first pellet was eaten (first pellet latency) were recorded during each training trial. Exposure to the effects of AM 251 and its vehicle was separated by 24 h and followed by a single test trial 24 h later in which rats were able to choose between vehicle- and AM251-associated flavours. A similar protocol was used for LiCl- and saline-treated rats. Each animal completed four training trails and four testing trials over 12 days.

During testing, 12 pellets from each flavour were arranged in an alternating fashion in order to provide a choice between treatment- and vehicle-associated flavours. When no aversion is present, vehicle- and treatment-associated flavours have an equal probability of being selected. To ensure that there was always a choice between vehicle- and treatment-associated flavours, rats were only permitted to eat 10 out of a possible 24 pellets. The probability of a single animal choosing the same flavour 10 times in a row solely due to chance alone is less than $1 \times 10^{-4}$. Rats in the 3 mg kg$^{-1}$ treatment and LiCl treatment groups were given 20% of the full dose for each LiCl- and AM 251-associated flavoured pellet eaten to avoid the possibility of extinction of a conditioned response. To avoid the possibility that AM 251 given in the presence of both flavours would impede the ability of animals to discriminate between the effects of treatment and vehicle conditions with their respective flavours, animals in the 5 mg kg$^{-1}$ treatment group were not injected on the day of testing. All animals completed each test trial within 20 min.

### *AM 251 and THC-induced hypothermia*

To determine how long AM 251 continued to antagonize $CB_1$ receptors in the hypothalamus, we examined the extent of THC-induced hypothermia in rats treated with AM 251 45 min, 48 h, and 96 h after administration. Briefly, silicone-coated temperature data loggers (SubCue Inc., Calgary, Canada) were surgically implanted into the abdominal cavity of male Lewis rats (300–350 g) under halothane anaesthesia (4% induction; 2–2.5% maintenance). Rats were allowed to recover for 3 days before being acclimatized to testing and handling procedures. Core body temperature readings were sampled every 15 min for 75 min during the course of each experiment.

Rats were randomly assigned into three independent groups. On day 1, at 10:45 h, all rats received an injection of AM 251 (5 mg kg$^{-1}$). After 45 min, group 1 was injected i.p. with THC ($n = 4$) to assess the hypothermic effects of the $CB_1$ receptor agonist in the presence of AM 251 (Costa *et al*., 1999; Rawls *et al*., 2002). The duration of AM 251's effect was tested in group 2 ($n = 5$) 48 h later, and group 3 ($n = 5$) 96 h later. Baseline temperature readings were taken 15 min prior to the onset of each experiment. Differences in temperature are shown as area under the curve over 75 min after administration.

### *Statistics*

Data were analysed with two-way independent measures ANOVA, two-way mixed design ANOVA, and one-way ANOVA where appropriate. Comparisons were made using paired and un-paired $t$-tests (two tailed), Newman–Keuls multiple comparison test, and Bonferroni post-test where appropriate.

## Results

### *Food intake*

AM 251 significantly reduced food intake in both daily and 5-day treated rats compared with vehicle-treated controls, $F_{(2,15)} = 24.33$, $P < 0.0001$. There was also a significant treatment by time interaction, $F_{(58,15)} = 5.93$, $P < 0.0001$ (Figure 1a). The source of the interaction was investigated by comparing differences between vehicle- and AM 251-treated rats at each time point using a one-way analysis of variance, and Newman–Keuls multiple comparison tests.

Reductions in food intake were significant for 4 days after the first dose of AM 251 (5 mg kg$^{-1}$) in the 5-day dosing schedule, compared with vehicle-treated controls, $P < 0.05$. Reductions in food intake tended to be greater in rats given the 5 mg kg$^{-1}$ dose compared with the 1 mg kg$^{-1}$ dose of AM 251; however, the extent of that reduction diminished between treatments, whereas reductions in daily food intake in rats treated daily with AM 251 (1 mg kg$^{-1}$) were consistent from treatment to treatment. Differences in food intake between 5 and 1 mg kg$^{-1}$ treatments were nonsignificant at all times, $P > 0.05$. Contrary to previous findings (Colombo *et al*., 1998; Bensaid *et al*., 2003; Hildebrandt *et al*., 2003; Ravinet-Trillou *et al*., 2003; Vickers *et al*., 2003) tolerance to the anorectic effects of AM 251 was not observed in either dosing paradigm. Consistent with previous findings (Hildebrandt *et al*., 2003; Vickers *et al*., 2003), rats treated with AM 251 became significantly hyperphagic shortly after treatment ended, $F_{(58,15)} = 5.93$, $P < 0.0001$. Overeating relative to vehicle-treated rats was significant on day 21, as well as during days 23–28 in both daily and 5-day dosing schedules, $P < 0.05$.

To determine whether there was a significant difference in overall food intake between daily and 5-day dosing schedules during and after treatment, the cumulative food intake data were analysed using a one-way independent measures ANOVA. Similar overall intakes were observed between rats treated daily ($762 \pm 31$ g) and rats treated every 5 days ($750 \pm 24$ g) over 15 days of treatment, $P > 0.05$, Newman–Keuls multiple comparison tests (Figure 1b). During treatment, rats in both dosing paradigms ate significantly less than vehicle-treated rats over the same period of time ($967 \pm 47$ g, $P < 0.001$). Differences in cumulative food intake between rats treated daily ($499 \pm 15$ g) and rats treated every 5 days ($512 \pm 8$ g) were also

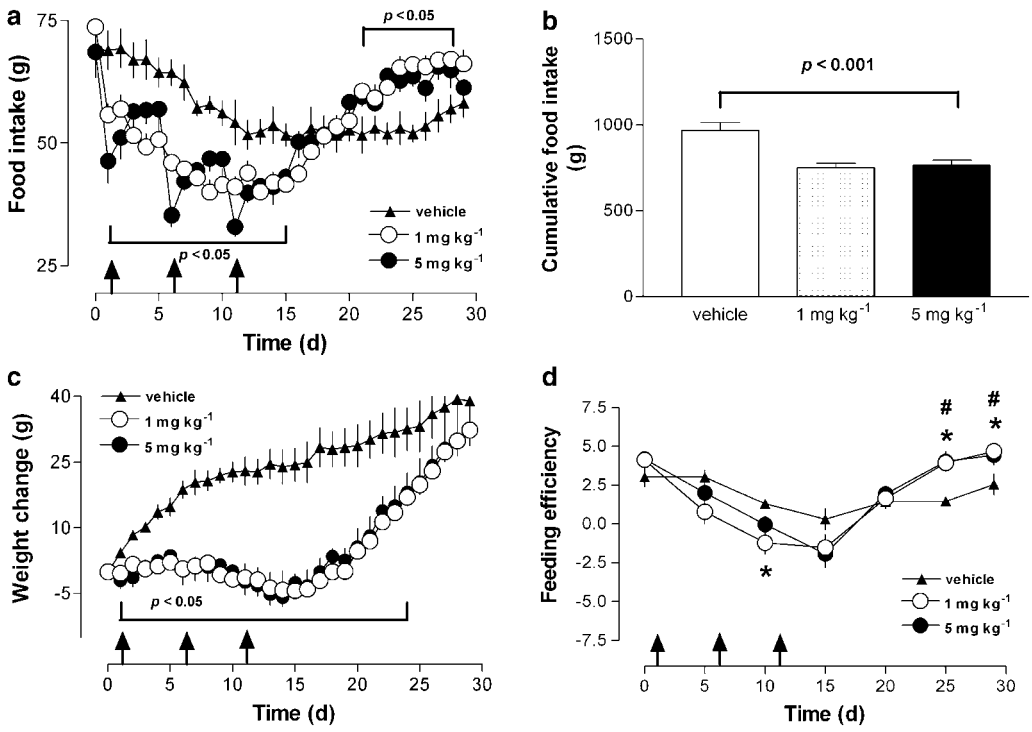


**Figure 1** (a) Daily food intake (mean ± s.e.m.; g) expressed in grams. Vehicle-treated rats (black triangles), 1 mg kg$^{-1}$ day$^{-1}$ AM 251 (open circles), 5 mg kg$^{-1}$ every 5 days AM 251 (black circles). Arrows indicate where 5 mg kg$^{-1}$ treatments were given. Each rat received identical amounts of AM 251 per body weight over 15 days. Significant differences in food intake between vehicle- and AM 251-treated rats are shown by the bars, $P<0.05$, Newman–Keuls multiple comparison test. Note the rebound hyperphagia that developed after treatment ended. $P<0.05$. (b) Cumulative food intake (g) from day 1 to day 15 during treatment (mean ± s.e.m.; g) with either vehicle (open bar) or AM 251 (1 mg kg$^{-1}$ day$^{-1}$, grey bar; 5 mg kg$^{-1}$ every 5 days, black bar). Note that the overall reduction in food intake was essentially the same between rats treated daily and rats treated every 5 days with AM 251 relative to vehicle-treated controls. $P<0.001$. (c) Changes in body weight (mean ± s.e.m.; g) starting the day before treatment with either vehicle (black triangles), or AM 251 (1 mg kg$^{-1}$ day$^{-1}$, open circles; 5 mg kg$^{-1}$ every 5 days, black circles). Note that weight loss is similar in both groups of rats given AM 251, even though treatment ends on day 11 in rats treated every 5 days and on day 15 in rats treated daily. (d) Feeding efficiency ($\Delta$ body weight/food intake $\times$ 100 mean ± s.e.m.) of vehicle (black triangles) and AM 251 (1 mg kg$^{-1}$ day$^{-1}$, open circles; 5 mg kg$^{-1}$ every 5 days, black circles) treated rats is shown in 5-day intervals during and after treatment. During treatment, feeding efficiency tended to be lower in AM 251-treated rats compared with vehicle-treated controls, suggesting that changes in metabolism and/or energy expenditure also contributed to the effect of AM 251 on body weight. However, such differences were only statistically significant in rats treated daily with AM 251 during days 5–10. $P<0.05$. Interestingly, feeding efficiency was significantly greater in rats treated daily and rats treated every 5 days with AM 251 relative to vehicle-treated rats after treatment ended, suggesting that rats treated with AM 251 compensated for decreases in feeding efficiency caused by AM 251. *$P<0.05$ (1 mg kg$^{-1}$ day$^{-1}$), $^{\#}P<0.05$ (5 mg kg$^{-1}$ every 5 days).

nonsignificant during days 21–28 after treatment ended, $P>0.05$ (Figure 1a). Vehicle-treated rats (427 ± 17 g) ate significantly less than AM 251-treated rats during the same period of time, $P<0.01$. Daily and 5-day treated rats were given the same amount of AM 251 over 15 days. The results demonstrate that the major effect on food intake by AM 251 occurred independently of the dosing strategy employed.

### *Body weight*

Reductions in body weight were very similar between daily and 5-day treated rats. AM 251 prevented weight gain in both dosing strategies. Figure 1c shows that differences in weight change between vehicle- and AM 251-treated rats were significant from day 1 to day 24, $P<0.05$, Newman–Keuls multiple comparison test. A two-way ANOVA performed on the cumulative weight change data showed that differences in weight change between vehicle- and AM 251-treated rats were significant between groups ($F_{(2,15)}=28.4$, $P<0.0001$) and over time ($F_{(29,15)}=38.2$, $P<0.0001$). The difference in weight gain in vehicle-treated rats during the first 15 days of the study also created a significant treatment by time interaction, $F_{(58,15)}=5.85$, $P<0.01$.

### *Feeding efficiency*

Rats treated with AM 251 tended to gain significantly less weight than vehicle-treated rats for every gram of food eaten, $F_{(2,15)}=32.5$, $P<0.0001$ (Figure 1d). Feeding efficiency data are presented as the change in body weight over total food intake (g) $\times$ 100 during 5-day intervals, starting 5 days prior to treatment. Although AM 251 tended to reduce feeding efficiency such differences were only statistically significant in rats treated daily with the $CB_1$ receptor antagonist during days 5–10 relative to vehicle-treated controls, $P<0.05$. After the end of the treatment, feeding efficiencies were significantly increased in both daily and 5-day treated rats from days 21 to 30 creating a significant treatment by time interaction,

$F_{(12,15)} = 13.1$, $P < 0.0001$. The results demonstrate that rats were able to compensate for reductions in feeding efficiency caused by AM 251 after treatment ended.

### *AM 251 and CTA*

Rats did not demonstrate any aversion to flavours associated with two highly anorectic doses of AM 251 (3; 5 mg kg$^{-1}$). In a free-choice test trial, the ratio between pellets selected from vehicle- and treatment-associated flavours is roughly 50/50 when no aversion is present. Figure 2a shows that rats were as likely to eat flavours associated with AM 251 as they were vehicle-associated flavours. In contrast, rats showed a robust aversion to flavours associated with LiCl after the very first training trial (single-trial learning), as previously observed (Garcia *et al*., 1976; 1985; Maggio & Koopmans, 1982), but no aversion to flavours associated with saline treatment. A two-way mixed design ANOVA performed on the percentage of flavoured pellets eaten showed that differences between treatments were highly significant, $F_{(2,12)} = 96.2$, $P < 0.0001$, whereas differences between trials were nonsignificant, $F_{(3,12)} = 0.02$, $P = 0.96$.

Significant differences between LiCl- and AM 251-treated rats were followed up using paired and unpaired two-tailed *t*-tests. Differences between AM 251- and LiCl-associated flavours were significant during each trial, $P < 0.0001$, whereas differences between 3 and 5 mg kg$^{-1}$ treatments were non-significant at all times, $P > 0.28$. The combined average of AM 251-associated flavours in the percentage of pellets eaten data was 49.8 ± 0.3%. In comparison, LiCl-associated flavours were selected only 1.0 ± 1.0% of the time over four trials. In the last three test trials, no flavoured pellets associated with LiCl treatment were eaten.

The lack of aversion to flavours associated with AM 251 was also evident in the data gathered during training trials. Figure 2b shows the average latency to eat the first pellet in minutes during trials 2–4. The results show that first pellet latency was significantly longer to flavours associated with LiCl treatment (16.4 ± 1.8 min) compared with saline control flavours, (3.2 ± 1.1 min, $P < 0.01$, two-tailed paired *t*-test). In comparison, rats showed similar latencies between vehicle- (1.6 ± 0.4 min) and AM 251 (3 mg kg$^{-1}$, 1.4 ± 0.4 min, 5 mg kg$^{-1}$, 1.2 ± 0.1 min)-associated flavours, $P = 0.52$. The results demonstrate a lack of aversion to flavours paired with the effects of AM 251. Data from the first training trial were excluded from this analysis on the basis that rats were naïve to their respective treatments at this time.

### *AM 251 and thermogenesis*

We tested the ability of AM 251 to block the hypothermic effects of THC given 45 min, 48 h, and 96 h after administration of the $CB_1$ antagonist. A two-way independent measures ANOVA and follow-up analysis performed on the cumulative change in temperature data showed that while AM 251 was highly effective at blocking the hypothermic effect of THC given 45 min later, $P < 0.01$ (unpaired *t*-test), it no longer antagonized the hyperthermic action of THC given 48 or 96 h later, creating a significant treatment × time interaction $F_{(2,2)} = 5.41$, $P = 0.01$ (Figure 3). The results demonstrate that a 5 mg kg$^{-1}$ dose of AM 251 no longer antagonized the hypothermic effects of a $CB_1$ receptor agonist after 48 h.

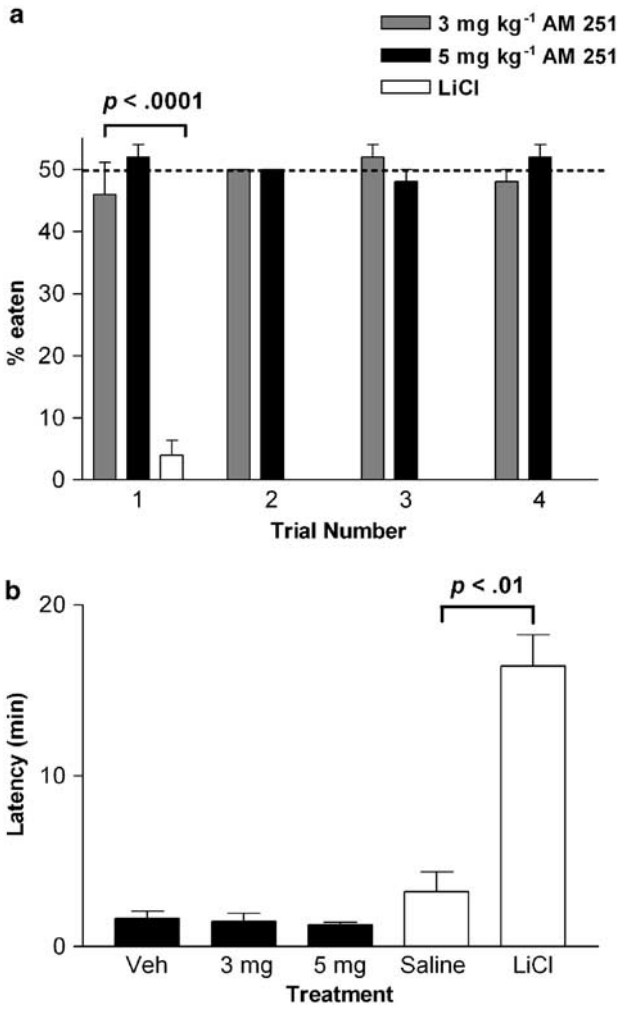


**Figure 2** (a) The percentage of pellets eaten data shows the selection of flavours associated with either AM 251 (2.5 mg kg$^{-1}$; grey bars; 5 mg kg$^{-1}$; black bars) or LiCl (open bars) treatment, relative to the selection of vehicle- or saline-associated flavours, respectively, during each test trial. Each rat was permitted to eat 10 out of a possible 24 flavoured pellets. Note that CTA occurs on the first trial for all LiCl-treated rats, but does not occur in AM 251-treated rats, even after four training and testing trials, $P < 0.0001$, unpaired *t*-test. Rats consistently chose saline-associated flavours over flavours associated with LiCl, whereas rats treated with AM 251 showed no preference for vehicle-associated flavours. (b) Differences in the latency (mean ± s.e.m.; min) before the first pellet was eaten between AM 251 or its vehicle (black bars) and LiCl or saline (open bars)-treated rats during training trials 2–4. Note the significant increase in the average latency to flavours associated with LiCl treatment. After the first training trial, rats avoided the flavour associated with LiCl treatment. Rats that refused to eat any flavoured pellets were timed out after 20 min, resulting in a significant increase in the average first pellet latency associated with LiCl injection. $P < 0.01$, paired *t*-test.

## Discussion

We show that a 1 mg kg$^{-1}$ dose of AM 251 given daily, or a 5 mg kg$^{-1}$ dose given every 5 days, produced matching reductions in food intake and body weight. Contrary to previous findings, reductions in food intake continued to be significant throughout treatment with no evidence of tolerance to the anorectic effect of AM 251 in either dosing strategy.

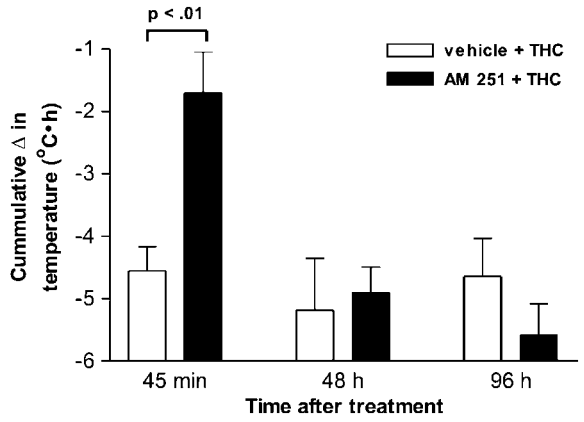


**Figure 3** The difference in temperature, from baseline, over 75 min after THC administration (mean ± s.e.m.; °C h) in rats treated with either vehicle (open bars) or AM 251 (black bars). Differences are shown as area under the curve. Note that AM 251 did not antagonize the effect of THC 48 or 96 h after administration. $P<0.01$, unpaired *t*-test.

The fact that a $5\,mg\,kg^{-1}$ dose of AM 251 did not antagonize the central effects of THC after 48 h suggests that the effect on food intake in the 5-day dosing strategy was not mediated by continued antagonism of $CB_1$ receptors in the central nervous system. The same dose of AM 251 significantly reduced food intake for 4 days without causing CTA, even after multiple training and testing trials. Consistent with findings by others, AM 251 also tended to reduce feeding efficiency, suggesting that reductions in body weight were related to changes in metabolism and/or increased energy expenditure.

In comparison, the majority of studies investigating the effect of $CB_1$ receptor antagonists on feeding behaviour report that decreases in daily food intake caused by chronic-daily administration of AM 251 or SR 141716A diminish over time, often to the point that differences between vehicle-and antagonist-treated animals are no longer significant after the first week of treatment (Colombo *et al*., 1998; Bensaid *et al*., 2003; Hildebrandt *et al*., 2003; Ravinet-Trillou *et al*., 2003; Vickers *et al*., 2003). For example, Wistar rats, weighing between 280 and 330 g, developed tolerance to the anorectic effect of daily SR 141716A ($2.5\,mg\,kg^{-1}$, i.p.) after only 3 days (Colombo *et al*., 1998), and obese mice treated orally with $3.0\,mg\,kg^{-1}$ of AM 251 developed tolerance to the anorectic effect of that compound after only 8 days (Hildebrandt *et al*., 2003).

Remarkably, in those studies and others (Ravinet-Trillou *et al*., 2003; Vickers *et al*., 2003), reductions in body weight relative to vehicle-treated animals continued to be significant throughout treatment, even though the daily food intakes of antagonist-treated animals were similar to those of vehicle-treated controls. Interestingly, tolerance also develops faster in lean, compared with obese, animals (Vickers *et al*., 2003). The observation that tolerance is linked to existing energy stores strongly suggests that peripheral factors are also involved in the development of resistance to the anorectic effect of $CB_1$ receptor antagonists. Consistent with this hypothesis, activity at $CB_1$ receptors increases *de novo* lipogenesis in the liver (Osei-Hyiaman *et al*., 2005) as well as in the adipose tissue (Cota *et al*., 2003; Ravinet-Trillou *et al*., 2004), possibly by inhibiting adenosine monophosphate-activated protein kinase (Kola *et al*., 2005), or through the induction of enzymes of the $\beta$-oxidation and tricarboxylic acid cycle (Jbilo *et al*., 2005). By inhibiting lipolysis, $CB_1$ receptor antagonists could increase the number of calories required to replace and maintain existing energy stores, thereby creating conditions in which increases in food intake fail to produce additional growth. Increases in adiponectin mRNA, a circulating hormone that leads to weight loss through increased oxygen consumption (thermogenesis), could give rise to similar conditions (Bensaid *et al*., 2003; Liu *et al*., 2005).

Consistent with the hypothesis that $CB_1$ receptor antagonists affect metabolic pathways in addition to the pathways that regulate feeding behaviour directly, treatment with SR 141716A ($10\,mg\,kg^{-1}$), or genetic ablation of the $CB_1$ receptor, also decreases feeding efficiency (Ravinet-Trillou *et al*., 2004; Jbilo *et al*., 2005). Feeding efficiency is a ratio that compares changes in body weight to the total number of calories ingested thereby providing an index of how well an organism is able to assimilate nutrients into body mass. Although it is likely that changes in the metabolism and energy expenditure also contributed to reductions in body weight in our study, the fact that feeding efficiency was only moderately reduced during both daily and 5-day dosing strategies suggests that the major effect of AM 251 on body weight in our study resulted from decreases in daily food intake. The reason that tolerance to the anorectic effect of AM 251 was not observed in our study was likely due to differences in the protocol. We employed a smaller dose of the $CB_1$ receptor antagonist ($1\,mg\,kg^{-1}$ per day) than is typically used in other studies (3–$10\,mg\,kg^{-1}$ per day) (Colombo *et al*., 1998; Bensaid *et al*., 2003; Hildebrandt *et al*., 2003; Ravinet-Trillou *et al*., 2003; Vickers *et al*., 2003; Liu *et al*., 2005), and in relatively large rats (480–580 g). Our results demonstrate that reductions in daily food intake by smaller, or less frequent, doses of AM 251 can be maintained in rats sustained on a highly palatable and nutrient-rich diet, possibly because of limited activity of the antagonist at $CB_1$ receptors in the pathways directly affecting lipolysis and energy expenditure.

We also found that reductions in food intake by AM 251 could not be attributed to feelings of malaise as measured by a highly sensitive, two-flavour CTA procedure. Given the sustained effect a single dose of AM 251 had on food intake, and based on work carried out by others (De Vry *et al*., 2004b; McLaughlin *et al*., 2005), one might expect that rats repeatedly trained to associate the physiological effects of AM 251 with a specific flavour would avoid that flavour in a free-choice test trial. Instead, we found that rats were as likely to select flavours associated with AM 251 as they were flavours associated with vehicle treatment. We also found no difference in the way rats approached AM 251- and vehicle-associated flavours, based on the time each rat took to begin eating over four training and testing trials. The results demonstrate that the anorectic effect of AM 251 in our study was not associated with malaise as measured by CTA at doses $\leqslant 5\,mg\,kg^{-1}$, even though CTA can be induced by $CB_1$ receptor antagonists when higher doses are used (De Vry *et al*., 2004b; McLaughlin *et al*., 2005).

To determine whether AM 251 was still acting on $CB_1$ receptors in the brain over a 5-day period, we examined how long AM 251 antagonized the effects of THC using a

thermoregulation assay, and found that AM 251 had no effect on hypothermia produced by an i.p. administration of the $CB_1$ agonist given 48 and 96 h later. $CB_1$ receptors are densely distributed in the anterior nucleus of the hypothalamus or preoptic area (Herkenham *et al*., 1991; Moldrich & Wenger, 2000), and thermosensitive neurons in the preoptic area are thought to be critical for thermoregulation (Boulant, 1981; Boulant, 2000). Because tolerance to the hypothermic effects of peripherally administered THC can be induced with intrahypothalamic administrations of the $CB_1$ agonist (Fitton & Pertwee, 1982), the major effect of $CB_1$ agonists on thermoregulation is thought to be mediated by $CB_1$ receptors in the hypothalamus. In our study, AM 251 had no significant impact on the effects of THC given 48 h later. Therefore, it is likely that $CB_1$ receptors in the central nervous system are no longer significantly antagonized by a $5\,mg\,kg^{-1}$ dose of AM 251 48 h after administration.

The observation that intermittent administration of AM 251 inhibits daily food intake and weight gain at least as well as daily dosing regimes in rats may have significant clinical implications for the use of $CB_1$ receptor antagonists in the treatment of human obesity. The advantages of an intermittent dosing strategy include targeting the peak anorectic effect of $CB_1$ receptor antagonist to times in which people are likely to overeat, and limiting the pharmacological dose and blockade of the $CB_1$ receptor to the shortest duration possible to limit unwanted side effects. It has long been known that people eat 8–13% more during weekends (St Jeor *et al*., 1983; de Castro, 2000). Data from our study suggest that weekly treatment with a $CB_1$ receptor antagonist given just prior to the weekend could enhance the efficacy of these agents by targeting their peak anorectic effect to times in which people are likely to overeat. Our data also demonstrate that only intermittent blockade of the $CB_1$ receptor is necessary to produce a prolonged inhibition of food intake. Mounting evidence suggests that the endogenous cannabinoid system plays important roles in gut function, suggesting that continued antagonism may lead to unwanted effects in this system. For example, daily administrations of $CB_1$ receptor antagonists have been associated with symptoms of nausea and diarrhoea in humans (Van Gaal *et al*., 2005) and endogenous activity at $CB_1$ receptors has been hypothesized to protect the gastrointestinal tract during inflammation as well as during abnormally high gastric and enteric secretions (Massa *et al*., 2004; Duncan *et al*., 2005). Minimizing the duration of antagonism at $CB_1$ receptors with a once or twice a week dosing strategy may result in fewer side effects and better patient compliance. However, additional studies will be needed to confirm these findings under experimental circumstances more closely related to those used in the treatment of human patients.

In summary, the matching reductions in food intake and bodyweight in rats treated either daily or infrequently with AM 251 demonstrate that a relatively low dose of the receptor antagonist is capable of producing significant changes in energy balance that are resistant to the acquisition of tolerance. These reductions are independent from noxious effects as measured by CTA and last longer than the pharmacological blockade of $CB_1$ receptors in the brain by AM 251.

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## References

BENOIT, S.C., AIR, E.L., WILMER, K., MESSERSCHMIDT, P., HODGE, K.M., JONES, M.B., ECKSTEIN, D.M., MCOSKER, C.C., SEELEY, R.J., WOODS, S.C. & SHELDON, R.J. (2003). Two novel paradigms for the simultaneous assessment of conditioned taste aversion and food intake effects of anorexic agents. *Physiol. Behav.,* **79,** 761–766.

BENSAID, M., GARY-BOBO, M., ESCLANGON, A., MAFFRAND, J.P., LE FUR, G., OURY-DONAT, F. & SOUBRIE, P. (2003). The cannabinoid CB1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. *Mol. Pharmacol.,* **63,** 908–914.

BOULANT, J.A. (1981). Hypothalamic mechanisms in thermoregulation. *Fed. Proc.,* **40,** 2843–2850.

BOULANT, J.A. (2000). Role of the preoptic-anterior hypothalamus in thermoregulation and fever. *Clin. Infect. Dis.,* **31,** S157–S161.

CHAMBERS, A.P., SHARKEY, K.A. & KOOPMANS, H.S. (2004). Cannabinoid (CB)1 receptor antagonist, AM 251, causes a sustained reduction of daily food intake in the rat. *Physiol. Behav.,* **82,** 863–869.

CHEN, R.Z., HUANG, R.R., SHEN, C.P., MACNEIL, D.J. & FONG, T.M. (2004). Synergistic effects of cannabinoid inverse agonist AM251 and opioid antagonist nalmefene on food intake in mice. *Brain Res.,* **999,** 227–230.

COLOMBO, G., AGABIO, R., DIAZ, G., LOBINA, C., REALI, R. & GESSA, G.L. (1998). Appetite suppression and weight loss after the cannabinoid antagonist SR 141716. *Life Sci.,* **63,** L113–L117.

COSTA, B., VAILATI, S. & COLLEONI, M. (1999). SR 141716A, a cannabinoid receptor antagonist, reverses the behavioural effects of anandamide-treated rats. *Behav. Pharmacol.,* **10,** 327–331.

COTA, D., MARSICANO, G., TSCHOP, M., GRUBLER, Y., FLACHSKAMM, C., SCHUBERT, M., AUER, D., YASSOURIDIS, A., THONEREINEKE, C., ORTMANN, S., TOMASSONI, F., CERVINO, C., NISOLI, E., LINTHORST, A.C., PASQUALI, R., LUTZ, B., STALLA, G.K. & PAGOTTO, U. (2003). The endogenous cannabinoid system affects energy balance *via* central orexigenic drive and peripheral lipogenesis. *J. Clin. Invest.,* **112,** 423–431.

COUTTS, A.A., IRVING, A.J., MACKIE, K., PERTWEE, R.G. & ANAVIGOFFER, S. (2002). Localization of cannabinoid CB(1) receptor immunoreactivity in the guinea pig and rat myenteric plexus. *J. Comp. Neurol.,* **448,** 410–422.

CROCI, T., MANARA, L., AUREGGI, G., GUAGNINI, F., RINALDICARMONA, M., MAFFRAND, J.P., LE FUR, G., MUKENGE, S. & FERLA, G. (1998). *In vitro* functional evidence of neuronal cannabinoid CB1 receptors in human ileum. *Br. J. Pharmacol.,* **125,** 1393–1395.

DARMANI, N.A. (2001). The cannabinoid CB1 receptor antagonist SR 141716A reverses the antiemetic and motor depressant actions of WIN 55, 212–2. *Eur. J. Pharmacol.,* **430,** 49–58.

DE CASTRO, J.M. (2000). Eating behavior: lessons from the real world of humans. *Nutrition,* **16,** 800–813.

DE VRY, J., JENTZSCH, K.R., KUHL, E. & ECKEL, G. (2004a). Behavioral effects of cannabinoids show differential sensitivity to cannabinoid receptor blockade and tolerance development. *Behav. Pharmacol.,* **15,** 1–12.

DE VRY, J., SCHREIBER, R., ECKEL, G. & JENTZSCH, K.R. (2004b). Behavioral mechanisms underlying inhibition of food-maintained responding by the cannabinoid receptor antagonist/inverse agonist SR141716A. *Eur. J. Pharmacol.,* **483,** 55–63.

DI MARZO, V. & MATIAS, I. (2005). Endocannabinoid control of food intake and energy balance. *Nat. Med.,* **8,** 585–589.

DI MARZO, V., GOPARAJU, S.K., WANG, L., LIU, J., BATKAI, S., JARAI, Z., FEZZA, F., MIURA, G.I., PALMITER, R.D., SUGIURA, T. & KUNOS, G. (2001). Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature,* **410,** 763–765.

DUNCAN, M., DAVISON, J.S. & SHARKEY, K.A. (2005). Endocannabinoids and their receptors in the enteric nervous system. *Aliment. Pharmacol. Ther.,* **27,** 667–683.

FITTON, A.G. & PERTWEE, R.G. (1982). Changes in body temperature and oxygen consumption rate of conscious mice produced by intrahypothalamic and intracerebroventricular injections of delta 9-tetrahydrocannabinol. *Br. J. Pharmacol.,* **75,** 409–414.

GARCIA, J., HANKINS, W.G. & RUSINIAK, K.W. (1976). Flavor aversion studies. *Science,* **192,** 265–267.

GARCIA, J., LASITER, P.S., BERMUDEZ-RATTONI, F. & DEEMS, D.A. (1985). A general theory of aversion learning. *Ann. NY Acad. Sci.,* **443,** 8–21.

HART, C.L., WARD, A.S., HANEY, M., COMER, S.D., FOLTIN, R.W. & FISCHMAN, M.W. (2002). Comparison of smoked marijuana and oral Delta(9)-tetrahydrocannabinol in humans. *Psychopharmacology (Berlin),* **164,** 407–415.

HERKENHAM, M., LYNN, A.B., JOHNSON, M.R., MELVIN, L.S., DE COSTA, B.R. & RICE, K.C. (1991). Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. *J. Neurosci.,* **11,** 563–583.

HILDEBRANDT, A.L., KELLY-SULLIVAN, D.M. & BLACK, S.C. (2003). Antiobesity effects of chronic cannabinoid CB1 receptor antagonist treatment in diet-induced obese mice. *Eur. J. Pharmacol.,* **462,** 125–132.

JBILO, O., RAVINET-TRILLOU, C., ARNONE, M., BUISSSON, I., BRIBES, E., PELERAUX, A., PENARIER, G., SOUBRIE, P., LE FUR, G., GALIEGUE, S. & CASELLAS, P. (2005). The CB1 receptor antagonist rimonabant reverses the diet-induced obesity phenotype through the regulation of lipolysis and energy balance. *FASEB J.,* **19,** 1567–1569.

KIRKHAM, T.C. & WILLIAMS, C.M. (2004). Endocannabinoid receptor antagonists: potential for obesity treatment. *Treat. Endocrinol.,* **3,** 345–360.

KIRKHAM, T.C., WILLIAMS, C.M. & DI MARZO, V. (2002). Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. *Br. J. Pharmacol.,* **136,** 550–557.

KOLA, B., HUBINA, E., TUCCI, S.A., KIRKHAM, T.C., GARCIA, E.A., MITCHELL, S.E., WILLIAMS, L.M., HAWLEY, S.A., HARDIE, D.G., GROSSMAN, A.B. & KORBONITS, M. (2005). Cannabinoids and ghrelin have both central and peripheral metabolic and cardiac effects *via* AMP-activated protein kinase. *J. Biol. Chem.,* **280,** 25196–25201.

KULKARNI-NARLA, A. & BROWN, D.R. (2000). Localization of CB1-cannabinoid receptor immunoreactivity in the porcine enteric nervous system. *Cell Tissue Res.,* **302,** 73–80.

LIU, Y.L., CONNOLEY, I.P., WILSON, C.A. & STOCK, M.J. (2005). Effects of the cannabinoid CB1 receptor antagonist SR141716 on oxygen consumption and soleus muscle glucose uptake in Lep(ob)/Lep(ob) mice. *Int. J. Obes. Relat. Metab. Disord.,* **29,** 183–187.

MAGGIO, C.A. & KOOPMANS, H.S. (1982). Food intake after intragastric meals of short-, medium, or long-chain triglyceride. *Physiol. Behav.,* **28,** 921–926.

MASSA, F., MARSICANO, G., HERMANN, H., CANNICH, A., MONORY, K., CRAVATT, B.F., FERRI, G.-L., SIBAEV, A., STORR, M & LUTZ, B. (2004). The endogenous cannabinoid system protects against colonic inflammation. *J. Clin. Invest.,* **113,** 1202–1209.

MCLAUGHLIN, P.J., WINSTON, K., SWEZEY, L., WISNIECKI, A., ABERMAN, J., TARDIF, D.J., BETZ, A.J., ISHIWARI, K., MAKRIYANNIS, A. & SALAMONE, J.D. (2003). 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.,* **14,** 583–588.

MCLAUGHLIN, P.J., WINSTON, K.M., LIMEBEER, C.L., PARKER, L.A., MAKRIYANNIS, A. & SALAMONE, J.D. (2005). The cannabinoid CB1 antagonist AM 251 produces food avoidance and behaviors associated with nausea but does not impair feeding efficiency in rats. *Psychopharmacology (Berlin),* **180,** 286–293.

MOLDRICH, G. & WENGER, T. (2000). Localization of the CB1 cannabinoid receptor in the rat brain. An immunohistochemical study. *Peptides,* **21,** 1735–1742.

OSEI-HYIAMAN, D., DEPETRILLO, M., PACHER, P., LIU, J., RADAEVA, S., BATKAI, S., HARVEY-WHITE, J., MACKIE, K., OFFERTALER, L., WANG, L. & KUNOS, G. (2005). Endocannabinoid activation at hepatic CB(1) receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J. Clin. Invest.,* **115,** 1298–1305.

PERTWEE, R.G. & TAVENDALE, R. (1977). Effects of delta9-tetrahydrocannabinol on the rates of oxygen consumption of mice. *Br. J. Pharmacol.,* **60,** 559–568.

PICKEL, V.M., CHAN, J., KASH, T.L., RODRIGUEZ, J.J. & MACKIE, K. (2004). Compartment-specific localization of cannabinoid 1 (CB1) and mu-opioid receptors in rat nucleus accumbens. *Neuroscience,* **127,** 101–112.

RAVINET-TRILLOU, C., DELGORGE, C., MENET, C., ARNONE, M. & SOUBRIE, P. (2004). CB1 cannabinoid receptor knockout in mice leads to leanness, resistance to diet-induced obesity and enhanced leptin sensitivity. *Int. J. Obes. Relat. Metab. Disord.,* **46,** 640–648.

RAVINET-TRILLOU, C., ARNONE, M., DELGORGE, C., GONALONS, N., KEANE, P., MAFFRAND, J.P. & SOUBRIE, P. (2003). Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.,* **284,** R345–R353.

RAWLS, S.M., CABASSA, J., GELLER, E.B. & ADLER, M.W. (2002). CB1 receptors in the preoptic anterior hypothalamus regulate WIN 55212–2 [(4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1*ij*]quinolin-6-one]-induced hypothermia. *J. Pharmacol. Exp. Ther.,* **301,** 963–968.

ROBBE, D., ALONSO, G. & MANZONI, O.J. (2003). Exogenous and endogenous cannabinoids control synaptic transmission in mice nucleus accumbens. *Ann. NY Acad. Sci.,* **1003,** 212–225.

RUTKOWSKA, M. (2004). The effect of AM 251, a cannabinoid CB1 receptor antagonist, on food intake in rats. *Acta. Pol. Pharm.,* **61,** 401–403.

SHEARMAN, L.P., ROSKO, K.M., FLEISCHER, R., WANG, J., XU, S., TONG, X.S. & ROCHA, B.A. (2003). Antidepressant-like and anorectic effects of the cannabinoid CB1 receptor inverse agonist AM251 in mice. *Behav. Pharmacol.,* **14,** 573–582.

ST JEOR, S.T., GUTHRIE, H.A. & JONES, M.B. (1983). Variability in nutrient intake in a 28-day period. *J. Am. Diet. Assoc.,* **83,** 155–162.

VAN GAAL, L.F., RISSANEN, A.M., SCHEEN, A.J., ZIEGLER, O. & ROSSNER, S. (2005). Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet,* **365,** 1389–1397.

VAN SICKLE, M.D., OLAND, L.D., HO, W., HILLARD, C.J., MACKIE, K., DAVISON, J.S. & SHARKEY, K.A. (2001). Cannabinoids inhibit emesis through CB1 receptors in the brainstem of the ferret. *Gastroenterology,* **121,** 767–774.

VICKERS, S.P., WEBSTER, L.J., WYATT, A., DOURISH, C.T. & KENNETT, G.A. (2003). Preferential effects of the cannabinoid CB1 receptor antagonist, SR 141716, on food intake and body weight gain of obese (fa/fa) compared to lean Zucker rats. *Psychopharmacology (Berlin),* **167,** 103–111.

ZHOU, D. & SHEARMAN, L.P. (2004). Voluntary exercise augments acute effects of CB1-receptor inverse agonist on body weight loss in obese and lean mice. *Pharmacol. Biochem. Behav.,* **77,** 117–125.

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