

Cannabinoid (CB)₁ receptor antagonist, AM 251, causes a sustained reduction of daily food intake in the rat

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Abstract

Cannabinoid (CB)₁ receptors are present throughout the nervous system, including several areas implicated in the control of food intake. Central and peripheral administration of CB₁ agonists increase food intake while CB₁ receptor antagonists reduce food intake. However, in some previous studies, tolerance to the anorectic effects of CB₁ antagonists develops within days. To further delineate the role of endogenous cannabinoid signaling in energy intake, we studied the effects of the CB₁ antagonist AM 251 (1.25, 2.5 and 5 mg/kg ip), the anandamide membrane transporter inhibitor VDM 11 (10 mg/kg ip), and the CB₁ agonists anandamide (1 mg/kg ip), and methanandamide (1 mg/kg ip), on food intake. A single administration of the CB₁ antagonist AM 251 significantly reduced food intake for a total of 6 days ($P < 0.05$). Reductions in food intake brought about by AM 251 were accompanied by reductions in weight gain for 6 days ($P < 0.05$). Contrary to expectations, VDM 11 did not increase food intake in this study. Anandamide was also unable to increase food intake; however, the more stable agonist methanandamide significantly increased food intake 3 h after administration ($P < 0.05$). These results support the role of CB₁ receptor antagonists in the treatment of obesity and suggest that the anorectic effect of AM 251 may last longer than previously reported. © 2004 Elsevier Inc. All rights reserved.

Keywords: Cannabinoids; Food intake; Body weight; VDM 11; Anandamide

1. Introduction

Cannabinoids refer to a class of ligands that activate CB₁ or CB₂ receptors [1,2]. Marijuana and the major plant cannabinoid delta(9)tetrahydrocannabinol (Δ^9 -THC) have been implicated in the control of feeding in both man [3] and animals [4,5]. In partially satiated animals, administration of naturally occurring cannabinoids, anandamide and 2-arachidonyl glycerol (2-AG), were shown to increase food intake and this action was blocked or reversed by the potent CB₁ receptor antagonist, SR 141716A, in a dose-dependent manner [6–8]. Administration of SR141716A, and the structurally and pharmacologically similar compound, AM 251, has been shown to reduce food intake [9–11], strongly

implicating the endocannabinoid system in this important behavior.

Several new inhibitors of anandamide and 2-AG reuptake are now available including AM 404, and its *o*-methyl derivative VDM 11. AM 404 inhibits the reuptake of anandamide and 2-AG, either by blocking the anandamide membrane transporter (AMT) [12–14], or by inhibiting the enzyme, fatty acid amide hydrolase (FAAH) [13], that degrades anandamide and, to a lesser extent, 2-AG [15]. In contrast, VDM 11, is a more selective uptake inhibitor, that, unlike AM 404, does not inhibit FAAH [16], and is totally devoid of any vanilloid receptor 1 (VR1) agonist activity [14], making it easier to interpret changes induced by VDM 11.

We chose to examine the effect of AM 251, VDM 11 and anandamide on food intake in partially satiated Lewis rats to further delineate the role of endogenous cannabinoid signaling in energy balance. To date, no study has examined

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the effects of an AMT inhibitor on food intake or the effects of a single administration of AM 251 on long-term daily food intake. Based on previous research showing that CB₁ agonists potentiate feeding behavior [17], and because AMT inhibitors have been shown to elevate circulating levels of anandamide [18], we hypothesized that rats given either VDM 11 or anandamide would show an increase in food intake relative to vehicle conditions. Conversely, by blocking CB₁ receptors with AM 251, we expected to see reductions in food intake relative to vehicle conditions.

2. Materials and methods

2.1. Experiment 1

2.1.1. Animals

Moderately obese male Lewis rats ($n=8$), weighing between 440 and 500 g at the beginning of the study, were individually housed in opaque plastic cylinder cages in a temperature-controlled environment between 20 and 22 °C, under a 12:12-h light–dark cycle (lights off at 1800 h). Vanilla-flavored Ensure Plus (Ross Laboratories, Saint-Laurent, Quebec, Canada) was used as food on account of its palatability, and to reduce the risk of spillage. A milky-sweet liquid, Ensure Plus is composed of 53.3% carbohydrate, 29% fat, 16.7% protein (1.41 kcal/g), which includes daily minerals and vitamins typical of a Western diet. Food was available from 1600 to 1700 h (prefeed) and 1800–0900 h daily; water was freely available at all times. Food and water were presented in glass bottles that attached to the outside of the cage. Animals were habituated to handling and testing procedures 3 weeks prior to testing on liquid diet. All experimental protocols 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.

2.1.2. Drugs

VDM 11 ((5Z,8Z,11Z,14Z)-*N*-(4-Hydroxy-2-methylphenyl)-5,8,11,14-eicosatetraenamide; Tocris Cookson) was supplied predissolved in anhydrous ethanol, 5 mg/ml, was dried, resuspended in a vehicle (2% dimethyl sulfoxide, 1% Tween 80 and 97% physiological saline), and administered intraperitoneally at a dose of 10 mg/kg [19]. AM 251 (*N*-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; Tocris Cookson) was also given at 5 mg/kg ip in the same vehicle. Drugs were aliquoted and placed in a freezer at –70 °C until use. Anandamide [*N*-(2-Hydroxyethyl)-5Z,8Z,11Z,14Z-eicosatetraenamide; Tocris Cookson] was supplied in a soya oil/water (1:4) emulsion vehicle (Tocrisolve, Tocris Cookson), and was administered at 1 mg/kg ip. Tocrisolve was used in the same volume (1 ml/kg) as a control. Drugs were given 7 days apart. All rats went through all conditions according to a counterbalanced design except for one rat that stopped

eating altogether for 2 days during the AM 251 (5 mg/kg) condition. Food for that animal was made available ad libitum, and its data was not included in the analysis.

2.1.3. Food intake

The initial food presented between 1600 and 1700 h (prefeed) was designed to produce partial satiety as previously described [6,7]. At approximately 1745 h, rats were given an intraperitoneal injection of either VDM 11 ($n=8$), AM 251 ($n=7$), vehicle ($n=8$), anandamide ($n=7$), or Tocrisolve ($n=7$). Food bottles were put back on at 1800 h. Intake measurements were taken daily as well as each evening at 1900, 2000, and 2100 h. Contrary to previous findings, anandamide had no significant effect on food intake at anytime during the study.

2.2. Experiment 2

Because of the lack of effect seen with anandamide, we also repeated our original protocol using a more stable analogue of the CB₁ agonist methanandamide [(*R*)-*N*-(2-Hydroxy-1-methylethyl)-5Z,8Z,11Z,14Z-eicosatetraenamide; Tocris Cookson; 1 mg/kg ip]. Briefly, naïve male Lewis rats ($n=8$), weighing between 462 and 502 g at the beginning of the study were individually housed with food and water as described above. Animals were assigned into either methanandamide or vehicle (Tocrisolve) conditions using a counterbalanced design. Drugs were given in a volume of 1 ml/kg at 1745 h; each treatment was separated by 72 h. Intake measurements were taken daily as well as each evening at 1900, 2000, and 2100 h.

2.3. Experiment 3

To determine whether the anorectic effect of AM 251 was dose dependent, we repeated our original protocol using two additional doses of the CB₁ receptor antagonist. Naïve male Lewis rats ($n=8$), weighing between 469 and 520 g at the beginning of the study, were individually housed in plastic cages, in a temperature-controlled environment between 20 and 22 °C, under a 12:12-h light–dark cycle (lights off 1900 h). Food was available from 1600 to 1700 h (prefeed) and 18:00–9:00 h daily; water was freely available at all times. Animals were assigned into vehicle, 1.25 or 2.5 mg/kg conditions via a counterbalanced design. Drugs were supplied and administered as previously described at 1745 h.

2.4. Experiment 4

Although animals were habituated to the diet 3 weeks prior to testing, the possibility of developing a conditioned taste aversion (CTA) was a potential explanation for the results using AM 251. To test whether a stimulus known to produce a CTA altered food intake under the conditions of our study, we examined food intake after injection of two doses of lithium chloride (LiCl; Fisher Scientific, USA; 1

mmol/kg; 0.3 mmol/kg ip) previously shown to produce CTA [20]. Briefly, male Lewis rats ($n=8$) used in Experiment 2 were randomly assigned into either 1 or 0.3 mmol/kg, or saline vehicle conditions. LiCl was dissolved in sterile distilled water in a volume of 1 ml/kg; physiological saline vehicle was also given 1 ml/kg. Drugs were given 3 days apart at 1745 h and food intake was measured 1 h later. Daily intakes were recorded for the next 3 days.

2.4.1. Statistics

Data are presented as mean \pm S.E.M. VDM 11, AM 251, and LiCl data were submitted to one-way and two-way repeated-measures ANOVAs, where appropriate. Specific comparisons were made by using Dunnett's multiple comparisons two-tailed test. One-tailed paired t tests were performed between anandamide, methanandamide, and corresponding vehicle conditions with Prism (GraphPad Prism version 3.03 for Windows, GraphPad Software, San Diego, CA, USA). All two-way repeated-measures ANOVAs and follow-up analysis (including one-way ANOVAs and two-tailed paired t tests) were performed with SPSS version 11 or 12.0 (SPSS, Chicago, IL) for Experiments 1 and 2.

3. Results

3.1. Early effects on food intake

Cumulative food intake differed between VDM 11 (10 mg/kg), AM 251 (5 mg/kg), and vehicle-control conditions after 1 h [$F(2,7)=8.76$, $P=.003$; Fig. 1], 2 h [$F(2,7)=5.21$, $P=.02$] and 3 h [$F(2,7)=239.0$, $P<.001$] of testing. Further analysis revealed that rats in the AM 251 condition ate significantly less than they did as vehicle controls at all three time points [1 h: AM 251 no intake vs. control 3.6 ± 0.8 g; $q(6)=4.01$; $P<.01$; 2 h: $1.3 \pm .8$ vs. 6.9 ± 1.3 g; $q(6)=2.86$,

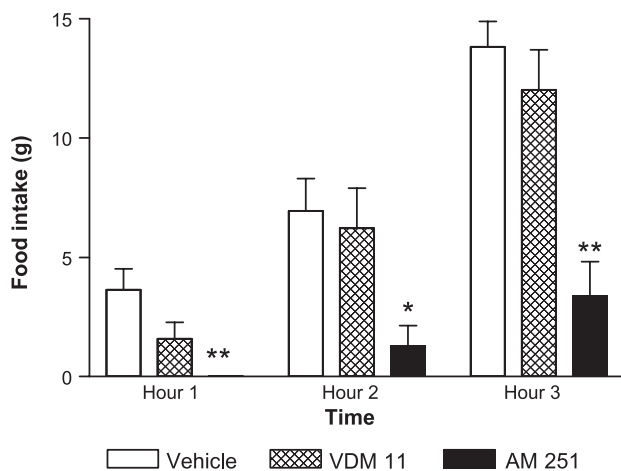


Fig. 1. Cumulative food intake over 3 h (mean \pm S.E.M.; g) starting after injections of either vehicle (clear bars), VDM 11 (hatched bars), or AM 251 (black bars). * $P<.05$, ** $P<.01$.

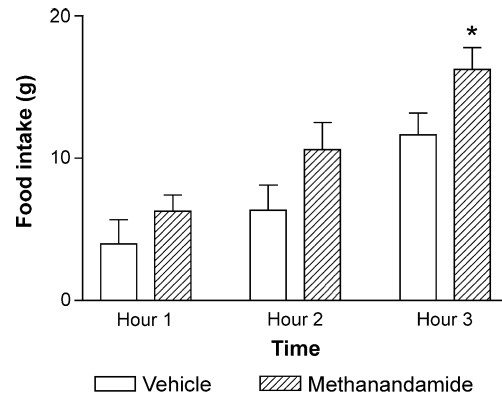


Fig. 2. Cumulative food intake over 3 h (mean \pm S.E.M.; g) starting after injections of either vehicle (clear bars) or methanandamide (hatched bars). * $P<.05$.

$P<.05$; 3 h: 3.3 ± 1.4 vs. 13.8 ± 3.3 g; $q(6)=19.75$, $P<.01$). Contrary to our initial expectations, the AMT inhibitor VDM 11 slightly reduced food intake during the first hour of testing [1 h: VDM 11 1.6 ± 0.7 g vs. control 3.7 ± 0.9 g; $P=.09$]. However, this trend was transient and failed to reach statistical significance. VDM 11 had no significant effect on food intake after 2 or 3 h [VDM 11 12.0 ± 1.6 g vs. control 13.8 ± 1.0 g; $P>.05$]. Anandamide (1 mg/kg) failed to significantly increase food intake (data not shown), while rats given methanandamide ate significantly more than rats given vehicle after 3 h [3 h methanandamide 16.2 ± 1.5 g vs. control 11.6 ± 1.5 g; $t=2.53$, $P=.02$; Fig. 2]. Under the conditions we described, neither dose of LiCl caused a significant change in food intake after 1 h (vehicle 6.0 ± 1.0 g; 0.3 mmol/kg LiCl 8.0 ± 0.8 g; 1 mmol/kg LiCl 5.7 ± 0.9 g).

3.2. Long-term effects on food intake

The anorectic effect of AM 251 continued far longer than a few hours after administration. Changes in daily food intake following a 5-mg/kg dose of AM 251 are presented in Fig. 3. The main effect of drug [$F(2,7)=28.65$, $P<.001$] and main effect of day [$F(8,7)=5.27$, $P<.001$] were highly significant. Further analysis revealed a significant effect of day in the AM 251 condition [$F(8,6)=8.70$, $P<.001$] that was nonsignificant in both VDM 11 (data not shown) and vehicle conditions ($P>.05$) producing a significant Drug \times Day interaction [$F(16,7)=3.84$, $P<.001$]. The reduction in daily food intake brought about by AM 251 was significant for a total of 6 days, starting on the day of injection [AM 251 36.7 ± 3.3 g vs. control 57.4 ± 2.4 g; $t(6)=4.13$, $P=.003$], and finishing the day before the next drugs were given [AM 251 47.0 ± 2.5 g vs. control 57.0 ± 2.2 g; $t(6)=2.53$, $P=.022$]. Neither dose of LiCl changed daily food intake relative to vehicle conditions (on Day 1: vehicle, 46.2 ± 2.2 g; 0.3 mmol/kg LiCl, 46.8 ± 2.2 g; 1.0 mmol/kg LiCl, 45.9 ± 1.9 g).

The reductions in food intake brought about by AM 251 were dose dependent (Fig. 4). The main effect of dose [$F(2,7)=9.86$, $P=.002$] and main effect of day [$F(9,7)=9.93$, $P<.001$] were both significant. Further

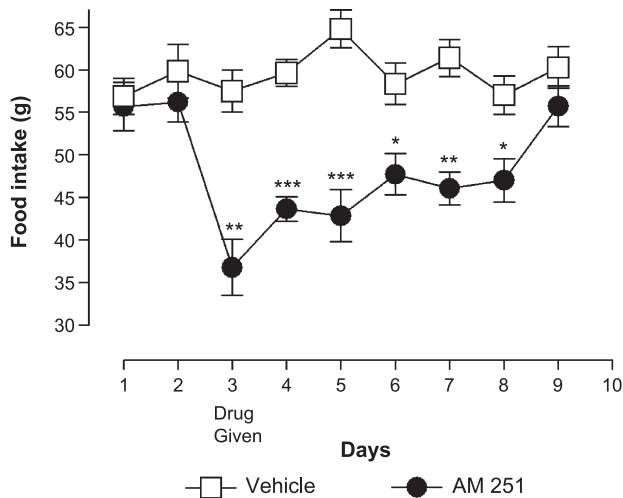


Fig. 3. Changes in daily food intake (g) starting 2 days before and finishing 7 days after administration of either vehicle (\square) or AM 251 (5 mg/kg, \bullet). Note the prolonged reduction in food intake after a single administration of AM 251. * $P < .05$, ** $P < .01$, *** $P < .001$.

analysis revealed a significant effect of day in rats given 1.25 mg/kg AM 251 [$F(8,7)=4.78$, $P < .001$] and 2.5 mg/kg AM 251 [$F(8,7)=8.47$, $P < .001$]. Because there was no effect of day during vehicle conditions, there was also a significant Drug \times Day interaction [$F(16,7)=2.66$, $P = .001$]. Reductions in food intake using 2.5 mg/kg of AM 251 were significant starting the day of administration [AM 251 36.0 ± 2.65 g vs. control 52.7 ± 1.8 g; $t(7)=6.68$, $P < .001$] and for the next 4 days [$t(7)=2.34$, $P \leq .05$]. In contrast, rats given the 1.25-mg/kg dose of AM 251 only showed significant reductions in food intake the day of administration [42.0 ± 1.3 vs. 52.7 ± 1.8 g; $t(7)=4.58$, $P = .003$].

Reductions in food intake were also accompanied by significant reductions in weight gain. Fig. 5 shows changes in weight gain over the course of the study. The main effect of dose was significant [$F(2,7)=20.00$, $P < .001$]. Further

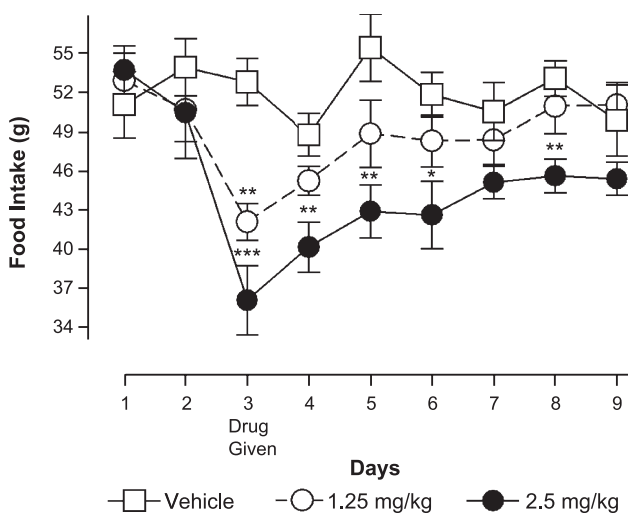


Fig. 4. Dose-dependent changes in daily food intake (g) starting 2 days before and finishing 7 days after administration of either vehicle (\square) or AM 251 (1.25 mg/kg, \circ or 2.5 mg/kg, \bullet). * $P < .05$, ** $P < .01$, *** $P < .001$.

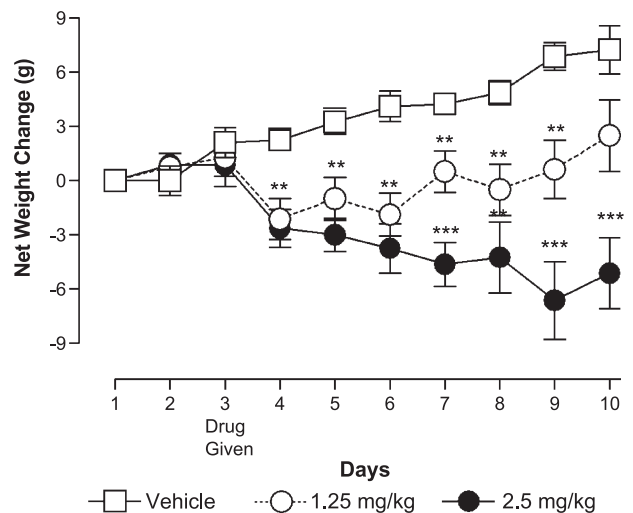


Fig. 5. Changes in weight gain or loss (g) starting 2 days before and finishing 8 days after administration of either vehicle (\square) or AM 251 (1.25 mg/kg, \circ or 2.5 mg/kg, \bullet). Note that even the lowest dose of AM 251 produced significant and sustained weight loss compared to the vehicle condition. ** $P < .01$, *** $P < .001$.

analysis showed significant changes in body weight in each of the three conditions over time [vehicle, $F(9,7)=6.00$, $P < .001$; 1.25 mg/kg, $F(9,7)=2.21$, $P = .032$; 2.5 mg/kg, $F(9,7)=6.86$, $P < .001$]. Because vehicle and drug conditions had opposite effects, there was also a significant Drug \times Day interaction [$F(9,7)=9.69$, $P < .001$]. On average, rats gained weight during vehicle conditions (7.3 ± 1.4 g over 10 days) and lost weight or failed to gain weight during the 1.25 mg/kg AM 251 (2.5 ± 2.0 g) and 2.5 mg/kg AM 251 (-5.1 ± 2.1 g) conditions.

4. Discussion

Results from this study show that the CB₁ receptor antagonist AM 251 significantly reduced food intake and body weight in a dose-dependent manner and for far longer than previously reported. Interestingly, in animals given the lowest dose of the drug (1.25 mg/kg), the duration of weight loss exceeded the duration of reduced food intake, similar to results described by others [9,10,21]. Contrary to our initial expectations, the AMT inhibitor VDM 11 caused only a slight reduction in food intake and anandamide failed to significantly increase eating over 3 h, although the metabolically more stable analogue of anandamide, methanandamide, significantly increased food intake after the same amount of time.

Hildebrandt et al. [9] showed that repeated daily administrations of AM 251 (3 mg/kg) led to nonsignificant changes in food intake after only 8 days in mouse. In contrast, we found that a single dose of AM 251 produced significant reductions in food intake for as many as 4 (2.5 mg/kg) and 6 days (5 mg/kg) in the rat. The anorectic effect of SR 141716A (10 mg/kg/day) has been shown to wane

after only 5 days [21]. A recent press release from Phase III clinical trials by Sanofi-Synthelabo [22] using SR 141716A (clinical name Rimonabant) in obese humans, reported significant weight loss only in conjunction with a calorie-restricted diet. These data suggest that the anorectic effect of CB₁ antagonists are being blunted by the acquisition of tolerance or desensitization within the endocannabinoid system. Alternatively, reductions in food intake could activate additional homeostatic pathways designed to defend against changes in set point. In line with this idea of active compensation, high doses of AM 251 and SR 141716A (approximately 10 mg/kg) may also lead to significant hyperphagia and a rapid return in body weight when treatment is stopped, whereas lower doses (approximately 3 mg/kg) may not [9,21]. The advantage of a low-dose weekly administration is thus threefold: first, it is less likely to cause significant rebound-hyperphagia; second, it could delay the acquisition of tolerance or desensitization; and third, weekly versus daily administration could minimize other unwanted side effects that might be produced by this kind of therapy.

The potential side effects of CB₁ antagonists from animal studies include the induction or enhancement of emesis [23,24]. Rats do not vomit but can display CTA. Although reductions in food intake by AM 251 may be explained by a CTA, the fact that rats had been adapted to the diet for at least 3 weeks, and some for 5 weeks, minimizes this likelihood. Furthermore, injecting LiCl at doses known to give CTA [20] failed to reduce food intake under identical conditions. Further research is needed to determine whether CTA is at all involved in the reduction in food intake by AM 251. In human trials, the most common side effects of SR141716A (5 and 20 mg doses) were nausea and dizziness [22]. Because of the widespread nature and complex physiology of the endocannabinoid system [2], it is perhaps not surprising that blocking CB₁ receptors may have unwanted effects. Two recent examples illustrate this point. The first comes from observations that mice treated with SR 141716A (and CB₁-deficient mice) show significantly more damage in response to an inflammatory stimulus compared with vehicle-treated controls [25]. Second, in an animal model of temporal lobe epilepsy, treatment with SR 141716A led to a significantly higher frequency of protracted seizures [26]. Clearly, CB₁ antagonists may pose risks if administered without careful monitoring. On the other hand, in addition to weight loss, treatment with CB₁ receptor antagonists may also correct insulin resistance as well as lower circulating leptin and free fatty acid levels [22,27].

Results from our study suggest that the duration of AM 251's anorectic effect exceeds the pharmacological profile described in mouse brain by Gatley et al. [28]. They reported that intravenously injected ¹²³I-labeled AM 251 radioactivity declined to about half its peak level after only 8 h in mouse brain. At the end of 2 days, less than 2.0% of the peak drug levels would still be present in the brain.

However, in our hands, food intake was significantly reduced for a total of 6 days following a single administration in rat. The mechanisms by which a single injection of AM 251 continues to reduce food intake after 4–6 days cannot be resolved with the current data. An intriguing possibility is that AM 251 is acting on CB₁ receptors expressed by fat stores. Recently, SR 141716A was shown to increase adiponectin (Acrap30) mRNA expression, an anorectic protein exclusively released by adipose tissue [29]. Adiponectin reduces body weight independent of feeding behavior, consistent with the observation that rats given AM 251 or SR 141716A continue to lose weight even after returning to baseline food intakes (current study and Refs. [9,10,27,29]). Differences in fat mass may also help explain why CB₁ antagonists are more effective in obese compared to lean controls [9,10,27].

The endocannabinoid system plays a powerful role in the regulation of energy balance. When animals are food deprived, endocannabinoid levels increase in relevant areas of the brain [8] as well as in the gut [7]. Signals from the gut to the brain are potentially modulated by CB₁ receptors that are expressed on vagal afferents [30]. Interestingly, their expression is down-regulated by the satiety factor cholecystokinin. In the hypothalamus, endocannabinoids appear to be under negative control by the anorectic hormone, leptin [31]. Defective leptin signaling is associated with increased endocannabinoid levels in the hypothalamus of obese mice and Zucker rats, and acute leptin treatment of normal rats reduced endocannabinoid levels in the hypothalamus, but not in the cerebellum. Furthermore, CB₁ expression is down-regulated in the extrahypothalamic regions of obese Wistar rats [32]. The expression of cannabinoids and their receptors in reward areas of the brain, such as the nucleus accumbens, may additionally contribute to the anorectic effect of SR 141716A by reducing the reward incentive for food [33–36]. These same areas confer the reward incentive for sex and are acted upon by drugs of abuse.

The reason anandamide did not increase food intake in our study is not clear, but may be the result of differences in protocol with previous studies [5,6]. The metabolically more stable analogue of anandamide methanandamide clearly did increase food intake in a manner consistent with previous data. Only one other laboratory has reported that anandamide has no effect on food intake [37]. However, in that study, animals were fed standard rat chow ad libitum, as opposed to having been given a prefeed and palatable diet [5–7]. Unlike the anorectic effect of CB₁ antagonists, CB₁ receptor agonists only increase food intake in pre- or partially satiated animals [7]. Furthermore, CB₁-mediated increases in food intake are more robust in animals given a palatable diet [4].

To date, the effects of an AMT inhibitor on food intake have not been investigated. Results from our study indicate that there was no increase in food intake as we had hypothesized. The modest reduction in food intake under

VDM 11 conditions was not significant. Recent evidence suggests that the release of anandamide is dependent on the same carrier protein responsible for its uptake [16]. Therefore, it is possible that an AMT inhibitor, such as VDM 11, could actually decrease the availability of anandamide by simultaneously blocking its release as well as its uptake.

In summary, the ability of redundant pathways involved in feeding behavior to compensate for one another and maintain body weight has been one of the biggest challenges in finding a treatment for obesity. Converging lines of evidence provide overwhelming support for the role of endogenous cannabinoids in the regulation of food intake. Our results suggest that CB₁ receptor antagonists could be effective agents in the treatment of obesity. Here, we show a single administration of AM 251 decreases food intake and body weight much longer than previously reported. The large reductions in daily food intake and body weight were dose dependent, but even with the lowest dose of drug used in this study, loss of body weight continued well beyond the period of reduced daily intake. Although anandamide did not significantly increase food intake in our hands, the more stable analog, methanandamide, did so. Future research will be needed to understand how the large effect of AM 251 on daily food intake is maintained for so long.

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