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The neutral cannabinoid CB_1 receptor antagonist AM4113 regulates body weight through changes in energy intake in the rat

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ABSTRACT

The aim of this study was to determine if the neutral cannabinoid CB_1 receptor antagonist, AM4113, regulates body weight in the rat via changes in food intake. We confirmed that the AM4113-induced reduction in food intake is mediated by CB_1 receptors using CB_1 receptor knockout mice. In rats, intraperitoneally administered AM4113 (2, 10 mg kg^{-1}) had a transient inhibitory effect on food intake, while body weight gain was suppressed for the duration of the study. AM4113-induced hypophagia was no longer observed once the inhibitory effect of AM4113 on body weight stabilized, at which time rats gained weight at a similar rate to vehicle-treated animals, yet at a lower magnitude. Pair-feeding produced similar effects to treatment with AM4113. Food intake and body weight gain were also inhibited in rats by oral administration of AM4113 (50 mg kg^{-1}). Dual energy x-ray absorptiometry (DEXA) was used to measure lean and fat mass. The AM4113 treated group had $29.3 \pm 11.4\%$ lower fat mass than vehicle-treated rats; this trend did not reach statistical significance. There were no differences in circulating levels of the endogenous cannabinoid 2-arachidonoyl glycerol (2-AG), glucose, triglycerides, or cholesterol observed between treatment groups. Similarly, 2-AG hypothalamic levels were not modified by AM4113 treatment. These data suggest that blockade of an endocannabinoid tone acting at CB_1 receptors induces an initial, transient reduction in food intake which results in long-term reduction of body weight gain.

1. Introduction

The endogenous cannabinoid (CB) system consists of CB_1 and CB_2 receptors, endogenous ligands for these receptors, and the enzymes responsible for the synthesis and degradation of these ligands (Sugiura et al., 2002; Matias and Di Marzo, 2006). Anandamide and 2-arachidonoyl glycerol (2-AG) were the first endogenous cannabinoid ligands to be discovered, and, like the exogenous ligands for these receptors, they display a range of actions including those on food intake and appetite (Kirkham and Williams, 2001). Cannabinoids affect appetite and body weight by acting at CB_1 receptors, where agonist stimulation increases food intake (Cota, 2007). Treatment of rodents with a CB_1 antagonist/inverse agonist, such as rimonabant, causes reductions in daily food intake, but this effect is transient, and food intake soon returns to the level of vehicle-treated controls (Colombo et al., 1998; Hildebrandt et al., 2003; Vickers et al., 2003; Ravinet Trillou et al., 2003; Bensaid et al., 2003; Liu et al., 2005) . This observation led to the hypothesis that the effect of CB_1 receptor antagonist/inverse agonists on body weight is maintained through actions on peripheral metabolic pathways (Ravinet Trillou et al., 2003; Cota et al., 2003; Jbilo et al., 2005; Horvath, 2006; Nogueiras et al., 2008; Cota et al., 2009; Koolman et al., 2010).

Endogenous cannabinoid signaling could affect metabolism by acting on lipogenic pathways, or on pathways that affect lipolysis and energy expenditure. CB_1 receptors are expressed in liver (Osei-Hyiaman et al., 2005), adipose tissue (Bensaid et al., 2003; Cota et al., 2003; Jbilo et al., 2005; Starowicz et al., 2008), gastrointestinal tract (Gómez et al., 2002; Duncan et al., 2005), pancreas (Starowicz et al., 2008) and skeletal muscle (Liu et al., 2005). In rodents, CB_1 receptor antagonist/inverse agonists increase in vivo resting energy expenditure (Liu et al., 2005; Herling et al., 2008; Kunz et al., 2008) and glucose uptake in isolated soleus muscle (Liu et al., 2005). CB_1 receptor agonists increase the expression of lipogenic transcription factors and de novo lipogenesis in liver (Osei-Hyiaman et al., 2005) and cultured adipocytes (Cota et al., 2003), effects that are blocked by a CB_1 receptor antagonist/inverse agonist.

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Pair-feeding studies, specifically designed to test the hypothesis that changes in metabolism maintain weight loss induced by a CB_1 receptor antagonist/inverse agonist, have produced conflicting results (Vickers et al., 2003; Ravinet Trillou et al., 2003; Thornton-Jones et al., 2006; Janiak et al., 2007; Irwin et al., 2008; Herling et al., 2008; Cota et al., 2009). In some studies, differences in body weight between pair-fed rodents, and rodents treated with a CB_1 receptor antagonist/inverse agonist implied the presence of an effect on metabolism (Ravinet Trillou et al., 2003; Herling et al., 2008; Cota et al., 2009). In others, pair-fed rodents weighed the same as treated animals, indicating that changes in body weight induced by a CB_1 receptor antagonist/inverse agonist result solely from the inhibition of food intake (Vickers et al., 2003; Thornton-Jones et al., 2006; Janiak et al., 2007; Irwin et al., 2008). The effects of a CB_1 receptor antagonist/inverse agonist on energy expenditure have also produced inconsistent findings. In one study, mice treated with SR141716 had higher basal oxygen consumption rates than vehicle-treated mice (Liu et al., 2005), but the body weight in these animals was the same as pair-fed mice. In another study, SR141716A significantly increased oxygen consumption in rat, but only for a brief time and only after the first treatment (Kunz et al., 2008).

We investigated whether the effect of a neutral CB_1 receptor antagonist, AM4113, on body weight in rat was due solely to effects on food intake or whether effects on metabolism may contribute to the effect. The potential advantage of a neutral CB_1 receptor antagonist is that the effects are specific to the pharmacological blockade of endogenous cannabinoid signaling (Chambers et al., 2007; Sink et al., 2008), without affects on constitutive receptor activity. Recently, it was shown that AM4113 inhibited food intake to a similar degree as the CB_1 receptor antagonist/inverse agonist AM251 in rat (Chambers et al., 2007; Sink et al., 2008), but unlike AM251, AM4113 did not potentiate vomiting in the ferret (Chambers et al., 2007) or promote nausea in rat (Sink et al., 2008). We examined the role that food intake plays in the actions of AM4113 on body weight by measuring food intake and body weight in rats that were pair-fed to an AM4113 treatment group. The effects of AM4113 on body composition and on fasting glucose and lipid levels were assessed using dual energy x-ray absorptiometry (DEXA) and blood analysis, respectively, to investigate which tissues and metabolic pathways were potentially altered by AM4113 administration. We also examined hypothalamic and plasma levels of the endogenous cannabinoid 2-AG following each treatment to determine whether AM4113 was modifying endocannabinoid levels as well as antagonizing CB_1 receptors to exert its effects. Furthermore, we investigated the effects of orally administered AM4113 to gain an insight into the therapeutic potential of a neutral cannabinoid antagonist.

2. Materials and methods

2.1. Compounds

The pyrazole-based neutral antagonist AM4113 (Chambers et al., 2007; Sink et al., 2008) was synthesized at Northeastern University. For intraperitoneal (i.p.) administration AM4113 was dissolved in DMSO using gentle heating and sonication before being diluted with Tween 80 and saline (4% DMSO; 1% Tween 80; 95% saline) to a final concentration of 2 and 10 mg ml^{-1} (Chambers et al., 2007). Injections were administered to mice at 100 µl 10 g^{-1} body weight and to rats at 100 µl 100 g^{-1} body weight. For oral (p.o.) administration AM4113 was dissolved in 4% DMSO before being diluted with extra light olive oil (Safeway, Calgary, Canada) to a final concentration of 25 mg ml^{-1} and was delivered in a volume of 200 µl 100 g^{-1} body weight.

2.2. Animal experiments

Animals were individually housed in transparent plastic cages, with sawdust bedding, in a temperature-controlled room maintained on a 12-h:12-h light/dark cycle and were allowed access to water ad libitum. Animal use for these studies was approved by the University of Calgary Animal Care Committee and all protocols were carried out in accordance with the guidelines of the Canadian Council on Animal Care.

2.3. Acute feeding experiments in CB_1 receptor knockout (−/−) and wild-type mice

Two breeding pairs of heterozygous $CB_1^{+/-C57BL/6N}$ mice were obtained from B. Lutz (University Medical Center, Mainz, Germany) and bred at the University of Calgary to obtain $CB_1^{-/-C57BL/6N}$ mice (Marsicano et al., 2002). Animals used in these studies were backcrossed to C57BL/6N for 6 generations and were used at the same age (10–16 weeks) and maintained under the same conditions as the wild-type mice. All $CB_1^{-/-}$ mice were genotyped using established protocols and were confirmed as homozygous gene-deficient animals ($CB_1^{-/-C57BL/6N}$) prior to inclusion in the study.

Female CB_1 receptor knockout mice weighing between 20 and 30 g at the start of the study were placed on a medium fat, palatable diet (51.4% carbohydrate, 31.8% fat, 16.8% protein; 4.41 kcal/g; Diet # D12266B, Research Diets, New Brunswick, NJ, USA) at least 4 days prior to the experiment. Food was available for 18 h each day starting at 16:30 h (lights off 18:00 h). One day prior to the experiment mice were assigned to either vehicle (wild-type mice, mean body weight ± S.E.M.; 26.3 ± 1.2 g, $n=5$; $CB_1^{-/-}$ mice: 22.1 ± 0.4 g, $n=6$) or 10 mg kg^{-1} AM4113 (wild-type mice: 26.5 ± 1.1 g, $n=5$. $CB_1^{-/-}$ mice: 22.3 ± 0.5 g, $n=6$) treatment groups. Mice were injected i.p. with 10 mg kg^{-1} AM4113 or vehicle immediately prior to the addition of food and their food intake was measured 1, 2, 3 and 18 h after injection.

2.4. Fourteen-day chronic feeding study in rats

Male Sprague–Dawley rats (Charles River, Montreal, Quebec; 180–280 g at the start of the study) were used to examine the effect of i.p. administered AM4113 on food intake and body weight. Animals were fed chocolate flavored Ensure plus liquid diet (53.3% carbohydrate, 29% fat, 16.7% protein; 1.41 kcal/g) (Abbot Laboratories, Abbott Park, IL, USA) to promote food intake and control for spillage. Rats were habituated to testing and handling procedures for 7 days prior to the start of the study. Food and water were presented in drip free inverted glass bottles that attached to the outside of the cage. Food was available for 18 h each day starting at 16:00 h (lights off 16:00 h). Prior to the first day of treatment, rats were assigned to either vehicle (1 ml kg^{-1}, mean body weight ± SEM.; 229 ± 14 g, $n=6$), 2 mg kg^{-1} (231 ± 12 g; $n=6$), or 10 mg kg^{-1} AM4113 (231 ± 11 g, $n=5$) treatment groups. Treatments were given i.p. once each day, for 14 days, immediately prior to the addition of food. An additional group of rats was treated with vehicle and pair-fed (232 ± 15 g, $n=5$) to rats in the 10 mg kg^{-1} treatment group. Food intake was time delayed in these rats by 1 day. Food intake and body weight were measured daily.

On day 15, after the 6 h period of food deprivation, under light anesthesia with isoflurane, body composition of each animal was analyzed using dual energy x-ray absorptiometry (DEXA) in conjunction with Hologic QDR software for small animals (Hologic QDR 4500, Holgic, Inc., Bedford, MA, U.S.A.). After DEXA analysis was complete rats were decapitated and trunk blood was collected in heparinized and non-heparinized tubes for the collection of plasma and serum, respectively. Heparinized tubes were immediately centrifuged for 10 min at 13,000 RCF after which 500 µl of plasma was aliquoted and flash-frozen in liquid nitrogen. At the same time the hypothalamus from each animal was isolated and flash-frozen. The level of 2-AG in plasma and hypothalamic samples was determined using the liquid chromatography-mass spectrometry (triple quadrupole) method described below. Non-heparinized tubes were placed on ice for at least 30 min before being centrifugated and aliquoted into two 300 µl