Available online at www.sciencedirect.com
SCIENCE DIRECT
Pharmacology, Biochemistry and Behavior 84 (2006) 353–359
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

The cannabinoid CB_1 receptor inverse agonist, rimonabant, modifies body weight and adiponectin function in diet-induced obese rats as a consequence of reduced food intake

Zoë D. Thornton-Jones [a], Guy A. Kennett [b], Karen R. Benwell [b], Dean F. Revell [b], Anil Misra [b], Daniel M. Sellwood [b], Steven P. Vickers [1,b], Peter G. Clifton [a,*]

[a] *Department of Psychology, John Maynard Smith Building, University of Sussex, Brighton, BN1 9QG, UK*
[b] *Vernalis Research Ltd, Oakdene Court, 613 Reading Road, Winnersh, Wokingham, RG41 5UA, UK*

Received 3 January 2006; received in revised form 9 May 2006; accepted 1 June 2006
Available online 30 June 2006

Abstract

The cannabinoid CB_1 receptor inverse agonist rimonabant induces hypophagia and body weight loss. Reduced body weight may potentially be due to decreased food intake or to direct metabolic effects of drug administration on energy expenditure. This study uses a paired-feeding protocol to quantify the contributions of energy intake to rimonabant-induced body weight loss. Diet-induced obese (DIO) rats were dosed with rimonabant (3, 10 mg/kg PO once daily) and matched with pair-fed controls. Food intake and body weight were measured daily. Blood samples and adipose tissue were collected on day 15 for measurement of plasma adiponectin and adiponectin mRNA levels. DIO rats treated with rimonabant and pair-fed controls showed very similar changes in body weight. Although tolerance developed to the anorectic effect of rimonabant, total food intake was significantly decreased over the 14-day study period and fully accounted for the observed reductions in body weight. Adiponectin mRNA and plasma adiponectin were elevated in vehicle-treated chow-fed animals compared to obese controls, and did not differ between rimonabant-treated and pair-fed animals. The similarities between rimonabant-treated and pair-fed animals in body weight loss and the absence of differences in measures of adiponectin activity between drug-treated and pair-fed animals suggest that the outcomes of this experiment were solely mediated by the drug-induced reduction in food intake.

Keywords: SR141716A; Rimonabant; Adiponectin; Paired-feeding; Body weight; Food intake; DIO rat

1. Introduction

Cannabinoids are important central modulators of feeding behaviour (review: Kirkham, 2005; Vickers and Kennett, 2005). For example, peripheral administration of the exogenous cannabinoid CB_1 receptor agonist Δ^9-tetra-hydrocannabinol (THC) promotes enhanced food intake (Williams and Kirkham, 2002). Peripheral or central administration of THC or the endocannabinoids 2-AG and anandamide increase food intake (Williams and Kirkham, 1999; Jamshidi and Taylor, 2001; Kirkham et al., 2002; Higgs et al., 2003; Verty et al., 2004a). Additionally, levels of these endocannabinoids in the limbic forebrain and hypothalamus have been shown to fluctuate as a function of nutritional status, with elevated levels following food deprivation and decreases during feeding (Kirkham et al., 2002). This suggests a fundamental role of the endocannabinoid system in appetite regulation. In support of this, CB_1 receptor knockout mice show reduced hyperphagia in response to starvation compared to their wild-type littermates (Di Marzo et al., 2001). CB_1 receptor knockout mice are also reported to be leaner than wild-type mice (Cota et al., 2003) and are resistant to diet-induced obesity (Ravinet Trillou et al., 2003).

Rimonabant (SR141716A) is a cannabinoid CB_1 receptor inverse agonist (Rinaldi-Carmona et al., 1995). Rimonabant

* Corresponding author. Tel.: +44 1273 678396; fax: +44 1273 678058.
E-mail address: pete@sussex.ac.uk. (P.G. Clifton).
[1] Now at: Renasci Consultancy Ltd., BioCity Nottingham, Pennyfoot St. Nottingham NG1 1GF, UK.

0091-3057/$ - see front matter © 2006 Published by Elsevier Inc.
doi:10.1016/j.pbb.2006.06.001

blocks the hyperphagic effects of cannabinoid agonists (Williams and Kirkham, 2002) and induces hypophagia when administered alone (Rowland et al., 2001; Higgs et al., 2003; Verty et al., 2004b). Rimonabant suppresses both the appetitive and consummatory components of feeding behaviour (Thornton-Jones et al., 2005). Central (ICV) administration of rimonabant also induces hypophagia (Verty et al., 2004a).

Chronic administration of rimonabant leads to a sustained reduction in body weight gain in lean and obese mice and rats (Colombo et al., 1998; Bensaid et al., 2003; Ravinet Trillou et al., 2003; Vickers et al., 2003). These effects are initially dependent on decreased food intake, although tolerance to the hypophagic effects of rimonabant develops quite rapidly. Tolerance to rimonabant-induced hypophagia is apparent within 4 days in lean rats (Colombo et al., 1998) but may take longer to develop in obese rats. Thus, Vickers et al. (2003) showed that obese Zucker rats chronically treated with rimonabant developed partial tolerance to the hypophagic effects after 13 days. However, whilst subsequent food intake with rimonabant was not significantly different from that of control subjects, consumption did remain lower for the entire 28 day study. It was unclear in these studies whether the effects of rimonabant on body weight loss were fully attributable to reductions in food intake. A 3-day pair-fed study suggested additional effects on body weight loss in rimonabant-treated diet-induced obese (DIO) mice beyond those observed in pair-fed controls (Ravinet Trillou et al., 2003). However, a 7-day pair-fed study in obese Zucker rats revealed no significant differences between pair-fed and rimonabant-treated subjects (Vickers et al., 2003).

A study by Bensaid et al. (2003) using obese Zucker rats confirmed the sustained effects of chronic rimonabant on body weight gain and its hypophagic actions. This study also showed enhanced gene expression of the adipokine, adiponectin, in animals chronically treated with rimonabant. Bensaid et al. (2003) found CB_1 receptor genes expressed in adipose tissue and proposed that the effects of rimonabant on body weight are mediated by direct effects on adipose tissue, perhaps through enhancement of adiponectin gene expression.

Adiponectin, also known as Acrp30, Adipo Q and ApM1 (Scherer et al., 1995; Hu et al., 1996), is the most abundantly expressed adipokine accounting for 0.01% of total human plasma proteins (Stefan and Stumvoll, 2002). In contrast to most adipokines, adiponectin plasma levels are inversely related to the degree of adiposity and are found to be low in patients with obesity (Arita et al., 1999; Weyer et al., 2001), type-2 diabetes (Hotta et al., 2000; Weyer et al., 2001) and coronary heart disease (Ouchi et al., 1999; Matsuzawa et al., 2003). Expression of adiponectin mRNA is also reduced in obese mice and humans (Hu et al., 1996) and in patients with type-2 diabetes (Statnick et al., 2000). Administration of adiponectin or a proteolytic fragment of adiponectin (globular domain) reduced body weight gain, however it is unclear whether these effects are dependent (Shklyaev et al., 2003) or independent (Fruebis et al., 2001) of food intake. Additionally food restriction leads to an elevation in plasma adiponectin levels in both mice (Berg et al., 2002; Combs et al., 2003) and humans (Hotta et al., 2000) and also provokes increased adiponectin gene expression (Zhang et al., 2002). Similarly, weight loss was associated with an increase in adiponectin gene expression (Milan et al., 2002) whilst access to an enriched diet lead to a decrease in gene expression and plasma levels prior to any changes in body weight (Naderali et al., 2003).

The present study had two important aims. First, given the contradictory results from earlier pair feeding studies we examined the effects of rimonabant in this paradigm using a more extended 14 day period and using diet induced obese rats, rather than obese Zucker rats. Second, in light of the known relationship between body weight and adiponectin gene expression, we determined whether adiponectin function is elevated following 14 days rimonabant treatment due to direct drug effects or as a consequence of drug-induced weight loss by comparisons between drug-treated animals and their pair fed controls.

2. Methods and materials

2.1. Animals

42 adult male Sprague–Dawley rats (Charles River, UK) were initially group housed in solid bottomed cages with dust free wood chippings and a cardboard tube. Water was available ad libitum and animals had access to either a high fat diet ($n=35$) (D12079B high fat diet, Research Diets D12079B, Western Diet, NJ, USA, 41% kcal from fat, energy density 19.7 kJ/g) or to standard laboratory diet ($n=7$) (Bantin and Kingman UK Ltd, Hull). Holding rooms were maintained under a 12-h light/dark cycle (lights off: 2000 h). Ambient temperature was 21±1 °C. A red light was the sole source of illumination during the dark period.

All procedures were conducted in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986.

2.2. Procedure

After a period of 21 weeks, animals were re-housed in individual cages and continued to have access to either the high fat diet ($n=35$) or standard laboratory chow ($n=7$) for a period of 10 days. Diet-induced obese (DIO) animals were then randomly allocated to one of the two drug treatment groups and each was matched to a pair-fed control of similar body weight. A further group of DIO animals was allocated to the vehicle control condition. DIO animals received rimonabant (3 and 10 mg/kg PO) or vehicle once daily for a 14-day period. Each pair-fed DIO control was dosed with vehicle and given a daily food allotment equal to that consumed by its drug-treated counterpart over the previous 24-h period. A check was also made to ensure that the previous ration had been consumed and this was always the case. A final group of age-matched animals only given access to standard laboratory chow was also dosed daily with vehicle. All weighing and dosing took place in the morning between 0900 and 1100 h.

Animals were killed by an overdose of CO_2 24 h after the last injection. Heart blood was collected for determining plasma

adiponectin levels. Plasma samples were frozen at −20 °C for subsequent analysis, then measured with an adiponectin Enzyme-Linked Immunosorbent Assay (ELISA) kit (K1002-1, B-Bridge International Inc., California, USA) in the Department of Pharmacology and Assay Technology, Vernalis Research Ltd, UK. Visceral adipose tissue was collected from the ventral and rostral areas of the abdominal cavity, immersed in RNAse Later (Sigma-Aldrich, UK) and frozen at −20 °C.

2.2.1. RNA preparation and real-time PCR (q-PCR)

Total RNA was extracted using the TRIzol (Invitrogen, UK) procedure (Chomczynski and Sacchi, 1987), according to the manufacturers instructions. Briefly, adipose tissue was homogenised in 1 mL of TRIzol reagent per 100 mg tissue and centrifuged at 8000×*g* for 25 min. Linear acrylamide (Ambion, UK) was then added to the supernatant followed by chloroform (Sigma, UK). RNA was then recovered from the aqueous phase by isopropanol (Sigma, UK) precipitation. The RNA pellet was washed with 75% ethanol and re-suspended in RNase-free water (Sigma, UK). RNA concentration was determined by absorption at A260. Total RNA was then treated with DNA-free (Ambion, UK) for 25 min at 37 °C to remove any traces of genomic DNA. 1 μg RNA was reverse transcribed with 100 ng random hexamers and 0.5 mM dNTPs in a final volume of 20 μL, using Superscript III reverse transcriptase (Invitrogen, UK). Reaction temperatures used were 25 °C for 5 min, and 50 °C for 1 h followed by an inactivation step of 70 °C for 15 min.

qPCR was conducted using Sybr Green I Mastermix (Applied Biosystems, UK) using an ABI PRISM 7700 Sequence Detection System. Each reaction was repeated across 7 plates using 1 μg of cDNA template per reaction in a final reaction volume of 25 μL with 25 pmoles of primer sets. Target gene expression was normalised against expression of the housekeeper gene glyceraldehyde-3-phosphate dehydrogenase (GAPD). No template (without RNA) and no amplification (without Superscript III) control reactions were run on each 96-well plate. Primers were designed using Primer Express software (ABI, UK). Primer sequences were as follows: Adiponectin forward, 5′-AGG TTG GAT GGC AGG CAT C-3′; Adiponectin reverse, 5′-GGC TCT CCT TTC CTG CCA G-3′; GAPD forward, 5′-GTC ATC ATC TCC GCC CCT T-3′; GAPD reverse, 5′-CTG AGT GGC AGT GAT GGC AT-3′. All primers were synthesised at 50 nM scale (Invitrogen, UK) and reconstituted at 12.5 pM/μL. Cycling parameters used for qPCR were: initial denaturing step at 95 °C for 15 min and 40 cycles consisting of 95 °C for 15 s, 60 °C for 1 min, 76 °C for 20 s, 80 °C for 20 s. Amplification products from the first run were sequenced to confirm primer authenticity and specificity.

2.3. Drugs

Rimonabant (5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl- *N*-1-piperidinyl-[1H]-pyrazole-3-carboxamide hydrochloride]) was synthesised in the Department of Chemistry, Vernalis Research Ltd, UK.

Injections of rimonabant were administered orally in a suspension of 1% methylcellulose in a volume of 3 mL/kg at doses of 0, 3 and 10 mg/kg. The drug solutions were freshly prepared for each day. All drug doses are expressed as free-base.

2.4. Data analysis

Body weight data were analysed by ANCOVA where dose was a between-subject factor and initial body weight was used as the covariate. A significant treatment × time interaction was examined by restricting the analysis to each level of time, followed by paired contrasts between treatment groups. Body weight changes are presented as percentage body weight gain compared to day 1. Food intake were analysed by a two-way ANOVA, since the inclusion of initial of body weight as a covariate did not reduce the relevant error term. Significant effects were investigated with a repeated ANOVA at each level of time.

PCR data were analysed using SDS 1.7 (Applied Biosystems). Subsequent calculations were automated using an Excel workbook entitled Data Analysis for Real-time PCR (DART-PCR) to enable rapid calculation of threshold cycles, amplification efficiency and resulting R_0 values from raw data exported from SDS 1.7 (Peirson et al., 2003). Expression of target gene was normalised against expression of GAPD for each data point. A mean of data points from all plates was taken for each individual. Any data points across plates that were 2 standard deviations away from an individual subject's mean were removed from any subsequent analysis. A mean of this data was then used to obtain group data. Differences in gene expression were assessed using a one-way ANOVA and an independent samples *t*-test was used to determine any difference between chow-fed and obese vehicle groups. Planned, orthogonal contrasts were then used to test key questions regarding adiponectin gene expression: 1) Do obese vehicle-treated subjects differ from obese rimonabant-treated and pair-fed subjects? 2) Do rimonabant-treated subjects differ from pair-fed subjects? A two-way ANOVA (factors dose and group) was also used to explore any effects and interactions between rimonabant-treated subjects and their pair-fed counterparts at both doses. Plasma adiponectin levels were analysed using a similar strategy.

3. Results

Absolute body weight over the 14-day study was dose-dependently reduced in DIO rats treated with rimonabant or paired feeding (Table 1; ANCOVA, $F_{5,35}=12.02$, $p<0.001$). Contrast analysis revealed highly significant decreases in both rimonabant-treated animals and their pair-fed counterparts ($p<0.001$) compared to the obese controls. However there were no differences between either rimonabant treated group and its respective paired control at the end of the study. The daily body weight change data (Fig. 1) showed a significant treatment × time interaction ($F_{65,468}=10.04$, $p<0.001$), with significant reductions in both rimonabant treated and pair-fed groups from Day 2 onwards. On Days 1 and 2 only, the group treated with 10 mg rimonabant showed a greater body weight loss than the pair fed control group (Fig. 1).

Table 1
The effects of chronic administration of rimonabant (0, 3, 10 mg/kg PO) and paired-feeding on body weight, food and water intake, adiponectin gene expression and plasma adiponectin concentrations

Initial body weight (g)		Final body weight (g)	Total food intake (g)	Total water intake (g)	Adiponectin mRNA (adiponectin R_0/GAPD R_0)	Plasma adiponectin (μg/mL)
Chow-fed control	598.1 (16.3)**	594.4 (15.5)**	320.7 (14.1)	411.2 (18.8)	5.0 (0.3)**	4.4 (1.1)*
Obese control	768.6 (19.4)	762.3 (17.3)	296.6 (7.3)	320.5 (22.5)	3.5 (0.1)	2.4 (0.2)
3 mg/kg daily rimonabant	765.7 (30.1)	726.3 (28.6)**	237.4 (11.5)**	316.4 (15.5)	4.2 (0.4)	2.4 (0.2)
10 mg/kg daily rimonabant	768.3 (29.9)	709.3 (25.9)**	203.7 (12.9)**	297.4 (14.6)	4.5 (0.2)	1.9 (0.1)
Pair-fed (3 mg/kg)	764.7 (23.8)	720.1 (23.9) **	237.4 (11.5)†	397.1 (59.9)	4.2 (0.2)	2.4 (0.3)
Pair-fed (10 mg/kg)	765.9 (28.9)	709.9 (21.8)**	203.7 (12.9)†	377.5 (43.3)	4.0 (0.2)	2.2 (0.3)

Data are mean±S.E.M. $n=7$ per group. *$p<0.05$, **$p<0.01$, Dunnett's t-test compared to vehicle-treated obese controls.
†Pair-fed groups: intake identical to relevant drug-treated group.

Total food intake over the study period was reduced in a dose dependent manner by both doses of rimonanbant (Table 1; $F_{2,17}=19.58$, $p<0.001$). There was also a significant effect of group on daily food intake (Fig. 2; $F_{3,24}=20.63$, $p<0.001$). Contrast analysis revealed significant decreases in intake compared to the controls for both rimonabant-treated groups (3 mg/kg: $p<0.01$, 10 mg/kg: $p<0.001$). A significant rimonabant-induced hypophagia was maintained throughout the study at the high dose with significant decreases in intake on all but days 11 and 14 although there was also a clear development of tolerance. Tolerance to the hypophagic effects of rimonabant also developed in the low dose group who furthermore showed no significant differences in intake compared to the vehicle group on days 4, 10, 11, 12 and 14 (Fig. 2). There was no overall effect of group on water intake (Table 1: $F_{5,36}$ 2.02, $p=0.1$). The water intake of the two DIO groups treated with rimonabant was very close to that of the DIO vehicle controls (Table 1), although there was some suggestion that restricted intake due to pair feeding might have increased water intake.

There was a significant effect of group on adiponectin gene expression (Table 1; $F_{5,41}=2.65$, $p<0.05$). Orthogonal comparisons revealed that adiponectin gene expression in chow-fed subjects treated with vehicle was significantly greater than in obese subjects treated with vehicle ($p<0.01$). Adiponectin expression in obese subjects treated with vehicle was not significantly different from that of rimonabant-treated and pair-fed subjects ($p=0.06$) 2) and there were no differences in gene expression between rimonabant-treated subjects and pair-fed subjects ($p=0.39$). There was a significant effect of group on plasma adiponectin levels (Table 1: $F_{5,41}=3.32$, $p<0.05$). This was due to a significant increase in adiponectin plasma levels in the chow-fed controls compared to the DIO controls ($p<0.01$), however there were no further significant differences between the groups (Table 1).

4. Discussion

This study demonstrates that DIO rats chronically treated with rimonabant (3, 10 mg/kg per day) show a dose-related decrease in body weight and total food intake over the 14-day study period. Pair-fed controls to the rimonabant-treated groups showed an almost identical pattern of body weight change. Although tolerance to the hypophagic effects of rimonabant developed towards the end of the 14-day study, the reduction in both cumulative food intake and body weight was maintained to

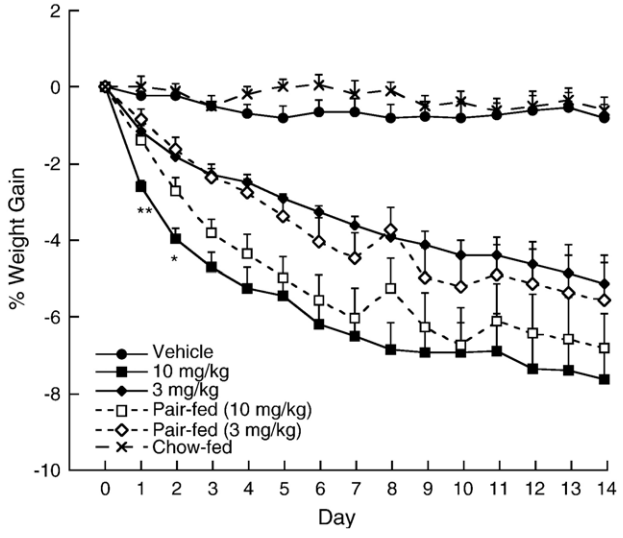

Fig. 1. Effects of chronic administration of rimonabant or paired-feeding on % body weight gain. Data are mean±S.E.M. *$p<0.05$, **$p<0.01$ 10 mg/kg rimonabant compared to pair-fed controls.

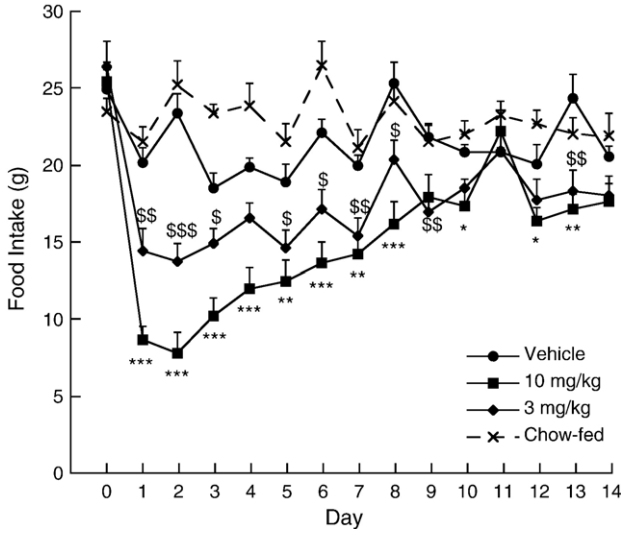

Fig. 2. Effects of chronic administration of rimonabant or paired-feeding on daily food consumption. Data are mean±S.E.M. */$$p<0.05$, **/$$$p<0.01$, ***/$$p<0.001$ compared to obese controls.

the end of the study period. Adiponectin gene expression in fat tissue at completion of the study period showed an enhanced level in vehicle-treated chow-fed animals compared to vehicle-treated obese animals. There were no differences in adiponectin gene expression between rimonabant-treated animals and their pair-fed counterparts. Plasma adiponectin showed a similar pattern of elevation in chow fed controls by comparison with all other treatment groups and an absence of differences between drug-treated and pair-fed groups.

The similarity of overall body weight changes in rimonabant-treated rats and their pair-fed controls suggests that the reduction of body mass resulted from a decrease in food intake. Ravinet Trillou et al. (2003) chronically administered rimonabant to DIO mice and included a 3-day pair-fed experiment in which the body weight loss in rimonabant-treated subjects was superior to pair-fed controls. This effect was also observed in our study where animals treated with 10 mg/kg rimonabant showed significantly decreased body weight gain on day 1 and day 2 compared to their pair-fed counterparts (Fig. 1), although this effect was not present in animals treated with 3 mg/kg rimonabant. However from day 3 of our study body weight gain did not differ between drug-treated and pair-fed groups and there was no overall difference between the rimonabant-treated groups and their pair-fed controls over the duration of the study. Therefore the effects of rimonabant on body weight in the present study appear to be primarily due to effects on food consumption although there may be a transitory additional effect of drug treatment during the first 1–2 days. An additional short pair-feeding study would be necessary to confirm this suggestion.

The conclusion that the effects of rimonabant on body weight depend on reduced energy intake is also consistent with the significant reduction in cumulative food intake over the 14-day study period. The deficit was 59 g in the 3 mg/kg group and 93 g in the 10 mg/kg group, which, given an energy density of 19.7 kJ/g (Method) equates to 1162 kJ and 1832 kJ respectively. Farrell and Williams (1989) conducted a study in which rats were food deprived and re-fed on several occasions. They concluded that food intake of about 20 kJ equated to a gram of body tissue, although this figure reduced somewhat on repeated deprivation and re-feeding. This would suggest that that the deficits in cumulative food intake over the study period equate to a loss of 58 g and 92 g of body weight in the 3 and 10 mg/kg rimonabant groups. These figures are very close to the actual figures reported in Table 1, again suggesting that direct metabolic effects of rimonabant were unimportant in relation to the overall body weight changes observed in this study.

CB_1 receptor knockout mice have also been shown to exhibit reduced food intake and body weight compared to wild-type controls (Cota et al., 2003). The reduction in body weight appears to be fully dependent on caloric intake in young mice but independent of intake in adult mice. The animals used in our study were more than 6 months old and had attained relatively stable weights (see Fig. 1—vehicle DIO and chow fed controls), yet our data indicate changes in body weight dependent on caloric intake and do not support the hypothesis that metabolic consequences of rimonabant have greater importance in older animals. This discrepancy may reflect developmental adaptations in the CB_1 receptor knockout mice or species differences.

Tolerance to the hypophagic effect of rimonabant has been consistently reported in the literature (see Introduction). Vickers et al. (2003) found that whilst tolerance to the anorectic effects of rimonabant was observed by day 13 in obese Zucker rats, food consumption was maintained below control levels for the remainder of the study. The development of tolerance was also clear in our study, although daily intake remained consistently, although not significantly, below that of DIO controls. At no point was there a rebound in food intake that would have allowed rimonabant-treated animals to recover their cumulative energy deficit.

The mechanisms that underlie development of tolerance to the anorectic effect of rimonabant and to the anorectic effect itself remain unclear. Comparable doses of rimonabant to those used in the present study can reduce saccharin preference in a conditioned taste aversion paradigm (De Vry et al., 2004) and potentiate lithium induced conditioned gaping (Parker and Mechoulam, 2003) in rats. Rimonabant also induces some motor stereotypies, including wet dog shakes and scratching (Vickers et al., 2003), which may also increase energy expenditure in addition to a possible disruption of food intake. A short term increase in energy expenditure of this type could potentially underlie the difference between the pair fed and 10 mg/kg rimonabant groups in the first 2 days of our study. Tolerance develops at least as rapidly to these effects as to the reduction in food intake (Vickers et al., 2003). It seems most likely that the anorectic effect depends on a reduced motivation to feed (Thornton-Jones et al., 2005) and the lack of effect on water intake in this study is consistent with that conclusion. The extent to which tolerance to this effect depends on a response to changed set point or to pharmacokinetic or pharmacodynamic effects has not been determined.

Analysis of adiponectin gene expression in adipose tissue revealed significantly elevated levels in chow-fed controls compared to DIO controls. This supports previously published data showing adiponectin gene expression to be lower in obese in comparison with lean subjects (Hu et al., 1996; Milan et al., 2002). Chronic administration of adiponectin mRNA levels following rimonabant treatment did not differ from chow-fed controls. This is consistent with the findings of a study by Bensaid et al. (2003) who showed adiponectin gene expression in obese Zucker (*fa/fa*) rats to be elevated after 4 days of treatment with rimonabant although the drug was administered ip in that study. Most interestingly in our study, adiponectin gene expression of pair-fed controls did not differ from that of rimonabant-treated animals on day 15. This is consistent with the hypothesis that any effects of rimonabant on adiponectin gene expression are likely to be an indirect consequence of reduced food intake and body weight following CB_1 receptor blockade rather than direct drug-induced changes on adipose tissue function.

There is some evidence that alterations in adiponectin gene expression are not solely dependent on weight change. Decreased expression and plasma levels have been demonstrated in rats with 2 days access to a fat-enriched, glucose-

enriched diet prior to any variation in body weight (Naderali et al., 2003). In our study gene expression was measured at a time by which the effects of rimonabant on food intake had tolerated out in the low dose group and possibly also in the high dose, therefore food restriction at this point was not severe. Data from the Bensaid study (2003) after 4 days of treatment indicates that large effects on adiponectin mRNA can be observed under some circumstances. Plasma adiponectin was also increased in chow-fed animals compared to obese controls but, as with adiponectin gene expression, there were no differences between obese controls, rimonabant-treated and pair-fed controls. However it is possible that we would have been able to observe such differences if additional plasma samples had been taken during the experiment.

In conclusion we suggest that the effects of rimonabant on body weight loss in our study were largely attributable to decreases in food intake since no differences between rimonabant-treated animals and pair-fed controls were observed. Furthermore adiponectin gene expression and plasma adiponectin levels did not differ between rimonabant-treated animals and their pair-fed counterparts. Both findings argue against any substantial direct effect of the drug on adipose tissue function in this study. The maintained reduction in body weight demonstrated in the present study, together with the reduction in responsiveness to food related cues during the appetitive phase of feeding behaviour (Thornton-Jones et al., 2005), suggest that the CB_1 receptor is an appropriate target for pharmacological treatment of human hyperphagia and obesity.

Acknowledgements

This work was supported by a MRC Collaborative Studentship to ZDT-J and co-funded by Vernalis Research Ltd.

References

Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 1999;257:79–83.

Bensaid M, Gary-Bobo M, Esclangon A, Maffrand JP, Le Fur G, Oury-Donat F, et al. 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 2003;63:908–14.

Berg AH, Combs TP, Scherer PE. ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends Endocrinol Metab 2002;13:84–9.

Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal Biochem 1987;162:156–9.

Colombo G, Agabio R, Diaz G, Lobina C, Reali R, Gessa GL. Appetite suppression and weight loss after the cannabinoid antagonist SR 141716. Life Sci 1998;63:113–7.

Combs TP, Berg AH, Rajala MW, Klebanov S, Iyengar P, Jimenez-Chillaron JC, et al. Sexual differentiation, pregnancy, calorie restriction, and aging affect the adipocyte-specific secretory protein adiponectin. Diabetes 2003;52:268–276.

Cota D, Marsicano G, Tschop M, Grubler Y, Flachskamm C, Schubert M, et al. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. J Clin Invest 2003;112:423–31.

De Vry J, Schreiber R, Eckel G, Jentzsch KR. Behavioral mechanisms underlying inhibition of food-maintained responding by the cannabinoid receptor antagonist/inverse agonist SR141716A. Eur J Pharmacol 2004;483:55–63.

Di Marzo V, Goparaju SK, Wang L, Liu J, Batkai S, Jarai Z, et al. Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature 2001;410:822–5.

Farrell DJ, Williams VJ. Calorimetric measurements made on rats during repeated periods of weight gain and weight loss. Comp Biochem Physiol 1989;94A:61–7.

Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, et al. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci U S A 2001;98:2005–10.

Higgs S, Williams CM, Kirkham TC. Cannabinoid influences on palatability: microstructural analysis of sucrose drinking after delta(9)-tetrahydrocannabinol, anandamide, 2-arachidonoyl glycerol and SR141716. Psychopharmacology (Berl) 2003;165:370–7.

Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000;20:1595–9.

Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 1996;271:10697–703.

Jamshidi N, Taylor DA. Anandamide administration into the ventromedial hypothalamus stimulates appetite in rats. Br J Pharmacol 2001;134:1151–4.

Kirkham TC. Endocannabinoids in the regulation of appetite and body weight. Behav Pharmacol 2005;16:297–313.

Kirkham TC, Williams CM, Fezza F, Di Marzo V. 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 2002;136:550–7.

Matsuzawa Y, Shimomura I, Kihara S, Funahashi T. Importance of adipocytokines in obesity-related diseases. Horm Res 2003;60(Suppl. 3):56–59.

Milan G, Granzotto M, Scarda A, Calcagno A, Pagano C, Federspil G, et al. Resistin and adiponectin expression in visceral fat of obese rats: effect of weight loss. Obes Res 2002;10:1095–103.

Naderali EK, Estadella D, Rocha M, Pickavance LC, Fatani S, Denis RG, et al. A fat-enriched, glucose-enriched diet markedly attenuates adiponectin mRNA levels in rat epididymal adipose tissue. Clin Sci (Lond) 2003;105:403–408.

Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation 1999;100:2473–6.

Parker LA, Mechoulam R. Cannabinoid agonists and antagonists modulate lithium-induced conditioned gaping in rats. Integr Physiol Behav Sci 2003;38:133–45.

Peirson SN, Butler JN, Foster RG. Experimental validation of novel and conventional approaches to quantitative real-time PCR data analysis. Nucleic Acids Res 2003;31:e73.

Ravinet Trillou C, Arnone M, Delgorge C, Gonalons N, Keane P, Maffrand JP, et al. Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. Am J Physiol Regul Integr Comp Physiol 2003;284:R345–53.

Rinaldi-Carmona M, Barth F, Heaulme M, Alonso R, Shire D, Congy C, et al. Biochemical and pharmacological characterisation of SR141716A, the first potent and selective brain cannabinoid receptor antagonist. Life Sci 1995;56:1941–7.

Rowland NE, Mukherjee M, Robertson K. Effects of the cannabinoid receptor antagonist SR 141716, alone and in combination with dexfenfluramine or naloxone, on food intake in rats. Psychopharmacology (Berl) 2001;159:111–116.

Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 1995;270:26746–9.

Shklyaev S, Aslanidi G, Tennant M, Prima V, Kohlbrenner E, Kroutov V, et al. Sustained peripheral expression of transgene adiponectin offsets the development of diet-induced obesity in rats. Proc Natl Acad Sci U S A 2003;100:14217–22.

Statnick MA, Beavers LS, Conner LJ, Corominola H, Johnson D, Hammond CD, et al. Decreased expression of apM1 in omental and subcutaneous adipose tissue of humans with type 2 diabetes. Int J Exp Diabetes Res 2000;1:81–8.

Stefan N, Stumvoll M. Adiponectin—its role in metabolism and beyond. Horm Metab Res 2002;34:469–74.

Thornton-Jones ZD, Vickers SP, Clifton PG. The cannabinoid CB1 receptor antagonist SR141716A reduces appetitive and consummatory responses for food. Psychopharmacology (Berl) 2005;179:452–60.

Verty AN, McFarlane JR, McGregor IS, Mallet PE. Evidence for an interaction between CB1 cannabinoid and melanocortin MCR-4 receptors in regulating food intake. Endocrinology 2004a;145:3224–31.

Verty AN, McGregor IS, Mallet PE. Consumption of high carbohydrate, high fat, and normal chow is equally suppressed by a cannabinoid receptor antagonist in non-deprived rats. Neurosci Lett 2004b;354:217–20.

Vickers SP, Kennett GA. Cannabinoids and the regulation of ingestive behaviour. Curr Drug Targets 2005;6:215–23.

Vickers SP, Webster LJ, Wyatt A, Dourish CT, Kennett GA. 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 (Berl) 2003;167:103–11.

Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 2001;86:1930–5.

Williams CM, Kirkham TC. Anandamide induces overeating: mediation by central cannabinoid (CB1) receptors. Psychopharmacology (Berl) 1999;143:315–7.

Williams CM, Kirkham TC. Reversal of delta 9-THC hyperphagia by SR141716 and naloxone but not dexfenfluramine. Pharmacol Biochem Behav 2002;71:333–40.

Zhang Y, Matheny M, Zolotukhin S, Tumer N, Scarpace PJ. Regulation of adiponectin and leptin gene expression in white and brown adipose tissues: influence of beta3-adrenergic agonists, retinoic acid, leptin and fasting. Biochim Biophys Acta 2002;1584:115–22.