Deorphanization of a G protein-coupled receptor for oleoylethanolamide and its use in the discovery of small-molecule hypophagic agents

Hilary A. Overton,[1,*] Adam J. Babbs,[1] Sheila M. Doel,[1] Matthew C.T. Fyfe,[1] Lisa S. Gardner,[1] Graeme Griffin,[2] Helen C. Jackson,[3] Martin J. Procter,[1] Chrystelle M. Rasamison,[1] Mads Tang-Christensen,[4] Peter S. Widdowson,[1] Geoffery M. Williams,[1] and Christine Reynet[1]

[1] (OSI)Prosidion, Windrush Court, Watlington Road, Oxford OX4 6LT, United Kingdom
[2] OSI Pharmaceuticals, Inc., 1 Bioscience Park Drive, Farmingdale, New York 11735
[3] RenaSci Consultancy Limited, Pennyfoot Street, Nottingham NG1 1GF, United Kingdom
[4] Rheoscience A/S, Glerupvej 2, 2610 Rødovre, Denmark
*Correspondence: hoverton@prosidion.com

Summary

The endogenous lipid signaling agent oleoylethanolamide (OEA) has recently been described as a peripherally acting agent that reduces food intake and body weight gain in rat feeding models. This paper presents evidence that OEA is an endogenous ligand of the orphan receptor GPR119, a G protein-coupled receptor (GPCR) expressed predominantly in the human and rodent pancreas and gastrointestinal tract and also in rodent brain, suggesting that the reported effects of OEA on food intake may be mediated, at least in part, via the GPR119 receptor. Furthermore, we have used the recombinant receptor to discover novel selective small-molecule GPR119 agonists, typified by PSN632408, which suppress food intake in rats and reduce body weight gain and white adipose tissue deposition upon subchronic oral administration to high-fat-fed rats. GPR119 therefore represents a novel and attractive potential target for the therapy of obesity and related metabolic disorders.

Introduction

The rapidly increasing prevalence of obesity in Westernized societies and in some developing countries has focused the efforts of healthcare providers and pharmaceutical companies on the search for new, efficacious, and safe treatments to counter this problem (Jandacek and Woods, 2004; Stein and Colditz, 2004). We are engaged in the search for agents that alter various physiological signaling systems, either through attenuating hunger signals or through eliciting premature satiety following meal ingestion, and that may act as novel anti-obesity therapies.

Recently, a number of fatty acids have been shown to act as ligands at G protein-coupled receptors (GPCRs) (Briscoe et al., 2003; Itoh et al., 2003; Hirasawa et al., 2004), and fatty-acid derivatives including anandamide (arachidonylethanolamide; AEA) and 2-arachidonyl glycerol may be endogenous ligands at the cannabinoid receptors CB1 and CB2 (Devane et al., 1992; Hanus et al., 1993; Mechoulam et al., 1995; Sugiura et al., 1995). Recent publications have demonstrated that the orphan receptor GPR55 responds to a similar series of fatty-acid ethanolamides and related compounds as do the cannabinoid receptors (A.J. Brown et al., 2005, Symposium on the Cannabinoids, Burlington, Vermont, International Cannabinoid Research Society, p. 16; S. Sjogren et al., 2005, p.106). This includes ''standard'' endogenous and synthetic cannabinoids as well as lipids with shorter chains/higher saturation such as palmitoylethanolamide (PEA). Due to this overlap of endogenous activating ligands, the adoption of the CB3 nomenclature for GPR55 may follow in due course. Although it is tempting to speculate that GPR35, which by clustering analysis is the closest relation to GPR55 (Fredriksson et al., 2003), may also be ultimately identified as a ''cannabinoid'' receptor, this presently remains to be established.

The CB1 receptor is known to play a role in food intake in rats and humans, and its modulation by antagonists such as rimonabant (SR-141716) is a promising approach for the control of obesity (Cota et al., 2003; Black, 2004). The endogenous fatty-acid ethanolamide oleoylethanolamide (OEA) has recently been described as a peripherally acting agent that suppresses food intake and, upon subchronic intraperitoneal administration, reduces body weight gain in rodent feeding models (Rodriguez de Fonseca et al., 2001; Fu et al., 2003; Gaetani et al., 2003; Oveisi et al., 2004; Nielsen et al., 2004; Piomelli et al., 2004). This present work provides evidence that OEA is an agonist at GPR119 (Fredriksson et al., 2003), an orphan GPCR expressed predominantly in the pancreas and gastrointestinal tract in humans and in the brain, pancreas, and gastrointestinal tract in rodents (Bonini et al., 2002).

In view of the gastrointestinal localization of GPR119, we hypothesized that the reported effects of OEA on feeding may be mediated, at least in part, by this receptor and that nonlipidic GPR119 agonists would therefore provide a novel means of pharmaceutical intervention to reduce food intake. We therefore expressed recombinant human and mouse GPR119 in a yeast assay system and used this to screen a proprietary small-molecule library of several hundred thousand compounds for novel agonists. An optimized agonist, PSN632408, was then used to test our hypothesis, and we report on the hypophagic effects of this molecule in rat models of obesity.

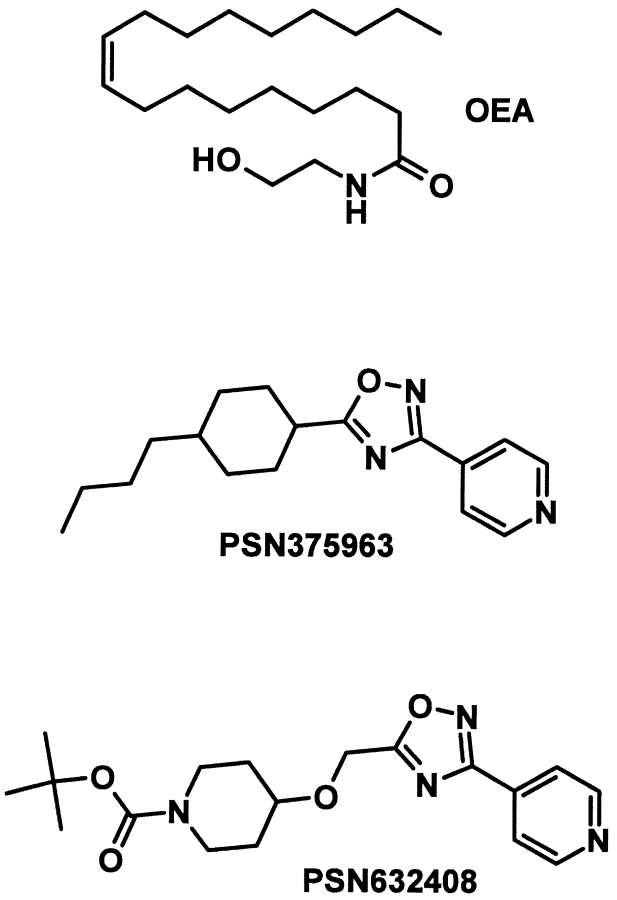

Figure 1. Structures of OEA, PSN375963, and PSN632408

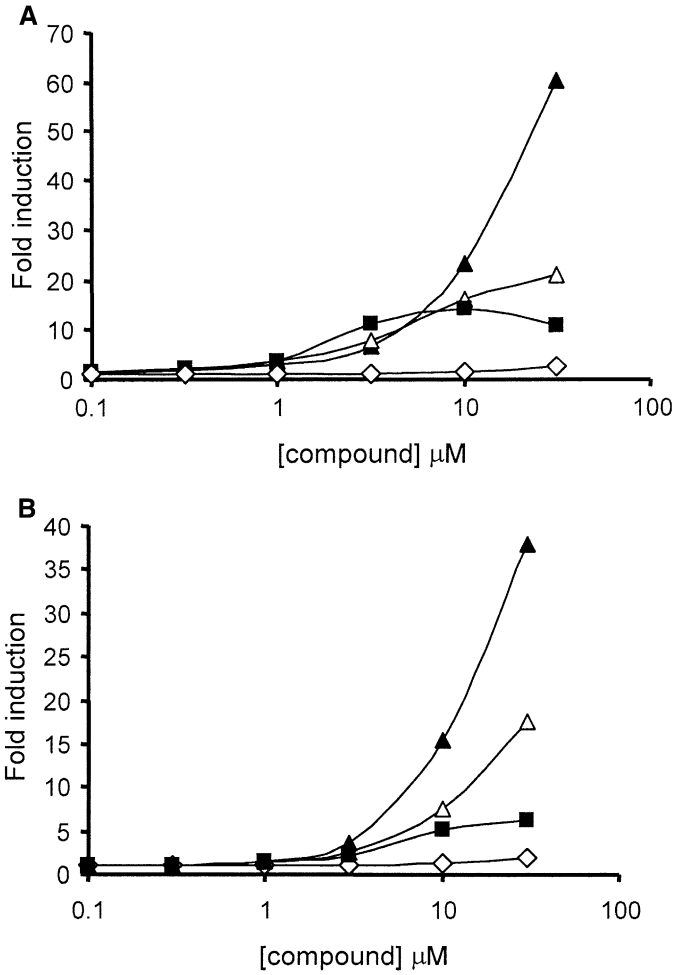

Figure 2. Fatty-acid ethanolamides are agonists at GPR119

Fold induction of signal (means; n = 4) by fatty-acid ethanolamides in yeast fluorimetric assay for (A) human GPR119 and (B) mouse GPR119.
OEA—filled triangles; PEA—open triangles; SEA—filled squares; AEA—open diamonds (see text for definitions).

Results and Discussion

Deorphanization of GPR119

In the course of our functional genomics program focused on orphan human GPCRs, one of the novel receptors identified was designated OSGPR116. Subsequently, receptors designated SNORF25 (Bonini et al., 2002) and GPR119 (Fredriksson et al., 2003) were described in the literature, and their sequences found to be identical to that of OSGPR116. The mRNA expression profile of OSGPR116 was similar to that described for SNORF25 (data not shown). For the sake of clarity, the receptor OSGPR116 is referred to as GPR119 in this paper.

Human and mouse isoforms of the receptor were cloned and expressed in a yeast-based functional assay (described in Experimental Procedures) and a search for endogenous ligands initiated. Since our homology clustering analysis had indicated that the closest relatives of this GPCR are the cannabinoid receptors, a number of cannabinoid ligands and fatty-acid ethanolamides were tested as potential agonists. The endogenous cannabinoid 2-arachidonyl glycerol and the synthetic cannabinoid ligands CP55940, WIN55212-2, methanandamide, and JWH-133 did not activate the receptor in the yeast-based assay (data not shown), while the endogenous cannabinoid arachidonylethanolamide (anandamide, AEA, C20:4, i.e., fatty-acid chain length of 20 carbons with 4 double bonds) produced a modest (2- to 3-fold) increase in fluorescence. In contrast, the more saturated fatty-acid ethanolamides oleoylethanolamide (OEA, C18:1, Figure 1), stearoylethanolamide (SEA, C18:0), and palmitoylethanolamide (PEA, C16:0) produced much greater increases in fluorescence (Figure 2), indicating that these are agonists at this receptor. The rank order of efficacy of these fatty-acid ethanolamides was identical at the human and mouse isoforms with OEA being the most efficacious in each case. No response to OEA was observed in an equivalent yeast strain in which the transforming plasmid lacked the GPR119 gene (data not shown), demonstrating that the OEA exerts its effect via the GPR119 receptor and not at some downstream point in the yeast signaling cascade. Linolenoyl ethanolamide (C18:3), linoleoyl ethanolamide (C18:2), and oleamide (the primary amide of oleic acid) also activated the human and mouse GPR119 receptors but with lower efficacies than OEA (data not shown).

Recently, oleoyl lysophosphatidylcholine (18:1-LPC) has been reported to be the most potent of a group of phospholipids acting as agonists at GPR119 (Soga et al., 2005). 18:1-LPC was tested in our yeast fluorimetric assay for human GPR119 and confirmed to be an agonist, although with a substantially lower activity than OEA (EC_{50} > 30 μM, compared to 3.2 μM for

Table 1. GPR119 agonism in yeast fluorimetric assay

	Human GPR119		Mouse GPR119	
	Emax[a,b]	EC_{50}, μM[b]	Emax[a,b]	EC_{50}, μM[b]
OEA	1	3.2 ± 0.33	1	2.9 ± 0.59
PSN375963	0.29 ± 0.03	8.4 ± 2.7	0.53 ± 0.04	7.9 ± 2.4
PSN632408	1.1 ± 0.04	5.6 ± 0.99	1.1 ± 0.03	7.9 ± 0.7

[a] Maximal fold induction of signal normalized to value for OEA.
[b] Values are means ± SEM.

OEA; 11-fold induction of signal with 100 μM 18:1-LPC compared to 30-fold with 100 μM OEA).

OEA, PEA, and SEA have all been described as agents capable of reducing food intake in rodent models (Rodriguez de Fonseca et al., 2001; Terrazzino et al., 2004). The discovery that these molecules act as agonists of an intestinally localized GPCR led us to hypothesize that GPR119 plays a role in the mediation of this hypophagic effect, and that small-molecule agonists could thus provide a novel means of pharmaceutical intervention to reduce food intake.

The identification of CB1 and CB2 (Matsuda et al., 1990; Munro et al., 1993) as two rather dissimilar receptors (about 44% amino acid homology) activated by a similar series of endogenous lipids demonstrated the existence of a subfamily of GPCRs now termed "cannabinoid receptors." Although additional cannabinoid receptors have been postulated and demonstrated to exist, either pharmacologically or through the use of CB1/CB2 receptor knockout mice (for example, Járai et al., 1999; Breivogel et al., 2001), the molecular identification of further cannabinoid receptors has not been forthcoming. The vanilloid receptor TRPV1, an ion channel, has been shown to be activated by anandamide (Zygmunt et al., 1999), but this receptor alone cannot account for all the non-CB1, non-CB2 receptor pharmacology described in the literature. GPR55 has recently been demonstrated to respond to fatty-acid ethanolamides, as described in the Introduction. Our identification of GPR119 as another receptor that responds to the same family of ligands (albeit preferentially activated by shorter chain fatty-acid ethanolamides such as OEA and PEA, and positively coupled to adenylate cyclase in contrast to CB1 and CB2) further suggests that the family of cannabinoid receptors is in fact significantly more extensive than the long-accepted CB1/CB2 receptor pairing.

Identification of small-molecule GPR119 agonists

The yeast-based fluorimetric assay was used to conduct a high-throughput screening campaign, leading to the identification of a number of small-molecule GPR119 agonists. One of the compounds identified, PSN375963 (Figure 1), showed similar potency to OEA at both the mouse and human GPR119 receptors, although its efficacy was lower (Table 1). Preliminary optimization of PSN375963 yielded PSN632408 (Figure 1; Fyfe et al., 2005), a compound similar in efficacy and potency to OEA at both human and mouse GPR119 receptors (Table 1) but without the lipophilicity associated with poor drug properties (Wenlock et al., 2003) that is present in both OEA and PSN375963.

GPR119 agonists increase intracellular cAMP

A stable HEK 293-derived cell line (HEK-OSGPR116) that expresses human GPR119 in a tetracycline-inducible manner was generated. Quantitative RT-PCR was used to demonstrate a concentration-dependent increase in the expression of GPR119 mRNA of two orders of magnitude when these cells were treated with tetracycline as described in Experimental Procedures (Figure 3A).

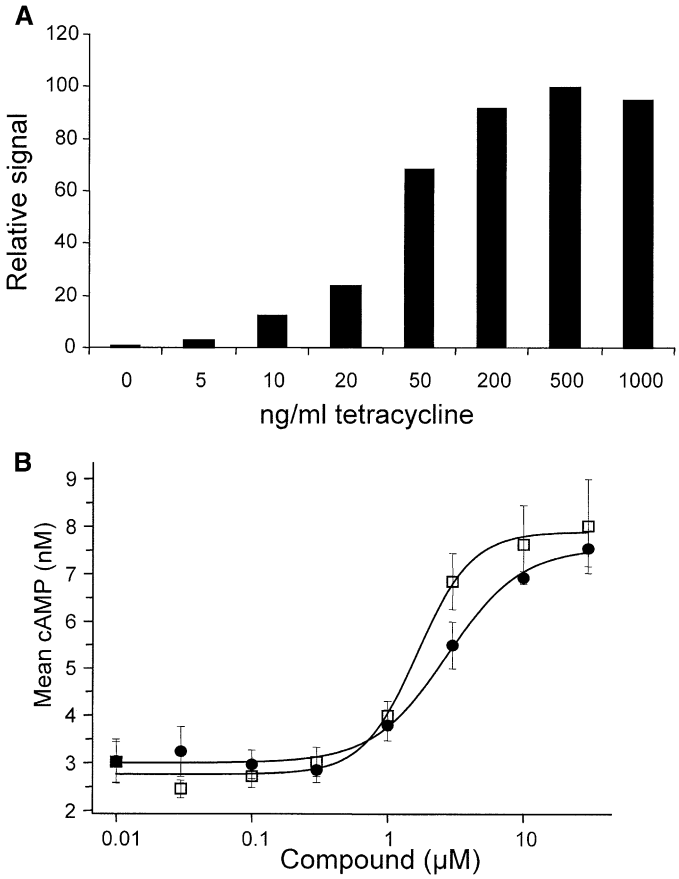

Figure 3. GPR119 agonists stimulate adenylate cyclase in mammalian cells

A) Quantitative RT-PCR analysis of hGPR119-specific mRNA levels in HEK-OSGPR116 cells treated with increasing concentrations of tetracycline.

B) Responses of intracellular cAMP levels to treatment of tetracycline-induced HEK-OSGPR116 cells with OEA (filled circles) or PSN632408 (open squares). Results expressed as means ± SEM.

The HEK-OSGPR116 cell line was used to investigate the effect of GPR119 agonists on intracellular levels of cyclic AMP (cAMP). Treatment with either OEA or PSN632408 produced concentration-dependent increases in cAMP level with mean EC_{50} values of 2.9 ± 0.46 μM for OEA and 1.9 ± 0.14 μM for PSN632408. Dose-response curves for a typical experiment are shown in Figure 3B. When an equivalent experiment was performed using control cells engineered to express chloramphenicol acetyl transferase instead of GPR119, neither OEA nor PSN632408 produced any effect on the level of intracellular cAMP (data not shown). These results demonstrate that agonists at GPR119 stimulate adenylate cyclase, most likely by signaling via $G_{\alpha s}$ coupling.

PSN632408 is a selective agonist at GPR119

PSN632408 was tested for agonist activity at the human cannabinoid receptors CB1 and CB2 using yeast-based reporter assays analogous to those developed for GPR119. No activation was seen at concentrations up to 100 μM (data not shown).

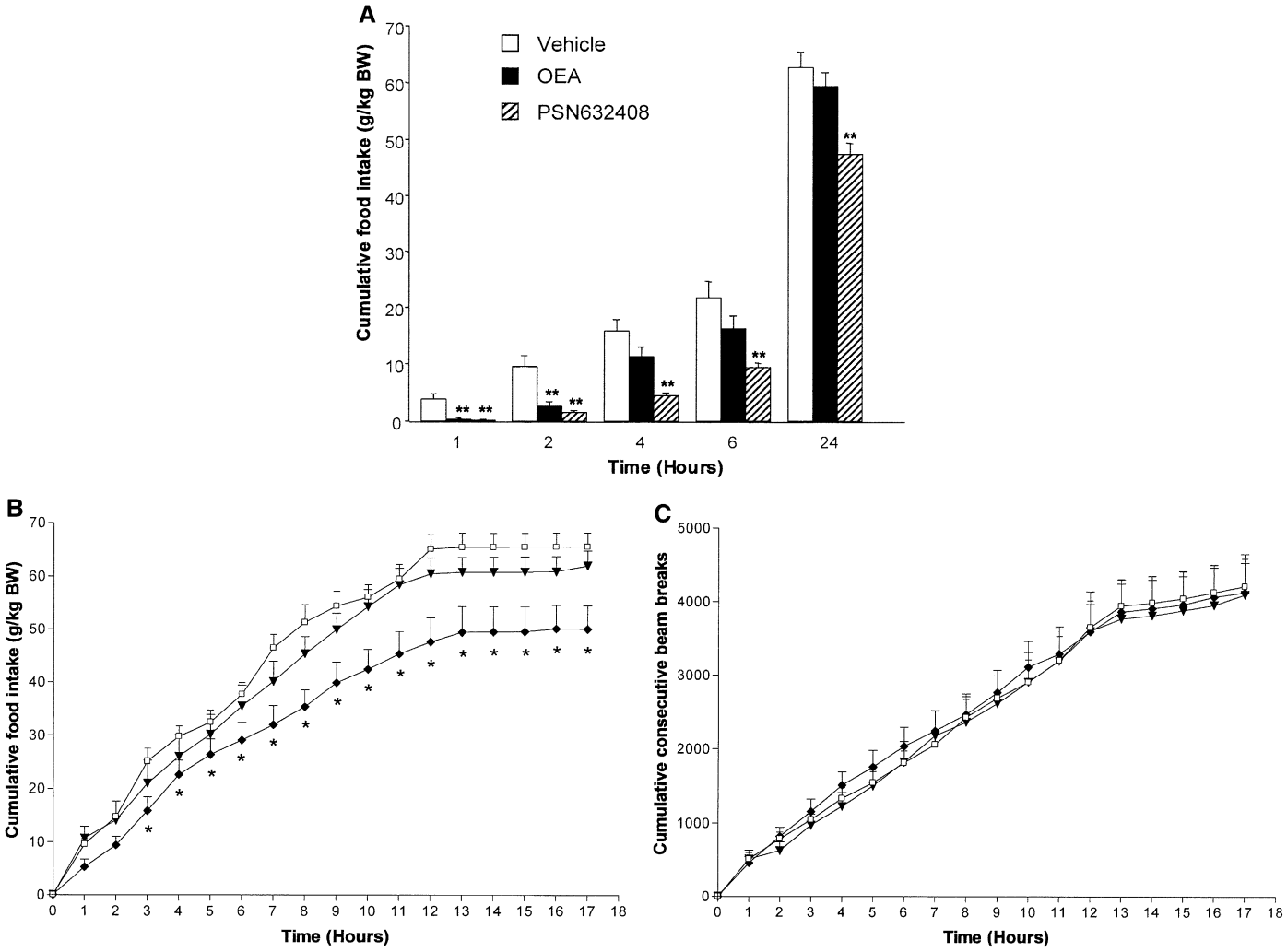

Figure 4. Acute dosing of rats with GPR119 agonists

A) Cumulative food intake (g/kg body weight) in weight-matched groups of 8 or 9 male Sprague-Dawley rats (weight range 352–425 g) after i.p. administration of vehicle, OEA (30 mg/kg), and PSN632408 (100 mg/kg). Results are expressed as means + SEM. Significant differences from the vehicle-treated controls are denoted by **$p < 0.01$ (one-way ANOVA followed by Dunnett's multiple comparison test).

B) Cumulative food intake (g/kg body weight) and (**C**) locomotor activity (cumulative consecutive beam breaks) in weight-matched groups of 10 or 11 male Sprague-Dawley rats (weight range 293–339 g) after i.p. administration of vehicle (open squares) or PSN632408 at 10 mg/kg (filled triangles) or 30 mg/kg (filled diamonds). Data are expressed as means + SEM. Significant differences from the vehicle-treated controls are denoted by *$p < 0.05$ (ANOVA followed by Fisher's post-hoc test).

Activation of human PPARγ was tested in a stable HT-29-derived reporter cell line. No activation was obtained upon treatment with PSN632408 at concentrations up to 10 μM (Figure S1A available with this article online).

The ability of PSN632408 to activate rat and human PPARα was assayed at Exygen Research (Penn State University, PA) using 3T3-derived stable reporter cell lines. No activation of rat PPARα was seen at concentrations up to 30 μM (Figure S1B). Similar results were obtained for human PPARα (data not shown).

The selectivity of PSN632408 was further evaluated at 10 μM against a set of 107 targets comprising GPCRs (61), ligand-gated ion channels (7), other receptors (5), ion channels (9), amine transporters (5), and enzymes (16) at Cerep (Celle L'Evescault, France) and MDS Pharma Services (Taipei, Taiwan). Included in the analysis were the following feeding-related receptors: cannabinoid CB1, cholecystokinin, ghrelin, leptin, glucagon-like peptide-1, 5-hydroxytryptamine, melanin concentrating hormone-1, melanocortin-4, and histamine-H3. At none of the 107 targets was inhibition greater than 30% observed. In addition, no binding was detectable at the rat TRPV1 receptor, which is activated by anandamide and which may also mediate some OEA effects (Zygmunt et al., 1999; Wang et al., 2005). PSN632408 was also tested in the hERG K^+ channel rubidium ion efflux assay (Cerep, Celle L'Evescault, France) and did not show any inhibition at 10 μM.

OEA and PSN632408 acutely reduce food intake in rats

The acute effects of GPR119 agonists on food intake were investigated using a rat feeding model as described in Experimental Procedures. Figure 4A shows the effects of intraperitoneal (i.p.) administration of OEA (30 mg/kg) and PSN632408 (100 mg/kg) on cumulative food intake in freely feeding male Sprague-Dawley rats. Both agents produced a reduction in

food intake. In the case of OEA, this reduction was statistically significant at 1 hr and 2 hr post-dosing only, although food intake remained lower than for the control group at later time points, giving a profile similar to that reported by Gaetani et al. (2003) and Proulx et al. (2005) using a 20 mg/kg intraperitoneal dose of OEA. In contrast, the cumulative food intake of animals dosed with PSN632408 remained significantly lower than the vehicle-treated group at all time points up to 24 hr. No overt adverse behavioral effects were observed with either compound. The hypophagic effect of PSN632408 is independent of stomach content since equivalent reductions in food intake were seen upon dosing with PSN632408 (100 mg/kg i.p.) in freely feeding and 18 hr fasted rats (Figure S2).

The effects of lower doses of PSN632408 (10 and 30 mg/kg i.p.) were then investigated using an automated system providing continuous concurrent measurements of food intake and locomotor activity. Hourly cumulative totals for these parameters have been plotted in Figures 4B and 4C. There was a slight trend, albeit not statistically significant, toward lower cumulative food intake at the 10 mg/kg dose, while the 30 mg/kg dose produced a statistically significant reduction at all time points from 3 to 17 hr post-dosing (Figure 4B). No rebound in food intake occurred: food intake in the PSN632408-treated rats showed no statistically significant difference from vehicle-treated controls in the 24–48 hr period (vehicle = 81.1 ± 4.9 g/kg body weight; 10 mg/kg PSN632408 = 83.1 ± 4.6 g/kg body weight; 30 mg/kg PSN632408 = 82.9 ± 3.2 g/kg body weight). Administration of PSN632408 did not significantly alter locomotor activity (Figure 4C), suggesting that the observed decrease in food intake induced by the 30 mg/kg dose was not simply due to nonspecific behavioral depression (or hyperactivity). It has been reported that a hypophagic dose of OEA (20 mg/kg i.p.) causes a statistically significant reduction in locomotor activity (Rodriguez de Fonseca et al., 2001; Proulx et al., 2005) and that the effect may be modulated by activation of TRPV1 by OEA (Wang et al., 2005; Proulx et al., 2005). Under the conditions of our experiment, we did not note any effect by PSN 632408 on locomotor activity, possibly because of its greater selectivity for GPR119 compared to OEA.

The possibility that the hypophagic response to PSN632408 may be due to the production of aversive signals was investigated by a conditioned taste aversion study (CTA; Tang-Christensen et al., 1998) in rats dosed intraperitoneally with a hypophagic dose (30 mg/kg) of PSN632408. No induction of CTA, as assessed by saccharin preference ratio, was produced (Figure S3A). Additionally, PSN632408 (30 mg/kg i.p.) did not increase kaolin intake over control levels in a kaolin consumption study (Figure S3B), hence there is no evidence of drug-induced malaise (Madden et al., 1999). The effects of this compound on food intake are therefore unlikely to be a result of nonspecific toxicity.

OEA has previously been reported to activate PPARα (Fu et al., 2003) and TRPV1 (Wang et al., 2005). Fu et al. (2003) have presented evidence that PPARα null mice are not susceptible to the effects of OEA (dosed intraperitoneally) upon food intake and body weight that are seen in their lean littermates, implicating PPARα as OEA's principal target. OEA was shown to alter expression of several PPARα target genes in the jejunum of wild-type but not PPARα null mice, including a repression of the inducible nitric oxide synthase gene, and it was proposed that the resulting reduction in nitric oxide could lead to stimulation of vagal afferents (Fu et al., 2003). A transcriptionally regulated effect such as this would be expected to have a slow onset and persist over several hours. Wang et al. (2005) have demonstrated that short-term food intake is reduced in response to OEA in wild-type but not TRPV1 null mice and postulate that TRPV1 is an additional target for OEA, mediating an immediate suppression of feeding via direct excitation of vagal sensory neurons. We have found that OEA also acts as an agonist at gastro-intestinally expressed GPR119, suggesting a third possible mechanism by which its hypophagic effects might be mediated. The relative contributions of these three potential targets to the hypophagic action of OEA is still to be determined.

PSN632408, a selective GPR119 agonist, does not bind to the TRPV1 receptor (or to a panel of other feeding-related receptors) and does not activate PPARα using a functional assay (see above). The hypophagia produced by PSN632408 is therefore most probably due to activation of the GPR119 receptor.

PSN632408 also produced acute hypophagic effects when dosed orally (Figure S4). At a dose of 100 mg/kg, food intake was significantly reduced compared to control from 4 hr post-dosing onward—total food intake over the first 24 hr post-dosing was 10.4% lower in animals treated with 100 mg/kg PSN632408 than in vehicle-treated controls ($p < 0.05$; ANOVA followed by Fisher's post-hoc test). A dose of 50 mg/kg PSN632408 also produced a reduction in food intake, and 24 hr food intake was 3.6% lower than the vehicle control, but this difference was not statistically significant. An oral dose of 100 mg/kg was therefore used for the subchronic dosing studies described below.

Effects of chronic administration of PSN632408

Diet-induced obese (DIO) rats, which have become chronically obese through access to high-energy, highly palatable food, represent models with similarities to the human obesity state. It was therefore of interest to determine whether a GPR119 agonist could modulate food intake and body weight in such animals.

PSN632408 (100 mg/kg orally) was administered daily for 14 days to male rats with a genetic predisposition to obesity (Levin et al., 1997; Levin and Keesey, 1998) fed with high-fat diet (HFD) for 13 weeks prior to start of dosing and throughout the experiment. Cumulative food intake was significantly lower in PSN632408-treated animals than in vehicle controls throughout the course of the experiment (Figure 5A). The mean daily food intake was decreased by 10% during the first week of dosing (controls = 21.05 ± 0.30 g versus PSN632408 = 18.89 ± 0.35 g; $p < 0.02$, Student's two-sample t test) and 15% during the second week (controls = 20.46 ± 0.37 g versus PSN632408 = 17.29 ± 0.29 g; $p < 0.01$, Student's two-sample t test). Body weight gain was significantly attenuated from day 6 onward with some evidence of weight loss (Figure 5B). Over the course of this short study, the vehicle-treated controls gained an average of 18.3 ± 3.3 g, while PSN632408-treated animals lost an average of 4.6 ± 3.4 g. (Mean initial body weights for the groups of animals are given in the legend to Figure 5.)

At the end of the experiment, body white adipose tissue deposits (mesenteric, retroperitoneal, epididymal, and subcutaneous inguinal fat) and intrascapular brown adipose tissue (IBAT) depots were removed and weighed. All of the white adipose tissue depots examined showed some reduction in mean weight in the PSN632408-treated rats compared to vehicle-treated

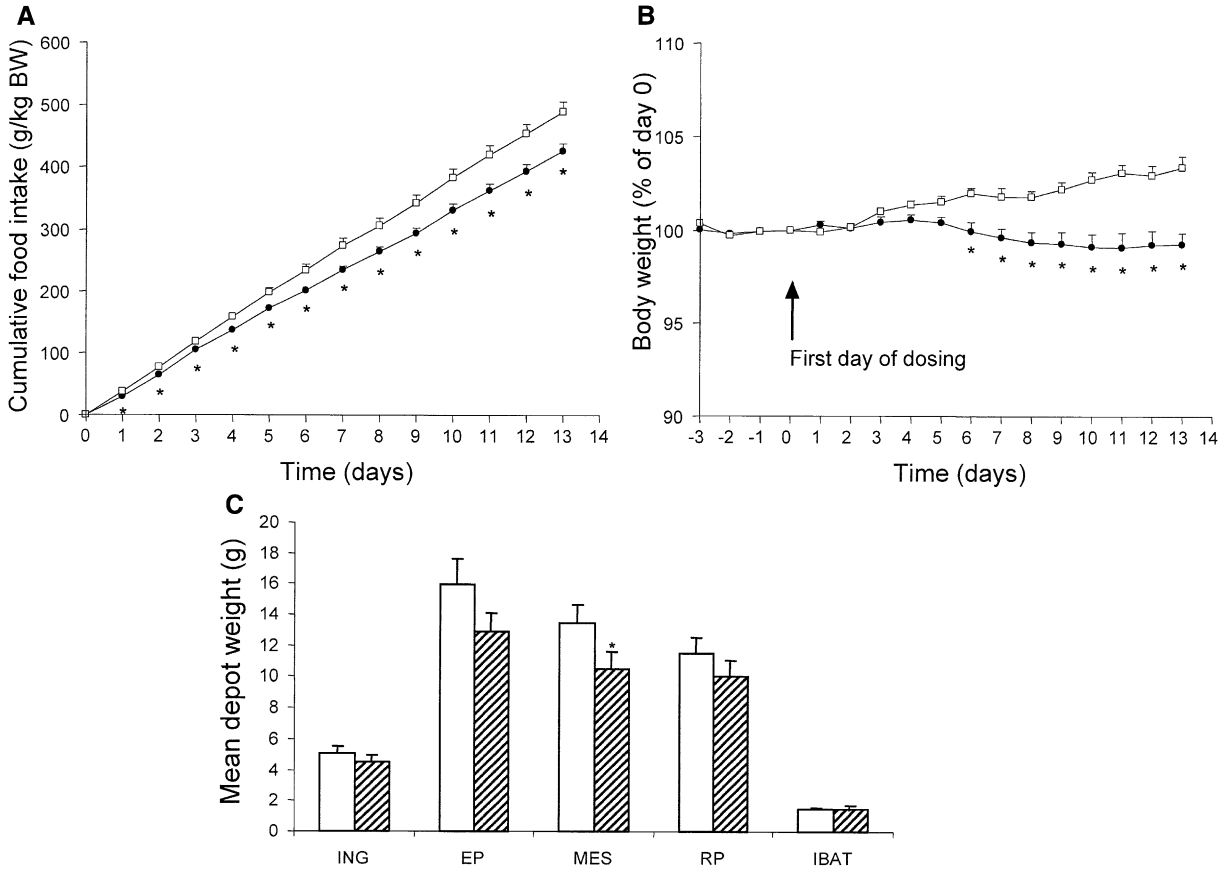

Figure 5. Subchronic dosing of DIO rats with PSN632408

A) Cumulative food intake (g/kg body weight) and (**B**) body weight (percentage of day 0 value) for groups of 9 or 10 rats dosed daily p.o. (commencing on day 0) with vehicle (open squares; mean body weight 545 ± 17 g on day 0) or with 100 mg/kg PSN632408 (filled circles; mean body weight 551 ± 14 g on day 0). Results are presented as means + SEM. Significant differences from the vehicle-treated controls are denoted by *$p < 0.05$ (ANOVA followed by Fisher's post-hoc test).

C) Weights of fat pad masses removed at day 14 (24 hr after last dose) from rats treated with vehicle (open bars) or PSN632408 (shaded bars). ING = inguinal, EP = epididymal, MES = mesenteric, RP = retroperitoneal, IBAT = intrascapular brown adipose tissue. Values expressed in g (mean + SEM). Significant differences from the vehicle-treated controls are denoted by *$p < 0.05$ (ANOVA factorial, Fisher's post-hoc test).

animals; this effect reached statistical significance in the case of the mesenteric fat depot. There was no effect on IBAT (Figure 5C).

A second subchronic study was conducted to examine the effects of PSN632408 using a different experimental paradigm, in which normal male Sprague-Dawley rats in the active growing phase are given a high-fat diet (see Experimental Procedures) for 10 days prior to dosing and throughout the experiment (Figure 6). Animals were dosed orally for 24 days with vehicle, PSN632408 (100 mg/kg), or sibutramine (5 mg/kg sibutramine hydrochloride hydrate), a prescribed weight-loss agent (Heal et al., 1998). For comparison, a fourth group was maintained on standard chow and dosed with vehicle. Figure 6A shows cumulative food intake over the course of the experiment—this is expressed as kJ/kg body weight to allow for the differences in diets. Cumulative food intake was significantly lower in PSN632408-treated animals than in vehicle controls from day 5 onward and in sibutramine-treated animals from day 2 onward (Figure 6A). Body weight gain was significantly reduced from day 3 onward in PSN632408-treated animals and from day 1 onward in sibutramine-treated animals (Figure 6B). During the first days of dosing, body weight gain in the sibutramine-treated group was markedly slower than in the PSN632408-treated animals. During the latter part of the study, however, the two groups gained weight at similar rates and by day 23 both groups had increased in body weight by the same proportion as chow-fed controls (131% ± 1.3% of starting weight for PSN632408, 132% ± 1.9% for sibutramine, and 131% ± 3.8% for chow-fed controls compared to 142% ± 2.2% for the high-fat diet-fed control group). This represents an overall weight gain of 102.9 ± 5.0 g for PSN632408 treatment and 104.2 ± 7.0 g for sibutramine treatment, compared to 135.6 ± 8.6 g for HFD-fed controls. (Mean initial body weights for the groups of animals are given in the legend to Figure 6.)

Analysis of white adipose tissue depots (Figure 6C) at the end of this experiment again showed decreases in weight following PSN632408 or sibutramine treatment for all sites examined. This reached statistical significance for epididymal and mesenteric depots and also for the total of the white adipose tissue depots examined in the PSN632408-treated rats and for mesenteric and total white adipose tissue depots in the sibutramine-treated rats. The intrascapular brown adipose tissue depots were not affected by PSN632408 or sibutramine treatment (data not shown).

Plasma leptin concentrations at the end of the study (Figure 6D) showed an identical trend to fat depot mass, with

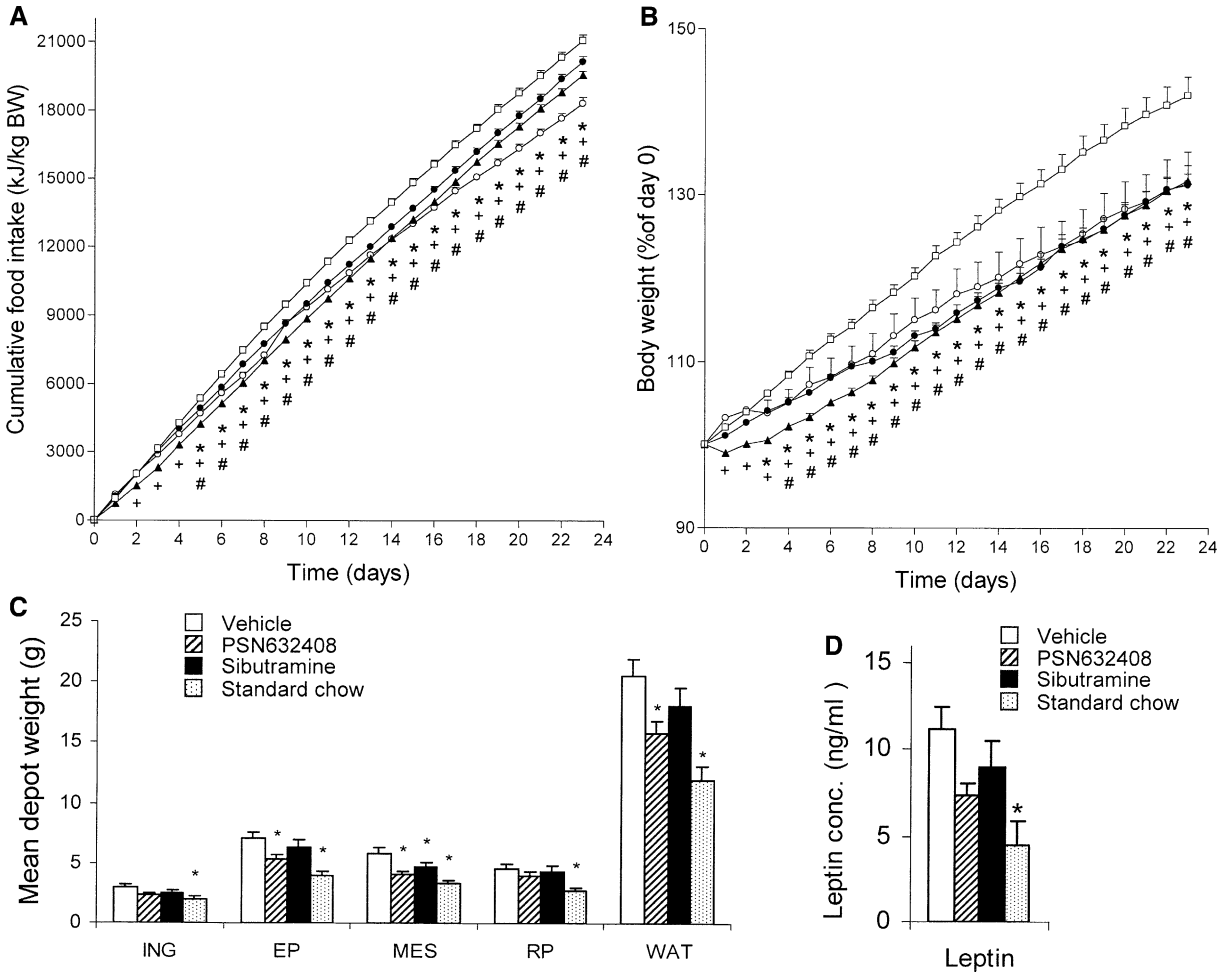

Figure 6. Subchronic dosing of normal rats on high-fat diet with PSN632408 and sibutramine

A) Cumulative food intake (kJ/kg body weight) and (**B**) body weight (percentage of day 0 value) for groups of 10 rats dosed daily p.o. (commencing on day 0) with vehicle (open squares; mean body weight 305 ± 7 g on day 0), 100 mg/kg PSN632408 (filled circles; mean body weight 306 ± 5 g on day 0), or 5 mg/kg sibutramine hydrochloride hydrate (filled triangles; mean body weight 303 ± 5 g on day 0). A further group of 4 rats on standard chow diet was dosed daily with vehicle (open circles; mean body weight 275 ± 5 g on day 0). Results are presented as means + SEM. Significant differences from the vehicle-treated HFD controls ($p < 0.05$; ANOVA followed by Fisher's post-hoc test) are denoted by * (PSN632408), + (sibutramine), and # (chow-fed controls).

C) Weights of fat pad masses removed on day 24 (24 hr after final dose) from rats treated with vehicle, PSN632408, sibutramine, or chow-fed controls, respectively. ING = inguinal, EP = epididymal, MES = mesenteric, RP = retroperitoneal, WAT = total of ING, EP, MES, and RP. Values expressed in g (mean + SEM). Significant differences from the vehicle-treated HFD controls are denoted by * ($p < 0.05$; ANOVA factorial, Fisher's post-hoc test).

D) Plasma leptin levels at day 24 (24 hr after final dose) from rats treated with vehicle, PSN632408, sibutramine, or chow-fed controls. Values expressed in ng/ml (mean + SEM). Significant differences from the vehicle-treated HFD controls are denoted by * ($p < 0.05$; ANOVA, Bonferroni's modified T-test).

PSN632408 and sibutramine treatment ameliorating the rises in leptin concentration measured in the HFD-fed animals compared to lean chow controls. Furthermore, measurements of plasma glucose and insulin levels at the end of the study showed a trend, albeit not statistically significant, toward improvement in insulin sensitivity in the sibutramine- and PSN632408-treated animals compared to HFD vehicle controls (plasma insulin: HFD controls = 279 ± 50 pM; PSN632408-treated = 210 ± 22 pM; sibutramine-treated = 256 ± 64 pM; lean controls = 142 ± 48 pM; plasma glucose: HFD controls = 5.39 ± 0.89 mM; PSN632408-treated = 3.61 ± 0.27 mM; sibutramine-treated = 3.78 ± 0.32 mM; lean controls = 3.86 ± 0.69 mM).

In conclusion, we have discovered that the naturally occurring feeding regulator OEA is an agonist at the GPR119 receptor, and that a selective, small-molecule agonist at this receptor acts to suppress feeding and reduce body weight gain and adiposity in rat models following oral dosing. Details of the mechanisms by which these effects are produced remain to be elucidated, but it is clear that this receptor system is a novel putative avenue for controlling caloric intake and body weight and hence has great potential value for discovering pharmaceuticals to alleviate the burdens of obesity and related metabolic disorders.

Experimental procedures

Cloning of human and mouse OSGPR116 receptors

The gene for the mouse OSGPR116 (GPR119) receptor was PCR amplified from mouse genomic DNA using the following primers: 5′TATATCGTCTC TCATGGAGTCATCCTTC3′ (forward) and 5′TATATTCTAGATTAGCCATCGA GCTCCGG3′ (reverse). The amplified product was digested with restriction enzymes BsmBI and XbaI, creating an NcoI-compatible overhang at the 5′ end of the product, and inserted into the yeast expression vector Cp4258 (Miret et al., 2002) at the NcoI/XbaI restriction sites. In the resulting construct,

expression of OSGPR116 is under the control of the PGK promoter while the N terminus of the receptor gene is fused to an 89 amino acid Mfα1-leader sequence, allowing its transport to the cell membrane. A similar protocol was followed for cloning the human OSGPR116 gene into the yeast expression vector. PCR amplification of the human gene was accomplished using human genomic DNA and the following primers: 5′CTCTTCGGTCTCTC ATGGAATCATCTTTCTCATTTGGAGTGATC3′ (forward) and 5′CTCTTCTC TAGACTTAGCCATCAAACTCTGAGCTGGAGATAGTG3′ (reverse). Amplified product was digested with the restriction enzymes BsaI and XbaI, creating an NcoI-compatible overhang at the 5′ end of the product, and inserted into the yeast expression vector Cp4258 at the NcoI/XbaI restriction sites. The sequences of the amplified human and mouse OSGPR116 coding regions were later confirmed to be identical to those given for GPR119 (Fredriksson et al., 2003) and SNORF25 (Bonini et al., 2002). For the sake of clarity, the receptors are referred to as GPR119 throughout this paper.

Yeast fluorescent reporter assay

Yeast cell-based reporter assays have previously been described in the literature (King et al., 1990; Campbell et al., 1999; Miret et al., 2002). *Saccharomyces cerevisiae* cells have been engineered such that the endogenous yeast G-α (GPA1) has been replaced with a G protein chimera. Additionally, the endogenous yeast GPCR, Ste3, has been deleted to allow for the heterologous expression of a mammalian GPCR of choice. In the yeast, elements of the pheromone signaling transduction pathway drive the expression of Fus1. By placing the β-galactosidase (LacZ) gene under the control of the Fus1 promoter (Fus1p), receptor activation can by monitored by a fluorescent read-out. Yeast cells were cotransformed with either a human or mouse GPR119 expression plasmid (as described above) carrying a LEU auxotrophic marker, and two Fus1p-LacZ reporter plasmids, with auxotrophic markers for URA and TRP. Transformants were grown on selective plates lacking leucine, uracil, and tryptophan. For the fluorimetric functional assay, recombinant yeast strains were grown to mid-log phase in synthetic defined medium without leucine, uracil, and tryptophan, pH 6.8 (Qbiogene Inc, Carlsbad, California) and mixed with test compounds in opaque 96-well plates. Dimethyl sulfoxide (DMSO) was present at 1% final concentration. After 4 hr incubation at 30ºC, fluorescein di (β-D-galactopyranoside) substrate at a final concentration of 80 μM and Triton X100 at a final concentration of 0.4% were added to each well. Incubation was continued for 60 min at 30ºC and sodium carbonate then added to a final concentration of 140 μM to terminate the reaction and enhance the fluorescent signal. The plates were read in a fluorimeter at 485/535 nm.

Mammalian cell line expressing GPR119

A stable cell line that expresses human GPR119 when induced with tetracycline (HEK-OSGPR116 cell line) was created using the Flp-In T-REx system (Invitrogen Ltd.) according to the manufacturer's protocol. Flp-In T-REx-293 cells were transfected with the plasmid pcDNA5/FRT/TO into which the GPR119 open reading frame had been inserted, and a population of stable transfectants was selected using hygromycin. A control cell line was derived in a similar manner using the plasmid pcDNA5/FRT/TO/CAT which contains the chloramphenicol acetyl transferase gene instead of GPR119. Cells were cultured in DMEM (Invitrogen #31966-021) supplemented with 10% fetal bovine serum, hygromycin (50 μg/ml) and blasticidin (15 μg/ml). Expression of the GPR119 gene was demonstrated by quantitative RT-PCR using the ABI Prism 7000 Sequence Detection System and primers with the following sequences:

5′AAAGATGGAACATGAGGAGCC3′ (forward), 5′GAGCTTTGAAGTCGC TGGGAG3′ (reverse) and 5′FAM-TGGCTGGAGGTTATCGATCCCCACG-TAMRA3′ (TaqMan probe).

Intracellular cyclic AMP determination

HEK-OSGPR116 cell monolayers were pretreated overnight with 10–20 ng/ml tetracycline, these conditions having been found to induce GPR119 expression at an optimal level for subsequent experiments. Treated monolayers were then incubated for 30 min at 37ºC with test compounds in stimulation buffer plus 1% DMSO. Cells were lysed and cyclic AMP (cAMP) content determined using the Perkin Elmer AlphaScreen (Amplified Luminescent Proximity Homogeneous Assay) cAMP kit. Buffers and assay conditions were as described in the manufacturer's protocol.

Acute feeding experiments

Acute feeding experiments were carried out using singly housed male Sprague-Dawley rats (Charles River Laboratories). Rats were maintained on a reverse-phase light-dark cycle and had free access to standard powdered rat diet. Prior to the start of the experiment, rats were allocated to weight-matched treatment groups. Compounds were administered during the 30 min prior to the start of the dark phase, either by intraperitoneal injection using 5% propylene glycol/5% Tween 80/90% saline as vehicle or by oral gavage using 25% Gelucire 44/14:75% water as vehicle. Food intake was monitored either by weighing feeding jars at 1, 2, 4, 6, and 24 hr after drug administration (Figures 4A and S2) or by an automated system providing online measurements of food intake and locomotor activity every fifth minute (Tang-Christensen et al., 2004) (Figures 4B, 4C, and S4).

Subchronic feeding experiments

Male DIO Levin rats (bred at Rheoscience) or normal male Sprague-Dawley rats (Charles River Laboratories) were housed singly under a 12 hr/12 hr light/dark cycle in temperature and humidity controlled rooms and offered an energy-dense high-fat diet (kcal 32% fat; 51% carbohydrate; 17% protein, #12266B; Research Diets, New Jersey) and water ad libitum. Weight-matched groups of 8–10 animals received single daily oral gavage (30 min before lights out) of test compound or vehicle control (25% Gelucire 44/14:75% water). Animals were habituated to the dosing paradigm by mock gavage for 3 days prior to the start of dosing. Sibutramine was dosed as 5 mg/kg sibutramine hydrochloride hydrate. Body weight and food intake were measured once daily. At 24 hr after the final dose terminal blood samples were taken for plasma glucose (GO assay kit, Sigma), insulin (Rat insulin ELISA kit, Mercodia, Sweden), and leptin (Rat leptin ELISA kit, Linco Res.) measurements. Adipose tissue depots were removed from the animals and weighed.

Supplemental data

Supplemental data include four figures and can be found with this article online at http://www.cellmetabolism.org/content/full/3/3/167/DC1/.

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