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REVIEW

# NPY receptors as potential targets for anti-obesity drug development

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The neuropeptide Y system has proven to be one of the most important regulators of feeding behaviour and energy homeostasis, thus presenting great potential as a therapeutic target for the treatment of disorders such as obesity and at the other extreme, anorexia. Due to the initial lack of pharmacological tools that are active *in vivo*, functions of the different Y receptors have been mainly studied in knockout and transgenic mouse models. However, over recent years various Y receptor selective peptidic and non-peptidic agonists and antagonists have been developed and tested. Their therapeutic potential in relation to treating obesity and other disorders of energy homeostasis is discussed in this review.

### Abbreviations

ARC, arcuate nucleus; d.d., dose-dependent; i.c.v., intracerebroventricular; i.p., intraperitoneal; NPY, neuropeptide Y; p.o., per oral; PP, pancreatic polypeptide; PVN, paraventricular nucleus; PYY, polypeptide YY

## Introduction

One of the most potent inducers of feeding in mammals is neuropeptide Y (NPY), a 36 amino acid neurotransmitter. NPY is part of a family of structurally related peptides that also includes the hormones polypeptide YY (PYY) and pancreatic polypeptide (PP). Whereas PYY and PP are mainly expressed in gut tissues, NPY is most abundantly expressed in the brain. In the human brain, NPY expression is highly concentrated in the basal ganglia and in the limbic system, particularly in the nucleus accumbens, as well as in the hypothalamus, a key brain area regulating energy homeostasis and appetite (Adrian *et al.*, 1983). The expression pattern of NPY in the human brain closely resembles its central distribution in rats and mice, in which most of the investigations into NPY functions have been carried out. Central administration of NPY to rats and mice induces hyperphagia, decreases energy expenditure, activates lipogenic enzymes in the liver and adipose tissues, collectively contributing to the development of obesity (Stanley *et al.*, 1986; Zarjevski *et al.*, 1993; Lin *et al.*, 2006). On the other hand, neutralization of endogenous NPY with NPY antibody induces a dose-dependent inhibition of feeding in rats (Dube *et al.*, 1994), demonstrating a role for NPY in normal feeding.

The NPY family of peptides signal through a family of G-protein coupled receptors known as Y receptors. The Y receptors couple to pertussis toxin-sensitive inhibitory G-proteins (Gi/Go), and activation of these receptors brings about inhibitory responses such as inhibition of adenylate cyclase and subsequent inhibition of cyclic AMP accumulation (Motulsky and Michel, 1988; Herzog *et al.*, 1992; Zhu *et al.*, 1992). Additionally, activation of Y receptors has been shown to inhibit $K^+$ and stimulate $Ca^{2+}$ channels (Aakerlund *et al.*, 1990; Bleakman *et al.*, 1991; Xiong and Cheung, 1994; Sun *et al.*, 2001), thereby imparting significant effects on cellular polarization and electrical signalling.

The known Y receptors – Y1, Y2, Y4, Y5 and y6 – have distinct tissue expression profiles and bind to members of the NPY family of peptides with different affinities. The Y1 receptor is implicated in mediating NPY-induced hyperphagia, vasoconstriction, proliferation of neuronal precursor cells and vascular smooth muscle cells, as well as preventing ethanol tolerance (Bhisikar *et al.*, 2009). Y2 receptors are mostly expressed presynaptically. Their activation by NPY,

PYY and N-terminal truncation products of these peptides, such as NPY3-36 and PYY3-36, leads to the inhibition of neurotransmitter release (Smith-White *et al.*, 2001; Wahlestedt and Hakanson, 1986). Y2 receptors are involved in promoting the proliferation and differentiations of adipocytes as well as stimulating angiogenesis of capillaries in adipose tissue (Kuo *et al.*, 2007). The Y4 receptor shows preferential binding to PP, with binding affinity in the picomolar range. This receptor modulates the vago-vagal reflex arc in the area postrema, and relays anorexigenic signalling from PP in the peripheral circulation to the hypothalamus (Katsuura *et al.*, 2002; Lin *et al.*, 2009), as well as being critically important in the regulation of reproductive functions (Sainsbury *et al.*, 2010). Y5 receptors are primarily expressed in the paraventricular nucleus (PVN) of the hypothalamus and interact synergistically with the Y1 receptor in regulating energy homeostasis (Gerald *et al.*, 1996; Kanatani *et al.*, 2000b; Mashiko *et al.*, 2009). The existence of Y3 receptors is postulated based on pharmacological studies in a number of tissues (Grundemar *et al.*, 1991; Lee and Miller, 1998), but has not been confirmed as a cloned entity. The y6 receptor only exists as a functional receptor in mouse and rabbit. It is completely absent in the rat genome and has a truncated structure in most mammalian genomes, including the human genome. Interestingly, despite the presence of a mutation in the y6 receptor coding sequence in humans, the level of transcription of the y6 receptor is considerable in several human tissues including skeletal muscle, small intestine, colon and adrenal gland (Gregor *et al.*, 1996; Matsumoto *et al.*, 1996; Rose *et al.*, 1997), suggesting a possible functional role for the mRNA itself or the truncated receptor in humans. In the mouse, y6 receptors are expressed in the hypothalamic suprachiasmatic nucleus (Weinberg *et al.*, 1996), suggesting a role for them in the regulation of diurnal rhythms and energy homeostasis.

Given the role of the NPY peptide family and their Y receptors in key physiological processes known to be dysregulated in a variety of disorders such as alcoholism, obesity and anorexia, the development of pharmacological agents targeted towards Y receptors is attractive not only for research applications but also as therapeutic agents in clinical settings. Indeed, a number of ligands for the Y receptors have been developed and tested in clinical trials. However to date, these pharmacological leads have not yet been translated into clinical tools. This review examines the agonists and antagonist against Y receptors that have emerged in the last 10 years, and investigates their potential applicability as research tools and in the clinic.

## The Y1 receptor

Initial studies using various peptide fragments (Wahlestedt *et al.*, 1986) pointed to the existence of two major forms of Y receptors, designated Y1 and Y2, the former of which was the first to be successfully cloned (Herzog *et al.*, 1992). Y1 receptors consist of 384 amino acids (Herzog *et al.*, 1992) and are pharmacologically distinguished from Y2 receptors by their ability to bind [Leu$^{31}$, Pro$^{34}$]NPY (Fuhlendorff *et al.*, 1990; Wahlestedt *et al.*, 1990). Indeed, ligand affinity analysis of a successfully cloned orphan G-protein-coupled receptor (Eva *et al.*, 1990) led to the identification of Y1, which binds the NPY family of ligands with the following rank order of potency: NPY = PYY > [Leu$^{31}$,Pro$^{34}$]NPY > > PP > PYY13-36 (Herzog *et al.*, 1992; Larhammar *et al.*, 1992).

In line with the previously reported distribution of NPY and predicted NPY binding sites (Adrian, 1978), Y1 receptor expression, detected using *in situ* hybridization and immunohistochemistry, was found in several thalamic nuclei, in the hippocampus, various amygdaloid nuclei and in the hypothalamus of the rat and mouse (Eva *et al.*, 1990; Kopp *et al.*, 2002; Kishi *et al.*, 2005). Evaluation of peripheral tissues revealed Y1 receptor mRNA in the colon (Goumain *et al.*, 1998), pancreatic β cells (Morgan *et al.*, 1998) and in the visceral adipose tissues of rats (Yang *et al.*, 2008). In humans, the Y1 receptor is expressed in the epithelium and mucosal nerves of the colon, in the kidney, adrenal gland, heart and placenta (Wharton *et al.*, 1993). Centrally, moderate levels of Y1 receptor mRNA were detected in the caudate nucleus, putamen, nucleus accumbens, amygdaloid nuclei and arcuate nucleus (ARC) and PVN of the hypothalamus of human brain (Jacques *et al.*, 1996).

The potential role of Y1 receptors in the pathophysiology of obesity, particularly in humans, was indicated by a polymorphism in the untranslated region of the Y1 gene, where a single cytosine to thymidine nucleotide substitution was associated with lower fasting triglyceride and significantly higher plasma high-density lipoprotein concentrations in 306 obese subjects (Blumenthal *et al.*, 2002). In contrast, pharmacological blockade of the Y1 receptor in rodents by central administration of the Y1 antagonists BIBP3226 (Kask *et al.*, 1998), LY357897 (Hipskind *et al.*, 1997) or 1229U91 (Kanatani *et al.*, 1996) resulted in significant attenuation of feeding, as did the administration of antisense oligodeoxynucleotides against Y1 receptors to the ventromedial hypothalamus of rats (Lopez-Valpuesta *et al.*, 1996). The Y1 receptor was also demonstrated to mediate the stimulatory effect of NPY on the proliferation of primary cultures of rat pre-adipocytes and 3T3-L1 pre-adipocytes *in vitro* (Yang *et al.*, 2008). Furthermore, chronic activation of the Y1 receptor by NPY or a Y1-selective agonist for 6 days led to a significant increase in body weight, fat accumulation and a reduction in fat oxidation in wild-type mice without hyperphagia (Henry *et al.*, 2005). Taken together, these evidences support a role of the Y1 receptor in the development of obesity.

In keeping with a potential role in the aetiology or treatment of obesity, germline ablation of Y1 receptors in genetically obese leptin-deficient *ob/ob* mice led to a significant improvement in the obesity syndrome, as characterized reductions in hyperphagia and body weight gain, demonstrating a role of Y1 in mediating the action of leptin deficiency and the associated elevation in hypothalamic NPYergic transmission (Pralong *et al.*, 2002). Paradoxically, while deletion of the Y1 receptor in male mice led to a reduction in body weight that persists for up to 20 weeks of age (Pralong *et al.*, 2002), we and others have demonstrated that the absence of Y1 receptors in mice led to the development of mild late-onset increases in adiposity without hyperphagia, and that this effect of Y1 receptor loss is more pronounced in female mice (Kushi *et al.*, 1998; Baldock *et al.*, 2007; Zhang *et al.*, 2010a), suggesting sexual dimorphism in Y1 receptor function (Zammaretti *et al.*, 2007). Additionally, the loss of

Y1 receptors in mice led to hyperinsulinaemia and altered ability to secrete insulin in response to glucose, and these effects may contribute to the obesity of Y1 deficient animals (Kushi *et al.*, 1998). Whereas specific adult-onset deletion of Y1 receptors in the hypothalamus did not alter any aspects of energy homeostasis (Baldock *et al.*, 2007), knockdown of Y1 receptors specifically in peripheral tissues led to a significant increase in lipid oxidation that was associated with protection against diet-induced obesity in the absence of changes in feeding behaviour or insulin levels (Zhang *et al.*, 2010a). Collectively, these results reveal likely functional redundancy of Y receptors that may be compensated throughout development after single gene deletion, and emphasize the need for Y1-specific agonists or antagonists to further delineate Y1 receptor functions.

## Agonists (Table 1)

*[D-Arg$^{25}$]NPY and [D-His$^{26}$]NPY.* Single D-amino acid substituted NPY analogues (Kirby *et al.*, 1993) and shortened cyclic NPY analogues (Kirby *et al.*, 1995) were reported to have improved Y1-receptor binding properties in preference to Y2 receptor binding, but were not evaluated for other Y receptors. Along with these peptides, Mullins and colleagues designed and synthesized additional cyclic NPY analogues and determined their affinity in binding assays in competition with $^{125}$I-PYY or $^{125}$I-PP (Mullins *et al.*, 2001). The single substituted analogues [D-Arg$^{25}$]NPY, [D-His$^{26}$]NPY and a cyclic analogue des-AA$^{11\text{-}18}$-[Cys$^{7,21}$, D-Lys$^{9}$ (Ac),D-His$^{26}$, Pro$^{34}$]NPY, showed preferential binding to Y1 receptors relative to Y2, Y4 and Y5 receptors, albeit with threefold, sevenfold and fourfold less efficiency, respectively, when compared with the native NPY (Mullins *et al.*, 2001). Despite this, intracerebroventricular administration of [D-Arg$^{25}$]NPY or [D-His$^{26}$]NPY to satiated Long-Evans rats at a dose of 1 nmol stimulated feeding comparably to the effect of NPY at 4 h following injection, while administration of des-AA$^{11\text{-}18}$-[Cys$^{7,21}$, D-Lys$^{9}$ (Ac),D-His$^{26}$, Pro$^{34}$]NPY did not significantly increase feeding (Mullins *et al.*, 2001). Further evidence of actions via Y1 receptors, the hyperphagic effect of [D-Arg$^{25}$]NPY was attenuated by co-administration of 1229U91, an antagonist of the Y1 receptor (Mullins *et al.*, 2001). As well as having effects on food intake, acute Y1 receptor activation by [D-Arg$^{25}$]NPY reproduced the NPY-induced effect to elevate plasma insulin levels in satiated and food deprived Long-Evans rats (Gao *et al.*, 2004). Interestingly, the increase in plasma insulin following application of Y1 but not Y5 agonist preceded the increase in feeding, suggesting distinct Y1-mediated mechanism for the regulation of feeding and insulin levels (Gao *et al.*, 2004).

Not only does acute administration of [D-Arg$^{25}$]NPY have effects that would be expected to increase adiposity, but chronic administration of this Y1 agonist recapitulated characteristics of NPY-induced obesity in mice, as did Y5 receptor activation (Henry *et al.*, 2005). Importantly, while the development of Y5 agonist-induced obesity required a combination of hyperphagia and nutrient partitioning, as indicated by a marked increase in respiratory quotient, repeated administration of [D-Arg$^{25}$]NPY led to weight gain by altering nutrient partitioning alone (Henry *et al.*, 2005). This finding demonstrates that hyperphagia may not be a primary mechanism by which Y1 receptor agonism promotes positive energy balance.

*[Phe$^{7}$, Pro$^{34}$]pNPY and [Arg$^{6}$, Pro$^{34}$]pNPY.* Soll and colleagues synthesized 30 analogues of NPY and chimeras of NPY/PP with amino acid substitutions in the N-terminal, C-terminal and β-hairpin turn region, with the aim of characterizing properties of Y1 receptor binding. This study resulted in the identification of seven peptide agonists that demonstrated higher affinity for the Y1 receptor and that had selectivity for Y1 and Y5 receptors, notably [Phe$^{7}$, Pro$^{34}$]pNPY and [Arg$^{6}$, Pro$^{34}$]pNPY (Soll *et al.*, 2001). According to their ability to inhibit cAMP synthesis in cells expressing human Y1, these peptides were classified as Y1 receptor agonists. [Phe$^{7}$, Pro$^{34}$]pNPY showed an approximately twofold increase in affinity for the Y1 receptor and a sevenfold decrease in affinity for the Y5 receptor, while [Arg$^{6}$, Pro$^{34}$]pNPY exhibited a 3780-fold preference towards the Y1 receptor relative to Y5 (Soll *et al.*, 2001). Subsequent binding studies confirmed that [Phe$^{7}$, Pro$^{34}$]pNPY and [Arg$^{6}$, Pro$^{34}$]pNPY are Y1-prefering ligands (Lecklin *et al.*, 2003). Administration of these peptides in 3.6 and 10 nmol doses into the third cerebral ventricle of guinea pigs stimulated food intake and increased the time spent eating and the number of meals eaten to the same extent as NPY within 4 h post-injection (Lecklin *et al.*, 2003).

*[Pro$^{30}$, Nle$^{31}$, Bpa$^{32}$, Leu$^{34}$]NPY28-36.* Outside the context of metabolism, the development of Y1-selective agonists is aimed towards detecting cells that predominantly express Y1 as opposed to Y2 receptors, as is characteristic of malignant tumours (Reubi *et al.*, 2001). Such a diagnostic tool could potentially help in the diagnosis or treatment of cancer. Albeit selective, previously reported Y1-agonists such as [Leu$^{31}$, Pro$^{34}$]NPY and [Phe$^{7}$, Pro$^{34}$]NPY are considered to be large peptides and have lower labelling efficacy relative to smaller ligands (Zwanziger *et al.*, 2009). Other ligands for Y1 which are small enough to be effectively labelled are antagonists, unable to induce the Y1 receptor internalization that is critical for reducing tumour to background ratio (Parker *et al.*, 2002; Bohme *et al.*, 2008). To address this, Zwanziger and colleagues designed and synthesized 19 analogues of NPY28-36 with amino acid substitution at positions 28–32 and 34. Of these peptide analogues, three competitively displaced $^{3}$H-NPY from Y1 receptors expressed on MCF-7 cells and showed selective binding towards the Y1 receptor in preference to Y2, Y4 and Y5 receptors (Zwanziger *et al.*, 2009). One peptide in particular, [Pro$^{30}$, Nle$^{31}$, Bpa$^{32}$, Leu$^{34}$]NPY28-36, was shown to be a strong agonist of the Y1 receptors, able to induced Y1 receptor internalization (Zwanziger *et al.*, 2009). Given the *in vitro* results, further reports on the *in vivo* effects of this novel Y1 agonist is highly anticipated.

## Antagonists (Table 1)

*J-104870.* Synthesized in 1999, J-104870 selectively displaced [$^{125}$I]PYY binding to human and rat Y1 receptors (Kanatani *et al.*, 1999). This compound has high specificity for the Y1 receptor and low affinity for the rat Y2 receptor and human Y4 and Y5 receptors, as determined by binding to CHO-K1dhfr2, LMtk2 and COS-7 cells recombinantly expressing these receptors on their cell surface (Kanatani *et al.*, 1999).

## Table 1

Effects of Y1 receptor agonists and antagonists on feeding behaviour and aspects of energy homeostasis

| | Agonist | Reference | Dose | Route | Significant effects | Organism | Condition | Duration |
|---|---|---|---|---|---|---|---|---|
| 1 | [D-Arg$^{25}$]NPY | Mullins *et al.*, 2001 | 1 nM | i.p. | ↑ Feeding | Long-Evans rats | *Ad libitum* | 4 h |
| | | Gao *et al.*, 2004 | 1 nM | i.c.v. | ↑ Feeding | Long-Evans rats | *Ad libitum* | 60, 120 min |
| | | | 1 nM | i.c.v. | ↑ Plasma insulin | Long-Evans rats | *Ad libitum* & fasting-induced | 15, 60, 120 min |
| | | | 1 nM | i.c.v. | ↓ Blood glucose | Long-Evans rats | *Ad libitum* & fasting-induced | 60, 120 min |
| | | Henry *et al.*, 2005 | 3 μg·day$^{-1}$ | i.c.v. | ↑ BW gain, adiposity, feeding<br>↑ Serum leptin<br>↓ Lipid oxidation | C57Bl/6 mice | *Ad libitum* | 6 days |
| 2 | [D-His$^{26}$]NPY | Mullins *et al.*, 2001 | 1 nM | i.p. | ↑ Feeding | Long-Evans rats | *Ad libitum* & fasting-induced | 4 h |
| 3 | [Phe$^{7}$, Pro$^{34}$]pNPY & [Arg$^{6}$, Pro$^{34}$]pNPY | Lecklin *et al.*, 2003 | 3.6 & 10 nM | i.c.v. | ↑ Feeding<br>↑ Duration & number of meals | Dunkin–Hartley guinea pigs | *Ad libitum* | 4 h |

| | Antagonist | Reference | Dose | Route | Significant effects | Organism | Condition | Duration |
|---|---|---|---|---|---|---|---|---|
| 1 | J-104870 | Kanatani *et al.*, 1999 | 200 μg | i.c.v. | ↓ Feeding | Sprague-Dawley rats | NPY-induced | 2 h |
| | | | 200 μg | i.c.v. | ↓ Feeding | Zucker *fa/fa* rats | *Ad libitum* | 2, 14, 24 h |
| | | | 100 mg·kg$^{-1}$ | p.o. | ↓ Feeding | Zucker *fa/fa* rats | *Ad libitum* | 2, 14, 24 h |
| | | Ishihara *et al.*, 2002 | 100 mg·kg$^{-1}$ | p.o. | ↓ BW, transient ↓ feeding | Zucker *fa/fa* rats | *Ad libitum* | 14 days |
| | | | 30, 100 mg·kg$^{-1}$ | p.o. | ↓ Adipocyte hypertrophy | Zucker *fa/fa* rats | *Ad libitum* | 14 days |
| 2 | J-115814 | Kanatani *et al.*, 2001 | 30, 100 μg | i.c.v. | ↓ Feeding | Sprague-Dawley rats | NPY-induced, palatable diet | 2 h |
| | | | 3, 10 mg·kg$^{-1}$ | i.v. | ↓ Feeding | Sprague-Dawley rats | NPY-induced, palatable diet | 2 h |
| | | | 10, 30 mg·kg$^{-1}$ | i.p. | ↓ Feeding | *db/db* mice | *Ad libitum* | 14 h overnight |
| | | | 10, 30 mg·kg$^{-1}$ | i.p. | ↓ Feeding | C57Bl/6 mice | *Ad libitum* | 14 h overnight |
| | | | 30 mg·kg$^{-1}$ | i.p. | ↓ Feeding | Y5$^{-/-}$ mice | NPY-induced | 2 h |
| 3 | Y1-718 | Kameda *et al.*, 2009b | 30 mg·kg$^{-1}$ | i.p. | ↓ Feeding | mdr1a$^{-/-}$ CF-1 mice | Fasting-induced | 0.5 h |
| 4 | BMS-193885 | Antal-Zimanyi *et al.*, 2008 | 30 μg | i.c.v. | ↓ Feeding | Sprague-Daley rats | NPY-induced (5 μg) | 1 h |
| | | | 50 μg | i.c.v. | ↓ Feeding | Sprague-Daley rats | NPY-induced (10 μg) | 1 h |
| | | | 1, 10, 30 nM | i.c.v. (PVN) | ↓ Feeding | Sprague-Daley rats | NPY-induced (0.1 nM) | 1 h |
| | | | 3, 10 mg·kg$^{-1}$ | i.p. | ↓ Feeding | Sprague-Daley rats | NPY-induced (10 μg) | 1 h |
| | | | 10, 20 mg·kg$^{-1}$ | i.p. | ↓ Feeding | Sprague-Daley rats | *Ad-libitum* | 15 h overnight |
| | | | 3, 10 mg·kg$^{-1}$ | i.v. | ↓ Feeding | Sprague-Daley rats | *Ad-libitum* | 3 h (dark) |
| | | | 10 mg·kg$^{-1}$ | i.p. | ↓ BW, feeding | Sprague-Daley rats | *Ad-libitum* | 44 days |

BW, body weight; i.c.v. intracerebroventricular; i.p. intraperitoneal; NPY, neuropeptide Y; p.o. per oral; PVN, paraventricular nucleus.

J-104870 inhibited NPY-induced calcium influx into the cell, indicative of antagonistic properties (Kanatani *et al.*, 1999).

Whereas intracerebroventricular treatment in satiated lean rats did not lead to any significant changes in feeding, intracerebroventricular and intraperitoneal administration of J-104870 inhibited feeding in rats with elevated hypothalamic levels of NPY, such as lean rats that were pretreated with intracerebroventricularly administered NPY and genetically obese Zucker *fa/fa* rats (Kanatani *et al.*, 1999), providing further evidence for the crucial participation of Y1 receptor signalling in NPY-ergic control of appetite. Importantly, treatment of obese Zucker *fa/fa* rats with J-104870 via the intraperitoneal route led to suppression of feeding for up to 24 h, at which time a drug concentration of 0.5 μM was found in the brain, indicating good brain penetration (Kanatani *et al.*, 1999). Chronic treatment of obese *fa/fa* Zucker rats with orally administered J-104870 for 2 weeks led to a transient reduction in food intake, suggesting activation of compensatory mechanisms in response to chronic Y1 blockade (Ishihara *et al.*, 2002). The authors suggested that this transient appetite-suppressive effect may be attributed to conditioned taste aversion. Despite this, the chronic 100 mg·kg$^{-1}$ dose of J-104870 led to a significant reduction in body weight while the chronic 30 mg·kg$^{-1}$ dose of the compound attenuated adipocyte hypertrophy in the absence of reduced body weight gain (Ishihara *et al.*, 2002). Although chronic oral administration of J-104870 did not attenuate the elevated plasma triglyceride, total cholesterol, glucose, insulin or corticosterone levels in these genetically obese *fa/fa* rats (Ishihara *et al.*, 2002), the overall findings from this study provide further evidence that manipulation of Y1 receptor function can influence energy balance via mechanisms independent of hyperphagia.

*J-115814.* J-115814 belongs to the diaminopyridine-class of Y1 receptor antagonists. Pharmacologically, J-115814 demonstrated high selectivity for the Y1 receptor, with binding affinity for the human Y2, Y4, Y5 and the mouse Y6 receptors being significantly lower, although it binds to Y1 receptors with lower affinity than NPY (Kanatani *et al.*, 2001). Intracerebroventricular or intraperitoneal administration of J-115814 to Sprague-Dawley rats at 10, 30 and 100 μg or 3 and 10 mg·kg$^{-1}$, respectively, attenuated NPY-induced feeding during a 2 h measurement (Kanatani *et al.*, 2001). In genetically obese *db/db* mice (Kanatani *et al.*, 2001) and diet-induced obese mice (Mashiko *et al.*, 2009), J-115814 significantly inhibited feeding at a minimum effective dose of 10 mg·kg$^{-1}$ when given intraperitoneally. Similarly, lean C57Bl/6J mice responded to the same minimum dose of J-115814, although to a lesser extent than the hyperphagic *db/db* mice (Kanatani *et al.*, 2001). The effect of J-115814 appeared to be specifically mediated by the Y1 receptor because intraperitoneal administration did not reduce NPY-induced food intake in Y1 receptor deficient mice as it did in wild-type and Y5 receptor deficient animals (Kanatani *et al.*, 2001). However, the potential clinical application of J-115814 as an anti-obesity compound is hampered by the finding that it has a potent interaction with the $I_{kr}$ potassium channel (Kameda *et al.*, 2009b), suggesting that it could have the undesirable side effect of cardiovascular toxicity (Fermini and Fossa, 2003).

*Y1-718.* With the aim of eliminating the inhibitory interaction of J-115814 at the $I_{kr}$ potassium channel, Kameda and colleagues generated a library of diaminopyridine class compounds with reduced molecular size and hydrophobicity. This work led to the identification of compound Y1-718 (Kameda *et al.*, 2009b). *In vitro* investigation of Y1-718 showed that it had potent antagonistic activity in a [$^{35}$S]GTPγS assay, was selective for Y1 over Y2, Y4 and Y5 receptors, and – unlike its predecessor, J-115814 – had negligible activity at the $I_{kr}$ potassium channel. Interestingly, Y1-718 was identified as having significant affinity for P-glycoproteins, an endogenous drug transport molecule (Ambudkar *et al.*, 2003). Indeed, intraperitoneal administration of Y1-718 at a 30 mg·kg$^{-1}$ dose to wild-type and mdr1a$^{-/-}$ mice which are deficient in the drug-transporting P-glycoproteins (Schinkel *et al.*, 1994) led to a brain-to-plasma drug concentration ratio of 0.9 and 0.27, respectively, demonstrating the P-glycoprotein-mediated entry of Y1-718 into the brain (Kameda *et al.*, 2009b). This finding is of particular importance given that Y receptor ligands will most likely need to gain access to the central nervous system in order to be of clinical benefit in the treatment of obesity or anorexia. Despite reduced entry into the brain, Y1-718 significantly inhibited fasting-induced feeding in mdr1a$^{-/-}$ mice at 30 min post administration (Kameda *et al.*, 2009b). To date, the chronic effects of the newly generated Y1-718 have not been reported, but these findings will be critical in ascertaining its potential suitability as an anti-obesogenic compound.

*BMS-193885.* BMS-1393885 is a dihydropyridine Y1 receptor antagonist (Poindexter *et al.*, 2002; Poindexter *et al.*, 2004; Antal-Zimanyi *et al.*, 2008). It has a high affinity for the human Y1 receptor stably expressed in Chinese hamster ovary cells and demonstrates a 200-fold lower potency at the α1 adrenergic receptor, with no affinity for the human Y2, Y4 or Y5 receptor. In SK-N-MC cells endogenously expressing human Y1 receptors, BMS-1393885 competitively inhibited PYY binding (Antal-Zimanyi *et al.*, 2008) and blocked NPY inhibition on forskolin-stimulated cAMP synthesis in Chinese hamster ovary cells expressing hY1 receptors, indicating an antagonistic action of this compound (Antal-Zimanyi *et al.*, 2008). Intraperitoneal or intravenous (i.v.) administration of BMS-1393885 revealed a 12 h half-life and also led to high brain concentrations, indicating good brain penetrability (Antal-Zimanyi *et al.*, 2008). Intracerebroventricular administration of 30 and 50 μg of BMS-1393885 to lean Sprague-Dawley rats significantly attenuated NPY-induced food intake, and bilateral infusion specifically into the PVN suppressed NPY-induced food intake at doses of 10 and 30 nmol (Antal-Zimanyi *et al.*, 2008). Rats receiving intraperitoneal injection of BMS-1393885 at 3 or 10 mg·kg$^{-1}$ showed a dose-dependent reduction in feeding induced by centrally injected NPY (Antal-Zimanyi *et al.*, 2008). Similarly, spontaneous nocturnal feeding was significantly reduced in rats treated with an intraperitoneal dose of 10 or 20 mg·kg$^{-1}$ or an i.v. dose of 3 or 10 mg·kg$^{-1}$ of BMS-1393885 (Antal-Zimanyi *et al.*, 2008). Chronic administration of BMS-1393885 for 44 consecutive days led to a significant suppression of feeding and prevented body weight gain in lean rats (Antal-Zimanyi *et al.*, 2008). Although these effects

are consistent with desired features of a potential anti-obesity therapeutic, and although BMS-1393885 did not induce any adverse effects in a set of behaviour paradigms, it is of concern that the compound is not orally available and also that a number of rats fell ill following 31 days of treatment with BMS-1393885, with adhesive peritonitis attributable to the poor tissue absorptive property of the drug (Antal-Zimanyi *et al.*, 2008).

*Y1-973.* Outside the scope of developing novel therapeutic leads for the treatment of obesity, the development of Y1-specific molecules has been directed towards the evaluation of the receptor engagement by drug candidates or to study the *in vivo* changes in Y1 receptor expression during disease states (Eva *et al.*, 2006). Towards this aim, Hostetler and colleagues synthesized and evaluated [$^{18}$F]Y1-973, a brain-penetrant molecule that was designed to allow detection by positron emission tomography (PET) of cells that express high levels of Y1 receptors, such as tumour cells. Y1-973 demonstrated sub-nanomolar affinity for Y1 in preference to Y2, Y4 and Y5 receptors (Kameda *et al.*, 2009a; Hostetler *et al.*, 2010). Following i.v. infusion of the compound into rhesus monkeys, [$^{18}$F]Y1-973 was rapidly detected in the brain by PET scanning, confirming efficient penetration of the blood-brain barrier. Using this methodology, [$^{18}$F]Y1-973 was detected in the striatum, cortex, thalamus and cerebellum, consistent with the previously reported pattern for Y1 receptor expression in the human brain (Caberlotto *et al.*, 1997). Specific [$^{18}$F]Y1-973 binding is reversible because Y1-718, a Y1 antagonist, dose-dependently reduced [$^{18}$F]Y1-973 uptake in the striatum area (Hostetler *et al.*, 2010). Further, autoradiography revealed high binding of [$^{3}$H]Y1-973 in the hippocampal dentate gyrus, cortical areas and caudate-putamen, and moderate binding in the hypothalamus and thalamus of human and rhesus monkey brains (Hostetler *et al.*, 2010). Taken together, these results show [$^{18}$F]Y1-973 to be the first Y1 receptor-specific PET tracer suitable for the *in vivo* detection of Y1 receptor binding, providing a novel tool with which to study the receptor-antagonist interaction for Y1 receptors. Additionally, given the specificity of Y1-793 for Y1 receptors and the considerable structural similarity to Y1-718 (both are diaminopyridine derivatives), it would be interesting to determine the potential of non-radiolabelled Y1-793 as a Y1 receptor antagonist.

## The Y2 receptor (Table 2)

The Y2 receptor is a 381 amino acid protein that is highly conserved between species, with more than 90% identity between orders of mammals and about 80% identity when comparing mammals and chicken (Gerald *et al.*, 1995; Berglund *et al.*, 2003). Interestingly, whereas the Y2 gene is localized in close proximity to the Y1 and Y5 gene cluster on chromosome 4q31-32, it has only approximately 30% overall amino acid identity to the Y1 receptor (Gerald *et al.*, 1995; Ammar *et al.*, 1996). Pharmacologically, the Y2 receptor binds to NPY and PYY with equally high affinity, but with low affinity for PP (Blomqvist and Herzog, 1997; Michel *et al.*, 1998). The most prominent pharmacological feature of the Y2 receptor is its high affinity to C-terminus fragments of NPY or PYY. Thus, NPY or PYY lacking 2, 13, 18 or even 22 amino acids from the N-terminus can bind to human, rat and mouse Y2 receptor with high to moderate affinity (Michel *et al.*, 1998). Importantly, whereas the more severely truncated forms of these peptides, such as NPY13-36 or PYY13-36 and NPY18-36 or PYY18-36, are normally produced pharmacologically, PYY3-36 and NPY3-36 can be produced endogenously by a specific protease – dipeptididyl peptidase-IV (DPPIV), itself a pharmacological target (Maes *et al.*, 2007) – that removes the first two amino acids from the N-terminus of the full length PYY or NPY (Mentlein, 1999; Unniappan *et al.*, 2006). This post-translational modification leads to an altered pharmacological profile, with the affinity to the Y1 receptor that is exhibited by the full-length peptides being lost in the truncated forms. It is important to note, however, that the affinity of NPY and PYY for Y2 receptors is unaltered by this modification. Thus, whereas conversion of NPY or PYY to the truncated forms NPY3-36 or PYY3-36 results in loss of Y1 binding, thus leaving PYY-mediated Y2 receptor activation in peripheral tissues physiologically unopposed, it does not lead to an enhanced net-activation of Y2 receptors. Moreover, losing C-terminus amino acid(s) due to specific proteolytic degradation, yielding products such as NPY3-35, PYY3-35, NPY3-34 or PYY3-34, renders the peptides completely inactive (Abid *et al.*, 2009). Unfortunately, none of the currently available assays measuring NPY or PYY levels in serum is able to distinguish between these carboxyl truncated variants, making it difficult to clearly determine the concentration of the active form of the peptides. This could also be one of the major reasons for the many discrepancies in different studies correlating concentrations of different forms of the peptides with certain conditions.

The Y2 receptor is mainly located presynaptically and involved in the suppression of transmitter release (Colmers *et al.*, 1991). In the central nervous system, Y2 receptor mRNA can be found within the hippocampus, hypothalamus and amygdala, as well as in specific nuclei of the brain stem (Parker and Herzog, 1999). Consistent with the high level of Y2 mRNA, autoradiography reveals that the Y2 receptor is the most prominent Y-receptor expressed in the central nervous system, representing approximately two-thirds of the total bind capacity for NPY (Lin *et al.*, 2005). Not surprisingly, Y2 receptors are involved in a large number of physiological functions induced by NPY family peptides such as angiogenesis (Zukowska-Grojec *et al.*, 1998), vasoconstriction (Pheng *et al.*, 1999; Malmstrom, 2001), effects on gastric emptying (Chen *et al.*, 1997), circadian rhythm (Golombek *et al.*, 1996; Huhman *et al.*, 1996; Gribkoff *et al.*, 1998) as well as in modulating emotional and stress-coping behaviours (Heilig, 2004). Importantly, activation of Y2 receptors has been linked to the induction of satiety, thus generating great interest in evaluating the anti-obesity potential of Y2 receptor agonism with compounds such as PYY3-36. This is in accordance with the particularly high levels of Y2 receptor expression found in the ARC of the hypothalamus and the area postrema in the brain stem (Parker and Herzog, 1999), both areas known to have a semi-permeable blood-brain barrier (Broadwell and Brightman, 1976), thus making Y2 receptors in these regions accessible to circulating PYY and PYY3-36. Recently, studies using germline and conditional Y2 receptor knockout models

## Table 2

Effects of Y2 receptor agonists and antagonists on feeding behaviour and aspects of energy homeostasis

| | Agonist | Reference | Dose | Route | Organism | Effects | Duration |
|---|---|---|---|---|---|---|---|
| 1 | C2-NPY | Hwa *et al.*, 1999 | 0.1, 0.3,1, 3 nmol per rat over 1 min infusion | i.c.v. | Satiated Long-Evans rats | ↔ Feeding | 2 h post-infusion |
| | | | | | | ↔ Brown adipose tissue temperature | 1 h post-infusion |
| 2 | NPY 13–36 | Hwa *et al.*, 1999 | 0.1, 0.3,1, 3 nmol per rat over 1 min infusion | i.c.v. | Satiated Long-Evans rats | ↔ Feeding | 2 h post-infusion |
| | | | | | | ↔ Brown adipose tissue temperature | 1 h post-infusion |
| 3 | N-acetyl[Leu$^{28}$, Leu$^{31}$]NPY(24–36) | Batterham *et al.*, 2002 | 100 fmol to 1 nmol per rat | intra-ARC injection | 24 h-fasted Wistar rats | ↓ Feeding | 2 h post-injection |
| | | | 100 fmol to 1 nmol per rat | intra-PVN injection | 24 h fasted Wistar rats | ↔ Feeding | 2 h post-injection |
| | | Henry *et al.*, 2005 | 3 μg·day$^{-1}$ continuous infusion via osmotic pump over 7 days | i.c.v. | C57BL/6 mice | ↓ Feeding, body weight and fat mass; no changes in energy expenditure, respiratory exchange ratio versus vehicle-infused control | |
| 4 | Polyethylene Glycol-Conjugated peptide consisting a peptide core (hPYY13-36) and a non-peptidic moiety (2-mercaptonicotinic acid) at N-terminus | Ortiz *et al.*, 2007 | 0.0094–0.94 μmol·kg$^{-1}$ | s.c. | 18 h-fasted C57BL/6 mice | ↓ d.d. feeding (abolished by Y2 antagonist BIIE0246) | Up to 72 h post-injection |
| | | | 0.07–0.74 mmol·kg$^{-1}$ | s.c. | 18 h-fasted Wistar rats | ↓ d.d. Feeding | Up to 48 h post-injection |
| | | | 0.0094–0.94 μmol·kg$^{-1}$ once daily administration for 14 days. | s.c. | DIO C57BL/6 mice | ↓ d.d. body weight | |
| | | | 0.31 μmol·kg$^{-1}$ once daily administration for 40 days | s.c. | DIO C57BL/6 mice | ↓ Body weight, feeding (transient), fed glucose levels | |
| | | | | | | ↑ Serum adiponectin levels, glucose tolerance | |

ARC, arcuate nucleus; d.d. dose-dependent; DIO, diet-induced obese; i.c.v., intracerebroventricular; PVN, paraventricular nucleus; s.c, subcutaneous.

revealed that in addition to effects on appetite control, the Y2 receptor is involved in the regulation of other components of energy homeostasis such as oxidative fuel selection and lipid metabolism (Zhang *et al.*, 2010b). More importantly from a therapeutic perspective, such regulation involves activation of central as well as peripheral Y2 signalling, for example, on adipocytes (Kuo *et al.*, 2007; Shi *et al.*, 2010). Additional, evidence emerging from knockout mouse models suggests that Y2 receptor antagonism may have benefits for bone health (Baldock *et al.*, 2002; Allison *et al.*, 2006). As such, future studies into effects of Y2 receptor agonism or antagonist will need to look more broadly at other physiological processes that are related to appetite and energy homeostasis.

The notion of Y2 receptor agonism (Table 2) as a potential anti-obesity treatment is supported by results from Batterham and colleagues demonstrating that PYY3-36 reduces food intake and/or body weight in animals and humans (Batterham *et al.*, 2002; 2003a). Peripheral administration of PYY3-36 in the concentration range normally seen postprandially dose-dependently inhibited feeding in 24 h fasted and freely feeding rats prior to the onset of the dark phase (Batterham *et al.*, 2002). These results are supported by other reports showing similar effects in fasted non-obese rats and mice (Adams *et al.*, 2004; Challis *et al.*, 2004; Martin *et al.*, 2004; Pittner *et al.*, 2004). Moreover, the anorectic effect of intraperitonally administered PYY3-36 was abolished in mice deficient of Y2 receptors (Batterham *et al.*, 2002), or in wild-type rodents in which PYY3-36 was co-administered with the Y2 receptor antagonist BIIE0246 (Abbott *et al.*, 2005; Scott *et al.*, 2005; Talsania *et al.*, 2005), demonstrating a Y2 receptor-dependent mechanism for PYY3-36-mediated anorexic effects. Although several Y2 agonists have been developed (Krstenansky *et al.*, 1989; Kirby *et al.*, 1993; Hwa *et al.*, 1999; Malis *et al.*, 1999; Balasubramaniam *et al.*, 2000; Batterham *et al.*, 2002), few of these have been examined in the context of food intake and energy metabolism after peripheral administration *in vivo*. Nevertheless, a recent study shows that subcutaneous injection of a Y2 receptor agonist – a polyethylene glycol-conjugated (PEGylated) peptide agonist consisting of a peptide core corresponding to residues 13–36 of human NPY and a non-peptidic moiety (2-mercaptonicotinic acid) at the peptide N terminus – reduced food intake in lean, 18 h fasted rodents, and this effect was abolished by pretreatment with the Y2 antagonist BIIE0246 (Ortiz *et al.*, 2007), lending support to the therapeutic potential of peripherally administered Y2 receptor agonists to reduce energy intake and potentially treat obesity. Importantly, intraperitoneal injection of PYY3-36 in diet-induced obese mice resulted in reduced food intake for up to 8 h compared with that of saline-injected control mice, suggesting that sensitivity to the anorectic effect of exogenous PYY3-36 remains present in the obese state, at least in an acute setting (le Roux *et al.*, 2006).

Chronic effects of PYY3-36 or Y2 receptor agonists on food intake and weight gain have been investigated in lean as well as in various obese rodent models. Whereas both unaltered (Challis *et al.*, 2004) and reduced (Batterham *et al.*, 2002) cumulative food intake and weight gain have been reported in lean rodents during 7 day intraperitoneal administration of PYY3-36, studies on obese rodent models – such as diet-induced obese mice or rats (Pittner *et al.*, 2004; Adams *et al.*, 2006; Vrang *et al.*, 2006; Ortiz *et al.*, 2007), *ob/ob* mice and *fa/fa* rats (Pittner *et al.*, 2004), have produced consistent results showing dose-dependent reductions in body weight and/or adiposity during chronic administration of PYY3-36 for periods ranging from 7 to 56 days (Pittner *et al.*, 2004; Adams *et al.*, 2006; Vrang *et al.*, 2006; Ortiz *et al.*, 2007). However, the anorectic effect of PYY3-36 seems transient, because the reduction in daily food intake or cumulative food intake during the immediate hours following injection is only apparent during the first 3–7 days of the treatment period (Adams *et al.*, 2006; Vrang *et al.*, 2006; Ortiz *et al.*, 2007; van den Hoek *et al.*, 2007). Receptor down-regulation and tolerance (Chelikani *et al.*, 2006), as well as redundancy and plasticity in the systems involved in regulation of energy homeostasis (Reidelberger *et al.*, 2008) have been suggested to contribute to the transient feeding responses seen during continuous infusion or repeated administration of PYY3-36. Importantly, the sustained reduction in body weight and/or adiposity in contrast to the transient reduction in food intake observed during chronic PYY3-36 treatment suggests that alterations in components of energy metabolism other than purely changes in energy intake contribute to the effect of Y2 agonism with PYY3-36 to reduce adiposity. Indeed, studies have shown that acute administration of PYY3-36 to lean or diet-induced obese rodents, while having little effect on total energy expenditure, significantly reduced respiratory exchange ratio. This change could not be accounted for by a reduction in food intake and persisted throughout the prolonged treatment period (Adams *et al.*, 2006; van den Hoek *et al.*, 2007), suggesting that enhanced lipid oxidation induced by PYY3-36 may contribute to its effects to reduce body weight and adiposity.

The role of PYY in the long-term regulation of energy homeostasis has been further evaluated using PYY knockout and transgenic mouse models. It is important to note that knockout or transgenic over expression of the PYY gene alters expression of both the full-length PYY as well as of PYY3-36, thus these genetically modified models may not be directly comparable with studies in which exogenous PYY3-36 was administered. The first reported PYY knockout model exhibited normal growth, body weight, energy expenditure and responsiveness to PYY3-36 (Schonhoff *et al.*, 2005). However in this mouse model, the PP gene was also affected, suggesting that these mice were double knockout for both PYY and PP. Moreover, the founder colony used in this study was on an FVB background, a mouse strain that has been shown to be resistant to obesity induced by feeding a high-fat diet or by carrying a transgene that normally leads to obesity on a C57Bl/6 background (Ludwig *et al.*, 2001; Chen *et al.*, 2005; Schonhoff *et al.*, 2005). Another study by Batterham and associates showed that both male and female homozygous PYY deficient mice were hyperphagic and developed marked obesity, suggesting that PYY ablation may cause obesity through increased food intake (Batterham *et al.*, 2006). Another PYY knockout model by Boey and colleagues demonstrated late-onset obesity in female mice on a chow diet and exacerbated diet-induced obesity in male and female mice without changes in either basal or fasting-induced food intake (Boey *et al.*, 2006). Interestingly, there was an increase in basal and glucose-induced serum insulin levels in both male and female PYY knockout mice in this study, raising the possibility of a role for hyperinsulinaemia in the develop-

ment of increased adiposity associated with PYY deletion. An additional PYY knockout model generated by Wortley *et al*. exhibited no difference from wild-type mice with respect to food intake or body weight on a standard chow diet, but exhibited exacerbated weight gain and fat gain on a high fat diet compared with wild types on the same diet (Wortley *et al*., 2007). The exacerbated diet-induced obesity of PYY knockout mice was not associated with any significant changes in food intake, energy expenditure, respiratory exchange ratio, locomotor activity or serum insulin levels relative to control mice, leaving the likely cause for obesity in this PYY knockout model unanswered (Wortley *et al*., 2007). In summary, three out of four PYY knockout models generated in different laboratories have shown progression towards obesity, with or without hyperphagia. These data collectively imply that lack of PYY contributes to the development of obesity.

In contrast to the effects of germline PYY deficiency, high levels of PYY expression during embryogenesis leads to neural tube defect in embryos (Yuzuriha *et al*., 2007), demonstrating a critical role for PYY in neural development. Therefore, only transgenic mice that have moderate to low PYY overexpression are viable. Interestingly, even small increases in PYY expression in transgenic mice induces marked resistance to diet-induced obesity and significantly attenuates the metabolic syndrome of genetically obese *ob/ob* mice in the absence of changes in body weight or basal and fasting-induced food intake (Boey *et al*., 2008). Additionally, PYY transgenic mice on the *ob/ob* background exhibited increased body temperature, enhanced hypothalamic expression of thyrotropin-releasing hormone mRNA and decreased brown adipose tissue depot weight, suggesting PYY-induced activation of the hypothalamo-pituitary-thyroid axis and increased thermogenic activity (Boey *et al*., 2008). These findings imply that PYY may have long-term benefits to reduce excess adiposity and ameliorate metabolic abnormalities associated with obesity through mechanisms independent of effects on food intake, notably stimulation of thyroid function. In keeping with this, PYY3-36 injection was shown to increase serum levels of thyrotropin in fasted rats (Oliveira *et al*., 2006).

The anorexic effects and therapeutic potential of PYY3-36 as an anti-obesity treatment have also been evaluated in humans and the results are in accordance with those obtained from rodent studies (Batterham *et al*., 2002; 2003a; Degen *et al*., 2005; Sloth *et al*., 2007a; 2007b; le Roux *et al*., 2008; Field *et al*., 2010). For instance, in normal-weight subjects a 90 min i.v. infusion of PYY3-36 at a dose producing PYY plasma levels similar to those observed post-prandially leads to a decrease in appetite and food intake for up to 12 h and a 33% decrease in cumulative energy intake in the 24 h period after the infusion (Batterham *et al*., 2002). More importantly, obese people remain responsive to the anorectic effect of exogenous PYY3-36, at least to an acute i.v. infusion (Batterham *et al*., 2003b; Sloth *et al*., 2007a; 2007b; Field *et al*., 2010), and this effect can be additive with other anorexic agents such as the glucagon-like peptide (GLP-1) receptor agonist oxyntomodulin (Field *et al*., 2010).

In contrast to focusing on the effect of PYY3-36 to induce satiety, few studies have examined effects of exogenous PYY3-36 in humans on other aspects of energy homeostasis. A recent study shows that i.v. infusion of PYY3-36 in lean and obese subjects increases thermogenesis and lipolysis in association with a reduced respiratory exchange ratio, suggesting enhanced lipid oxidation (Sloth *et al*., 2007a). The increased thermogenesis by PYY3-36 administration in humans (Sloth *et al*., 2007a) is in accordance with the increased core body temperature observed in PYY transgenic mice (Boey *et al*., 2008). Moreover, the influence of PYY3-36 in promoting lipolysis and increasing lipid oxidation observed in humans (Sloth *et al*., 2007a) has also been observed in rodents (Adams *et al*., 2006; van den Hoek *et al*., 2007). These effects are consistent with a report that high post-prandial levels of PYY are associated with increased lipid oxidation as indicated by a reduction in respiratory quotient value (Guo *et al*., 2006). However, whereas this evidence collectively suggests the potential therapeutic value of exogenous PYY3-36 in the treatment of obesity, nausea and vomiting appear to be common side effects associated with i.v. PYY3-36 infusion, particularly at higher doses (Degen *et al*., 2005; Gantz *et al*., 2007; Sloth *et al*., 2007a; le Roux *et al*., 2008). Supraphysiological doses of i.v. PYY3-36 cause nausea without additional reduction in food intake, and the authors of that study suggested that two PYY thresholds may exist: exceeding the first threshold results in reduced food intake without nausea, and exceeding the second threshold causes nausea without further reduction in food intake (le Roux *et al*., 2008). Interestingly, subcutaneously administered PYY3-36 at a dose that was well tolerated by volunteers did not reduce food intake but significantly enhanced circulating free fatty acid levels (Sloth *et al*., 2007b), suggesting effects on fat metabolism. Thus, it would be interesting to examine whether the PYY3-36-induced effects to increase energy expenditure and fat oxidation occur at lower doses to those required to induce an anorexic effect, and whether this can still have beneficial effects in the context of obesity.

Currently, studies on chronic effects of peripherally administered PYY3-36 in humans are lacking. Thus, it remains to be determined whether the potentially anti-obesity therapeutic benefits that have been observed with acute PYY3-36 administration persist during prolonged administration, whether it would lead to weight loss and fat loss, and whether it would be well tolerated. Furthermore, given the negative role of Y2 receptor agonism in the regulation of bone metabolism mentioned above, and because weight loss itself is associated with bone loss due to weight bearing effects (Ozcivici *et al*., 2010), the long-term effects of PYY3-36 and other Y2 receptor agonists on bone mass may need particular attention. Finally, the short half-life of PYY3-36 in the circulation of less than 20 min will limit its clinical use as anti-obesity drug, and a modified version of the peptide with greater stability and longer-lasting effects will need to be developed to avoid the need for frequent administration.

In contrast to the extensive research into Y2 receptor agonism as a potential obesity treatment, effects of Y2 receptor antagonism have received relatively little attention. Among the few Y2 receptor antagonists developed so far (Grouzmann *et al*., 1997; Doods *et al*., 1999; Bonaventure *et al*., 2004; Lunniss *et al*., 2010), BIIE0246 is the one most frequently studied. However, these studies have mainly focused on investigations into the mechanisms of action of

Y2 receptor ligands, PYY and PYY3-36, rather than investigating any direct effects of BIIE0246 on energy homeostasis. On the other hand, Y2 receptor knockout mouse models have served as an extremely useful tool to study the physiology of this receptor and have provided some important insights into the effects of Y2 receptor antagonism on energy homeostasis.

The first study of a germline Y2 receptor knockout mouse reported increased food intake, fat mass and body weight accompanied with leptin resistance as indicated by an attenuated response to leptin in female mice (Naveilhan *et al.*, 1999). Another Y2 deficient mouse model from our group showed that female germline Y2 receptor knockout mice also had increased food intake; however, with reduced body weight, whereas male Y2 knockout mice had transiently reduced food intake and a sustained decrease in body weight associated with decreased adiposity at 16 weeks of age (Sainsbury *et al.*, 2002a; Zhang *et al.*, 2010b). The discrepancies between these germline knockout models may arise from the different background of the two mouse strains as well as the different strategies used to target the Y2 gene, hence affecting the completeness of Y2 deletion (Herzog, 2003). Importantly, evidence emerging from more recent studies on conditional Y2 receptor knockout models suggests that interpretation of changes in body weight and body composition observed in different Y2 receptor knockout models needs to consider the possibility of differential effects for central versus peripheral, and/or hypothalamic versus non-hypothalamic, effects of this Y receptor. Thus, adult-onset hypothalamus-specific Y2 receptor deletion – induced by injection of a recombinant adeno-associated viral vector expressing Cre-recombinase into the hypothalamus of adult $Y2^{lox/lox}$ mice – led to significant increases in daily food intake, weight gain and fat gain (Shi *et al.*, 2010). Furthermore, specific deletion of Y2 receptors expressed only in NPY-expressing neurons in adult mice resulted in a significant increase in NPY mRNA expression with a concomitant decrease in proopiomelanocortin (POMC) mRNA expression in the ARC (Shi *et al.*, 2010), providing direct evidence that Y2 receptors on NPY-ergic neurons act as an auto-receptor regulating NPY expression and directly or indirectly influence neighbouring POMC neurons in the ARC. Importantly, female mice with conditional Y2 receptor deletion in hypothalamic NPY-ergic neurons showed increased adipose tissue mass, hepatic steatosis and a greater capacity for fatty acid synthesis in muscle (Shi *et al.*, 2010), demonstrating the obesogenic effect of selective blockade of Y2 receptor signalling in NPY neurons. Collectively, findings from conditional Y2 receptor knockout models are consistent with the notion that PYY3-36 and other Y2 receptor agonists can act as a satiety and anti-obesogenic factor by interacting with hypothalamic Y2 receptors, and that lack of hypothalamic Y2 signalling results in increased food intake, fat gain and weight gain. These findings also suggest that lack of Y2 receptor signalling in non-hypothalamic tissues (for instance, in adipose tissue) could contribute to weight loss, as was observed in one (Sainsbury *et al.*, 2002b; 2003; Zhang *et al.*, 2010b) but not all (Naveilhan *et al.*, 1999) germline Y2 receptor knockout models.

Interestingly, crossing Y2 knockout mice onto the *ob/ob* background attenuated the increased adiposity, hyperinsulinaemia and hyperglycaemia typical of *ob/ob* mice without affecting food intake or body weight gain (Naveilhan *et al.*, 2002; Sainsbury *et al.*, 2002b). Y2 receptor deletion has also been shown to confer protection against obesity and associated metabolic conditions induced by high fat feeding (Sainsbury *et al.*, 2006) and chronic corticosterone administration (Sainsbury *et al.*, 2002a). Furthermore, increased body weight and adiposity in female mice due to ovariectomy, a model mimicking menopause in women, was normalized by global Y2 receptor ablation but not by hypothalamic-specific Y2 receptor deletion (Allison *et al.*, 2006). These studies suggest that the anti-obesity effects of peripheral Y2 receptor deletion may outweigh any possible obesogenic effects produced by blocking the anorectic and weight-reducing effects of Y2 agonists in the hypothalamus, and thus that Y2 receptor antagonism may overall be more beneficial than Y2 agonism for treating obesity.

It is important to note that studies with Y2 receptor knockout models have also revealed a significant role for Y2 receptors in the regulation of bone metabolism. Germline Y2 receptor knockout mice have a twofold increase in trabecular bone volume as well as greater trabecular number and thickness compared with control mice, an effect due to increased osteoblast activity and an increased rate of bone mineralization and formation (Baldock *et al.*, *2002*). The increased bone mass in germline Y2 receptor knockout mice coincides with the reduced fat mass in these animals, suggesting potential energy partitioning changes between fat and lean tissues caused by Y2 receptor deletion. In support of this hypothesis, Y2 receptor deletion abolishes the fasting-induced reduction in activity of the hypothalamo-pituitary-somatotropic axis (Lin *et al.*, 2007) and restores the low serum levels of insulin-like growth factor-1 in *ob/ob* mice (Sainsbury *et al.*, 2006), suggesting a role of Y2 signalling in regulating activity of the somatotropic axis, activation of which is known to promote the accretion of lean mass at the expense of fat mass (Ho *et al.*, 1996). This regulation of the somatotropic axis by Y2 receptors is likely to occur in the hypothalamus, because Y2 receptors have been shown to co-localize with growth hormone releasing hormone (GHRH) neurons in the ARC and ventromedial hypothalamus, and hypothalamus-specific Y2 receptor deletion prevented fasting-induced inhibition of hypothalamic GHRH expression (Lin *et al.*, 2007). Interestingly, hypothalamus-specific Y2 receptor deletion recapitulated the high bone mass phenotype observed in germline Y2 knockout mice (Baldock *et al.*, 2002), demonstrating the key role of hypothalamic Y2 receptors in regulating bone metabolism. Importantly, the potential of hypothalamic Y2 receptors as a target for novel anabolic treatments for osteoporosis was demonstrated in a study showing that hypothalamic Y2 receptor deletion in gonadectomized sex-hormone deficient adult male and female mice prevented ongoing bone loss, an effect attributable to activation of an anabolic osteoblastic bone formation that counterbalances the persistent elevation of bone resorption seen in gonadectomized wild-type animals (Allison *et al.*, 2006). These studies suggest that whereas Y2 receptor agonists such as PYY3-36 that can access the hypothalamus may be beneficial in treating obesity, they are likely to have detrimental effects on bone mass in the long-term and analyses of bone density should be incorporated into trials of Y2 agonists. Thus pharmaceutical agents that antagonize Y2 receptors in the hypothalamus as well as in non-hypothalamic sites (e.g.

in peripheral tissues) could confer dual anti-obesity and anti-osteoporotic quantities.

## The Y4 receptor

The Y4 receptor is substantially different from other Y receptors, sharing only 30% primary sequence identity with them (Yan *et al.*, 1996; Darby *et al.*, 1997). The conservation of Y4 between human and mouse (76%) is also relatively low when compared against Y1 (94%) and Y2 (94%) receptors, suggesting rapid evolutionary divergence of this receptor. Despite this, rat and mouse Y4 receptors show similar pharmacology and tissue expression profiles to their human homolog. While the Y4 receptor exhibits affinity for all three members of the NPY family, it preferentially binds to PP and shows improved binding affinity to NPY and PYY analogues that incorporate PP residues, namely [Pro$^{34}$]PYY and [Leu$^{31}$, Pro$^{34}$]NPY (Bard *et al.*, 1995; Darby *et al.*, 1997). *In situ* hybridization on rat brain has revealed the presence of Y4 receptor mRNA in the brain stem, specifically in the area postrema, in the dorsal motor nucleus of the vagus nerve, and in the nucleus tractus solitarius (Larsen and Kristensen, 1997; Parker and Herzog, 1999). Consistent with this mRNA expression, Whitcomb and colleagues described a dense population of high-affinity PP receptors in the dorsal vagal complex of the caudal brain stem of rats (Whitcomb *et al.*, 1990), the primary site receiving afferent neuronal signals from peripheral organs and also containing an incomplete blood-brain barrier, permitting the entry of small peptide hormones (Pardridge, 1983). The presence of Y4 receptors has also been reported by *in situ* hybridization and immunocytochemistry techniques in the lateral hypothalamic area, specifically in orexin-containing neurons (Campbell *et al.*, 2003). Northern blot analyses showed that in humans, Y4 receptors are predominantly expressed in peripheral tissues such as the heart, gastrointestinal tract, skeletal muscle, pancreas, testis and uterus (Bard *et al.*, 1995; Yan *et al.*, 1996). Significant amounts of Y4 receptor mRNA, however, are also present in the human brain, with the highest level of expression being observed in the hypothalamus (Bard *et al.*, 1995).

Elucidation of the function of Y4 receptors has been facilitated by pharmacological agents and transgenic and knockout mice. Over-expression of PP, the endogenous ligand for Y4 receptors, reduces body weights and adiposity in association with reduced food intake and a decreased rate of gastric emptying in mice on a lean background (Ueno *et al.*, 1999), while peripheral administration of exogenous PP to wild-type mice produced a dose-dependent reduction in food intake accompanied by a decreased rate of gastric emptying, increased energy expenditure, increased colonic muscle contraction and faecal output (Asakawa *et al.*, 1999; 2003; Balasubramaniam *et al.*, 2006; Moriya *et al.*, 2010). Similarly, in genetically obese *ob/ob* mice, PP administration reduced food intake and body weight gain while increasing energy expenditure and reducing the rate of gastric emptying (Katsuura *et al.*, 2002; Asakawa *et al.*, 2003). Moreover, repeated administration of PP improved the insulin resistance and hyperlipidaemia of *ob/ob* mice and Shionogy *ob/ob* mice with fatty liver (FLS-*ob/ob* mice), and attenuated the liver enzyme abnormalities of the latter model (Asakawa *et al.*, 2003). These findings suggest that PP promotes negative energy balance, likely via effects on appetite and gut function.

Interestingly, however, mice lacking Y4 receptors do not exhibit the obese phenotypes that might have been expected given the effects of PP administration to reduce energy balance as described above. Indeed, germline Y4 receptor knockout mice on a lean background exhibit reduced food intake, reduced adiposity and/or reduced body weight (Sainsbury *et al.*, 2002c). It is postulated that compensatory mechanisms for the global loss of Y4 receptors throughout development may contribute to the conflicting phenotype of PP transgenic and Y4 deficient mice. Indeed, circulating PP concentrations were significantly elevated in the germline Y4 receptor knockout mice, to levels comparable with PP-over-expressing mice (Ueno *et al.*, 1999; Sainsbury *et al.*, 2002c). Thus, in the absence of Y4 receptors, excess PP may activate other receptors besides Y4, such as Y5, albeit with lower affinity, thereby inducing PP-like effects. Similar to the lack of obesity in Y4 receptor knockout mice on a lean background, germline Y4 receptor knockout does not reduce the hyperphagia, increased body weight nor excess adiposity of genetically obese leptin-deficient *ob/ob* mice, although it does rescue the infertility and depressed activity of the hypothalamo-pituitary-gonadotropic axis of these mice (Sainsbury *et al.*, 2002c). These findings suggest that whereas PP can reduce food intake and energy balance in lean and obese mice, signalling through Y4 receptors does not contribute to the hyperphagia and obesity of leptin-deficient mice, in which other mechanisms such as increased Y1 and Y5 signalling may be at play. On the other hand, although PP is not expressed in the brain, the enhanced NPY signalling known to occur in the brain of *ob/ob* mice – as well as in normal animals during energy deficit – (Sainsbury and Zhang, 2010) may lead to Y4-mediated down-regulation of the gonadotropic axis and prevention of pregnancy under conditions of low energy supply (Sainsbury *et al.*, 2010).

The effects of peripheral PP administration to mice were abolished by vagotomy or deficiency of Y4 receptors (Asakawa *et al.*, 2003; Lin *et al.*, 2009; Moriya *et al.*, 2010), demonstrating the critical role of the vagus nerve and Y4 receptors in mediating effects of PP. Similarly in humans, insulin-induced PP release was attenuated in vagotomized patients with duodenal ulcers or by the administration of atropine, a competitive antagonist for the muscarinic acetylcholine receptor (Schwartz, 1978). Circulating PP was demonstrated to cross the blood-brain barrier through a semi-permeable region in the area postrema and to bind to Y4 receptors in dorsal vagal complex (Whitcomb *et al.*, 1990; Katsuura *et al.*, 2002. It is thought that Y4 agonism in this area of the brain stem may mediate the transmission of afferent neuronal activity, leading to autonomic regulation of gastrointestinal functions. Indeed, peripheral administration of PP was shown to activate several different types of orexigenic and anorexigenic hypothalamic neurons in wild-type but not in Y4 receptor deficient mice, emphasizing the involvement of Y4 receptor signalling. Importantly, these PP-activated neurons include the orexigenic brain-derived neurotrophic factor and orexin as well as the anorexigenic protein product of the POMC gene, alpha-melanocyte stimulating hormones, suggesting that PP may up-regulate anorexigenic and down-regulate orexigenic pathways in the

central nervous system (Lin *et al.*, 2009; Sainsbury *et al.*, 2010). Consistent with this, hypothalamic mRNA levels of the orexigenic NPY, ghrelin and orexin in the hypothalamus were reduced while that of the anorexigenic urocortin and POMC were elevated in response to Y4 agonism with PP in mice (Asakawa *et al.*, 2003). Notably, these changes in the mRNA expression in response to PP injection was observed in wild-type, but not Y4 receptor deficient mice, demonstrating the critical role of the Y4 receptor in these processes (Lin *et al.*, 2009; Sainsbury *et al.*, 2010). Thus, Y4 agonism by PP may regulate food intake by suppressing orexigenic pathways and stimulating anorexigenic pathways.

In keeping with an effect of PP to induce negative energy balance, low circulating PP levels is associated with conditions of obesity in both children (Zipf *et al.*, 1981; Reinehr *et al.*, 2006) and adults (Lassmann *et al.*, 1980; Marco *et al.*, 1980; Glaser *et al.*, 1988), whereas fasting and post-prandial PP levels are significantly elevated in the circulation of patients with anorexia nervosa (Alderdice *et al.*, 1985; Takeno *et al.*, 1990; Kinzig *et al.*, 2007). These results indicate that PP is involved in the pathophysiology of obesity and may be a suitable anti-obesogenic agent. Indeed, short-term i.v. administration of PP to lean human subjects led to sustained suppression of appetite and food intake up to 24 h post-infusion (Batterham *et al.*, 2003b; Jesudason *et al.*, 2007. Additionally, an i.v. infusion of PP that restored basal and meal-stimulated PP levels in people with obesity or obesity due to Prader–Willi syndrome attenuated the hyperphagia seen in these people, possibly through enhanced satiation (Berntson *et al.*, 1993). More importantly from a clinical perspective, administration of PP at doses that reduces food intake does not induce nausea in humans, unlike PYY (Batterham *et al.*, 2003b; Jesudason *et al.*, 2007; le Roux *et al.*, 2008).

These reports, along with results from rodent studies, have highlighted the potential benefit of PP as a satiety-promoting agent and have suggested that agonism of Y4 receptors may be a potential anti-obesity strategy. However, the use of PP as a therapy is limited by several constraints. Firstly, native PP has a half-life of only approximately 6 min, limiting its bioactivity *in vivo* (Adrian, 1978; Balasubramaniam *et al.*, 2006). Additionally, the weak but significant interaction between PP and Y5 receptors (Gerald *et al.*, 1996) raised the possibility that PP administration could induce unwanted Y5-mediated side effects. These factors have prompted the development of Y4 selective ligands with improved half-life.

## *Agonists (Table 3)*

Balasubramaniam and colleagues reported a series of second-generation peptidomimetic analogues of PP. These peptide analogues were developed to improve the affinity and selectivity of two previously described analogues of the NPY C-terminal fragments, [Cys$^{31}$, Trp$^{32}$, Nva$^{34}$]NPY31-36 and 1229U91, towards Y4 receptors.

*[Cys$^{31}$, Trp$^{32}$, Nva$^{34}$]NPY31-36.* [Cys$^{31}$, Trp$^{32}$, Nva$^{34}$]NPY31-36 is a C-terminal truncated product of NPY and was synthesized in 1996 (Balasubramaniam *et al.*, 1996). As a monomer, [Cys$^{31}$, Trp$^{32}$, Nva$^{34}$]NPY31-36 showed weak affinity towards Y receptors, possibly due to lack of stability and thus poor bioactivity. Because it has been suggested that native NPY may exist as a homodimer in solution (Saudek and Pelton, 1990; Cowley *et al.*, 1992), a strategy to dimerize this analogue was pursued and achieved by the formation of disulphide bridges between its cystine residues. Indeed, dimerization was found to improve affinity of this peptide for Y receptors. However, its value as a potential therapeutic agent is limited due to its mix of antagonistic and agonistic effects on Y1 and Y4 receptors respectively (Balasubramaniam *et al.*, 2006).

*BVD-74D.* The second-generation of PP analogues retained the dimeric features of [Cys$^{31}$, Trp$^{32}$, Nva$^{34}$]NPY31-36 but also included optimization of the spacing and orientation of the peptide chains (Balasubramaniam *et al.*, 2006). The dimerization strategy involved incorporation of a variety of diaminodicarboxylic acids, a family of amino acid derivatives that contain a modifiable spacer group and two identical carboxylic groups in place of the conventional but chemically and metabolically labile disulphide bridges formed between two cystine residues (Hiebl *et al.*, 1999). These diaminodicarboxylic acids include diaminoadipic acid (Adp), diaminopimelic acid (Pimpalnerkar *et al.*, 1998) and diaminosuberic acid (Sub), which present di-, tri- and tetramethylene group, respectively, that can be used as spacers. Three parallel dimer designs of cystine-substituted diaminodicarboxylic analogues with the otherwise identical primary sequence of [Cys$^{31}$, Trp$^{32}$, Nva$^{34}$]NPY31-36 were synthesized, yielding compounds named 2A and 4A as well as their D-/L-isomers 2B and 4B, respectively, plus compound 3 in a D- and L-isomer mix. To present differential orientations of the peptide dimers, linear tandem dimer, 5, and trimer analogue, 6, were also synthesized. Lastly, substitution of the Trp$^{32}$ and Nva$^{34}$ residues of [Cys$^{31}$, Trp$^{32}$, Nva$^{34}$]NPY(31–36) with Tyr and Leu were introduced to the diamino-dicarboxylic derivatized peptide dimmer to produce compound 7 (also known as BVD-74D) (Balasubramaniam *et al.*, 2006; 2007; Li *et al.*, 2010).

The parallel dimers 2A, 2B, 3, 4A, 4B and the linear tandem dimer and trimer 5 and 6 displayed an enhanced affinity and a moderate improvement in selectivity for the Y4 receptor in comparison to the parent molecule [Cys$^{31}$, Trp$^{32}$, Nva$^{34}$]NPY(31–36), but not relative to PP. Functional analysis demonstrated that these compounds – with the exception of 4B – were able to inhibit forskolin-stimulated cAMP synthesis in Chinese hamster ovary cells that express recombinant human Y4 receptor, indicating that these pentapeptide dimers act as agonists of the Y4 receptor (Balasubramaniam *et al.*, 2006).

Interestingly, the results from competitive binding and cAMP assays obtained for these compounds, all of which have varying spatial arrangement, dimer orientation and chirality of the side chains, are comparable. The exception is 4B, an L- or D-isomer of 4A. This particular disparity highlights the importance of chirality, and puts forward an interesting prospect for the D-enantiomers of analogue 2 (either 2A or 2B, because these isomers were successfully separated by liquid chromatography method but was not able to be identified). Because mammalian enzymes are known to exist only as L-isomers and to process only L-substrates (Gerber *et al.*, 2005), D-enantiomers of analogue 2 may be less

## Table 3

The effects of Y4 receptor agonists on feeding behaviour and aspects of energy homeostasis

| | Agonist | Reference | Dose | Route | Significant effects | Organism | Condition | Duration |
|---|---|---|---|---|---|---|---|---|
| 1 | PP | Asakawa *et al.*, 1999 | 0.3, 3 nmol per mouse | i.c.v. | d.d. ↑ feeding | ddy mice | *Ad libitum* | 20 min, 1, 2, 4 h |
| | | Asakawa *et al.*, 1999, 2003 | 0.3, 3 nmol per mouse | i.p. | d.d. ↓ feeding, gastric emptying | ddy mice | Fasting-induced | 20 min, 1, 2, 8, 24 h |
| | | Asakawa *et al.*, 2003 | 0.3 nmol per mouse | i.p. | ↓ Feeding, gastric emptying | *ob/ob* mice | Fasting-induced | 20 min, 1, 2 h |
| | | | 3 nmol per mouse | i.p. | ↓ Feeding, gastric emptying | *ob/ob* mice | Fasting-induced | 20 min, 1, 2, 8 h |
| | | | | | ↑ Oxygen consumption | *ob/ob* mice | Fasting-induced | 1–2 h |
| | | | 3 nmol per mouse × 2 per day | i.p. | ↓ BW gain, feeding, ↓ Blood glucose, ↓ Cholesterol, triglycerides, FFA | *ob/ob* mice | *Ad libitum* | 6 days |
| | | | 3 nmol per mouse × 2 per day | i.p. | ↓ BW gain, feeding, ↓ Fat mass, liver weight, ↓ blood cholesterol | FLS-*ob/ob* mice | *Ad libitum* | 14 days |
| | | Lin *et al.*, 2009 | 200, 300, 500 μg·kg$^{-1}$ | | ↓ Feeding | C57/Bl6-129SvJ mice | Fasting-induced | 24 h |
| | | Moriya *et al.*, 2010 | 1, 3 mg·kg$^{-1}$ | | ↑ Fecal output, diarrhoea score | C57Bl/6 mice | *Ad libitum* | 1, 2, 3, 4 h |
| | | Berntson *et al.*, 1993 | 50 pmol·kg$^{-1}$·h$^{-1}$ 90 min | | ↓ Feeding (–12%) | Prader–Willi human | *Ad libitum*, food removed post-administration | 60 min |
| | | Batterham *et al.*, 2003b | 10 pmol·kg$^{-1}$·min$^{-1}$ 90 min | i.v. | ↓ Hunger score | Human | Lean, overnight fasting, buffet | 9 h |
| | | | | | ↓ Feeding | Human | | 2, 12, 24 h |
| | | Jesudason *et al.*, 2007 | 5 pmol·kg$^{-1}$·min$^{-1}$ 90 min | | ↓ Hunger score, feeding | Human, pancreatic tumour | Lean, overnight fasting, buffet | 30 min |
| 2 | BVD-74D | Balasubramaniam *et al.*, 2006 | 50, 100, 150 nmol per mouse | i.p. | d.d. ↓ feeding | C57Bl/6 mice | Fasting-induced | 4 h |
| | | | 100 nmol per mouse | i.p. | ↓ Feeding | C57Bl/6 mice | Fasting-induced | 2, 4 h |
| | | Li *et al.*, 2010 | 10 mg·kg$^{-1}$ | i.p. | ↓ Feeding | C57Bl/6 mice | Fasting-induced | 24 h |
| | | | 10 mg·kg$^{-1}$ | i.p. | ↓ BW regain | C57Bl/6 mice | Fasting-induced | 8 h |
| | | | 10 mg·kg$^{-1}$ | i.p. | ↓ Feeding | C57Bl/6 mice | HF, fasting-induced | 4 h |
| | | Balasubramaniam *et al.*, 2007 | 50 nmol | i.p. | ↓ Feeding | C57Bl/6 mice | Fasting-induced, +i.p. BT-48 | 2, 4, 8, 24 h |

BW, body weight; d.d., dose-dependent; HF, high-fat diet; i.c.v., intracerebroventricular; i.p., intraperitoneal; i.v., intravenous; PP, pancreatic polypeptide.

susceptible to enzymatic degradation and thus have a longer half-life than native PP (Adrian, 1978). On the other hand, it has been hypothesized that the improved Y4 receptor affinity and selectivity of analogue 2 relative to [Cys$^{31}$, Trp$^{32}$, Nva$^{34}$]NPY31-36 may be due to the multiple methylene groups in the diamino-dicarboxylic acid spacer, facilitating monomer interaction with cell surface receptors (Balasubramaniam *et al.*, 2006).

The positive outcomes of competitive binding and cAMP assays were further improved by the introduction of amino acid substitutions in compound 7 (BVD-74D), particularly Tyr$^{32}$, which is thought to promote ligand stability through hydrogen bonding from the hydroxyl side chain of the tyrosine residue (Leban *et al.*, 1995; Jois and Balasubramaniam, 2003; Balasubramaniam *et al.*, 2006). BVD-74D demonstrated picomolar affinity for the Y4 receptor, and a 40- and 80-fold increase in affinity in comparison to compounds 4A, 4B and the L- and D- peptide dimers of compound 7 without the Tyr$^{32}$ and Leu$^{34}$ substitution. More importantly, intraperitoneal administration of 50–150 nmol of BVD-74D to normal chow-fed wild-type C57BL/6 mice produced dose-dependant attenuation of re-feeding lasting for more than 2 h following 16–18 h of food-deprivation compared with saline-injected controls (Balasubramaniam *et al.*, 2006; Li *et al.*, 2010). This effect of BVD-74D was not seen after administration of lower doses of 1 mg·kg$^{-1}$ (equivalent to 10 nmol) and 25 nmol of the compound (Balasubramaniam *et al.*, 2006; Li *et al.*, 2010). Additionally, a 100 nmol dose of BVD-74D effectively reduced food intake for up to 4 h in high fat-fed wild-type and FLS-*ob/ob* mice, while their body weights were not significantly different from that of saline-injected control animals (Li *et al.*, 2010). The lack of long-lasting effect of acute BVD-74D administration on body weight raises questions as to its potential use *in vivo*, and while no negative side effects were reported for a high effective dose of this compound relative to PP the minimum reported effective dose of PP is 0.3 nmol (Asakawa *et al.*, 2003), it remains to be determined whether repeated administration of the compound has a significant and long-lasting effect on body weight, adiposity and feeding behaviour. Interestingly, combined administration of BVD-74D with a Y2-selective agonist, BT-48, increased and prolonged the anorectic effect of either compound compared with their effects when applied independently (Balasubramaniam *et al.*, 2007).

It is important to note that the effect of BVD-74D to reduce food intake was not seen in Y4-deficient mice, confirming that the hypophagic action of this compound was specifically mediated by the Y4 receptor. However, BVD-74D was less potent at inhibiting forskolin-stimulated cAMP synthesis and at reducing food intake in comparison to a 10 nmol dose of PP, despite the comparable affinity of BVD-74D and PP for Y4 receptors ($K_i$ BVD-74D = 0.05 ± 0.01 nM, $K_i$ PP = 0.08 ± 0.01 nM) (Balasubramaniam *et al.*, 2006). It must be considered that while BVD-74D demonstrated a lack of selectivity for Y2 and Y5 receptors even at a high concentration, BVD-74D has a non-negligible affinity for the Y1 receptor and a weak agonistic capacity in Y1-expressing cells. This and previously demonstrated anorectic effects of peripherally-administered Y1 antagonists (Kanatani *et al.*, 2001; Sparta *et al.*, 2004) suggest that the potential agonism of Y1 receptors by BVD-74D may explain the discrepancy between PP-induced and BVD-74D-induced effects on food intake in this study.

Based on these early *in vitro* and *in vivo* results, the second-generation of PP analogues synthesized and described by Balasubramaniam and colleagues shows potential as a foundation for the development of future Y4-selective agonists for the possible clinical management of obesity. However, while the affinity of these analogues for Y1, Y2 Y4 and Y5 receptors has been evaluated, when testing in mice the presence of functional y6 receptors must be taken into consideration, as y6 may be mediating some of the observed effects of these compounds, particularly given the Y4-like pharmacology of y6 receptors (Gregor *et al.*, 1996; Weinberg *et al.*, 1996). Lastly, based on the short-lasting effects of these compounds after acute administration, it appeared that these peptide analogues do not greatly improve upon the short half-life of PP. It will thus be of interest to investigate the chronic effect of these peptide analogues on food intake, body weight and adiposity in future studies.

## The Y5 receptor

The Y5 receptor was first isolated and cloned from rat hypothalamic cDNA on the speculation that an additional Y receptor subtype existed that was pharmacologically similar to but distinct from the Y1 receptor and able to mediate feeding responses to NPY that are not mediated by Y1 (Wahlestedt and Reis, 1993; Gerald *et al.*, 1996; Hu *et al.*, 1996).

Consistent with its hypothesized role in feeding, *in situ* hybridization histochemistry showed high levels of Y5 mRNA in areas of the rat brain that are implicated in the regulation of feeding, including the lateral hypothalamus area and overlapping with Y1 mRNA expression in the ARC, PVN and suprachiasmatic nucleus of the hypothalamus (Gerald *et al.*, 1996; Parker and Herzog, 1999). Y5 mRNA was also reported in the amygdala, an area of the brain that is primarily involved in memory processing and emotional functions, suggesting a possible role for this Y receptor in mediating emotional aspects of feeding behaviour (Gerald *et al.*, 1996). In the mouse brain, like in the rat brain, Y5 receptor mRNA was also detected in the ARC, adding further weight to the functional candidature of Y5 in the mediation of feeding behaviour (Naveilhan *et al.*, 1998). In contrast to its high transcript levels, however, competitive binding assays on rat brain slices using the Y1- and Y5-preferring ligand $^{125}$I-[Leu$^{32}$, Pro$^{34}$]PYY in the presence of Y1 and Y4-blockade by BIBP3226 or GW1229 demonstrated low density of Y5 receptor translation in hypothalamic neurons relative to other areas that exhibited higher levels of Y5-specific binding, including the ventral hippocampus, area postrema and nucleus tractus solitarius (Dumont *et al.*, 1998). Immunohistochemistry for Y5 receptor promoter-controlled Cre expression revealed strong expression in the mouse suprachiasmatic nucleus, PVN and dorsomedial hypothalamic nucleus (Oberto *et al.*, 2007). Additionally, Y5-immunoreactive neurons were detected in abundance in the hippocampus and hypothalamus, where they overlap with neurons expressing gonadotropin-releasing hormone, neurophysins, corticotropin-releasing hormone and γ-amino butyric acid (GABA) (Grove *et al.*, 2000; Campbell *et al.*, 2001). Taken together, these findings show that Y5

mRNA is expressed in numerous regions of the mouse and rat brain, albeit protein expression and Y5 binding in hypothalamic nuclei appears to be relatively low.

Interestingly, the Y1 and Y5 receptor genes are transcribed in opposite directions from a common promoter region on human chromosome 4q31-q32 (Herzog *et al.*, 1997; Nakamura *et al.*, 1997). This close proximity of the two Y receptor genes suggests that they have evolved from a gene duplication event. Furthermore, the transcription of both genes from opposite strands of the same DNA sequence suggests that transcriptional activation of one will have an effect on expression of the other. Because both Y1 and Y5 receptors are known to play an important role in the regulation of food intake and energy homeostasis (Kalra and Kalra, 2004; Brothers and Wahlestedt, 2010), the coordinate expression of their specific genes may be important in the modulation of NPY-ergic activity (Herzog *et al.*, 1997).

Pharmacologically, human NPY, PYY and PP (hNPY, hPYY, hPP) are equally potent at activating Y5 receptors and inhibiting forskolin-stimulated cAMP synthesis in transfected cells (Gerald *et al.*, 1996; Hu *et al.*, 1996). Y5 receptors are also activated by various analogues of NPY and PYY, including hPYY3-36, porcine NPY2-36 (pNPY2-36) and p[Leu$^{31}$, Pro$^{34}$]NPY, but not by peptide fragments or analogues that are poor orexigenic agents, such as pNPY13-36 (Gerald *et al.*, 1996; Hu *et al.*, 1996). Furthermore, [D-Trp$^{32}$]NPY was demonstrated to be a weak but selective Y5 agonist, effectively producing a 4.5-fold increase in food intake at a dose of 2 nmol per rat when compared with empty-treated control rats (Gerald *et al.*, 1996). Additionally, activation of Y5 receptors by intracerebroventricular administration of 3 nmol [D-Trp$^{32}$]NPY in satiated Long-Evans rats led to dose-dependant depression of body temperature and a significant reduction in energy expenditure (Hwa *et al.*, 1999), further confirming a critical role of this receptor in the regulation of energy homeostasis. In support of this, intracerebroventricular administration of phosphothioate end-protected antisense oligodeoxynucleotides against Y5 receptors led to the inhibition of spontaneous, fasting-induced, and NPY-induced feeding in rats (Schaffhauser *et al.*, 1997; Tang-Christensen *et al.*, 1998). Interestingly, although showing a blunted response to centrally administered hNPY- and hPYY3-36-induced feeding (Marsh *et al.*, 1998), Y5$^{-/-}$ mice develop late-onset obesity and significant hyperphagia in association with down-regulation of the anorexigenic POMC gene and up-regulation of NPY expression in the hypothalamus under fasting conditions (Marsh *et al.*, 1998; Kanatani *et al.*, 2000b; Higuchi *et al.*, 2008), suggesting that potential compensatory effects of germline Y5 deletion may mask the true role of Y5 receptors in the regulation of energy homeostasis. Additionally, it is possible that enhanced activation of Y1 receptors in Y5$^{-/-}$ mice can compensate for the lack of Y5 receptor signalling. Indeed, co-administration of NPY and 1229U91, a potent Y1 and Y4 receptor antagonist and agonist, respectively, to Y5$^{-/-}$ mice completely abolished NPY-induced hyperphagia, leading to the conclusion that the central effects of NPY are mediated by both Y1 and Y5 receptors (Marsh *et al.*, 1998). Furthermore, the modest or lack of improvement in the obesity syndrome of genetically obese *ob/ob* mice by separate inactivation of Y1 or Y5 receptors, respectively (Marsh *et al.*, 1998; Pralong *et al.*, 2002) supports the notion that interaction between Y1 and Y5 receptors exists in the regulation of feeding behaviour and energy homeostasis (Kanatani *et al.*, 2000b).

Collectively, these results demonstrate the role of Y5 receptors in the central mediation of feeding behaviour, and imply that development of highly specific and highly potent antagonists to one or both of the Y1 or Y5 receptors would be an attractive avenue to pursue for the medical management of obesity. However, the predominantly central distribution of Y1 and Y5 receptors presents a challenge for the design of a compound able to permeate the blood-brain barrier. The following compounds were developed with the aim of combining specificity for Y5 receptors with the ability to access Y5 receptors in the central nervous system.

## *Agonists (Table 4)*

*D-[Trp$^{34}$]NPY.* Based on [D-Trp$^{32}$]NPY, a prototypic Y5-selective agonist with mixed properties (Gerald *et al.*, 1996; Matos *et al.*, 1996; Hwa *et al.*, 1999), several other C-terminally substituted analogues of NPY were synthesized to optimize selective potency towards Y5 receptors (Balasubramaniam *et al.*, 1994; Parker *et al.*, 2000). The variant [D-Trp$^{34}$]NPY showed particularly improved affinity for and potency at the Y5 receptor relative to [D-Trp$^{32}$]NPY, with significantly reduced potency at the Y1, Y2, Y4 and y6 receptors (Parker *et al.*, 2000). *In vivo* investigation demonstrated that the orexigenic potency of [D-Trp$^{34}$]NPY exceeded that of [D-Trp$^{32}$]NPY (Parker *et al.*, 2000). Acute intracerebroventricular administration of the peptide stimulated feeding in satiated Long-Evans rats (Parker *et al.*, 2000), and chronic intracerebroventricular administration to mice recapitulated many aspects of the obesity syndrome including hyperphagia, weight gain, hyperinsulinaemia and hyperleptinaemia in mice that were allowed to eat *ad libitum* (Mashiko *et al.*, 2003). While preventing [D-Trp$^{34}$]NPY-induced hyperphagia via pair feeding normalized body weight and food intake in [D-Trp$^{34}$]NPY-treated mice, increased adipose tissue weight, hypercholesterolaemia and hypertriglyceridaemia were not ameliorated (Mashiko *et al.*, 2003). Furthermore, administration of [D-Trp$^{34}$]NPY to pair-fed mice reduced the activity of hormone-sensitive lipase in white adipose tissues and reduced mRNA expression of uncoupling protein-1 in brown adipose tissues (Mashiko *et al.*, 2003). The effects of [D-Trp$^{34}$]NPY suggest that the obesity induced by Y5 receptor agonism involves changes in the regulation of energy expenditure and fuel partitioning as well as in appetite control (Mashiko *et al.*, 2003).

*[Ala$^{31}$, Aib$^{32}$]-incorporated peptide fragments.* Other Y5-specific agonists that have been reported include [Ala$^{31}$, Aib$^{32}$]-pNPY, [hPP$^{1-7}$, Ala$^{31}$, Aib$^{32}$]hNPY and [cPP$^{1-7}$, NPY$^{19-23}$, Ala$^{31}$, Aib$^{32}$, Gln$^{34}$]hPP, which are based on NPY and NPY/PP chimeras and feature a β-turn induced by the dipeptide Ala-Aib (aminoisobutyric acid) in the peptide region that mediates receptor binding (Cabrele *et al.*, 2000). Whereas introduction of the Ala-Aib-induced β-turn to the native NPY and [cPP$^{1-7}$, NPY$^{19-23}$, Ala$^{31}$, Aib$^{32}$, Gln$^{34}$]hPP did not improve binding selectivity for and agonistic activity on the human Y5 receptor, [cPP$^{1-7}$, NPY$^{19-23}$, Ala$^{31}$, Aib$^{32}$, Gln$^{34}$]hPP demonstrated a threefold increase in binding affinity relative to NPY (Cabrele

## Table 4

Effects of Y5 receptor agonists and antagonists on feeding behaviour and aspects of energy homeostasis

| | Agonist | Reference | Dose | Route | Significant effects | Organism | Condition | Duration |
|---|---|---|---|---|---|---|---|---|
| 1 | [D-Trp$^{32}$]NPY | Gerald *et al.*, 1996 | 2.0 nmol | i.c.v. | ↑ Feeding | Sprague-Dawley rats | *Ad libitum* | 4 h |
| | | Matos *et al.*, 1996 | 40 μg | i.c.v. | ↑ Feeding | Wistar rats | *Ad libitum* | 2 h |
| | | Hwa *et al.*, 1999 | 3.0 & 5.0 nmol | i.c.v. | d.d. ↑ Feeding | Long-Evans rats | *Ad libitum* | 2 h |
| | | | 0.3, 1.0, 3.0 & 5.0 nmol | i.c.v. | d.d. ↓ BAT temperature | Long-Evans rats | *Ad libitum* | 1 h |
| | | | 3.0 nmol | i.c.v. | 30% ↓ energy expenditure | Long-Evans rats | *Ad libitum* | 1 h |
| | | Balasubramaniam *et al.*, 1994 | 10 μg | i.c.v. | ↓ Feeding | Sprague-Dawley rats | NPY-induced | 1 h |
| | | Small *et al.*, 1997 | 12 nmol | i.c.v. | ↓ Feeding | Wistar rats | NPY-induced | 1 h |
| 2 | [D-Trp$^{34}$]NPY | Parker *et al.*, 2000 | 1.0–3.0 nmol | i.c.v. | ↑ Feeding | Long-Evans rats | *Ad libitum* | 1, 2, 4 h |
| | | Mashiko *et al.*, 2003 | 5.0 & 10.0 μg·day$^{-1}$ | i.c.v. | ↑ BW, adiposity, ↑ Cumulative food intake | C57Bl/6J mice | *Ad libitum* | 7 days |
| | | | 10.0 μg·day$^{-1}$ | i.c.v. | ↑ Plasma total cholesterol, ↑ HDL- & non-HDL-cholesterol, ↑ TG, insulin, leptin | C57Bl/6J mice | *Ad libitum* | 7 days |
| | | | 5.0 μg·day$^{-1}$ | i.c.v. | ↑ Plasma total cholesterol, ↑ TG, insulin, leptin | C57Bl/6J mice | *Ad libitum* | 7 days |
| | | | 5.0 μg·day$^{-1}$ | i.c.v. | ↑ Plasma insulin, leptin, ↑ BAT UCP-1, UCP3, ↑ Liver weight, ↑ Liver SREBP-1c, ↑ Liver & skeletal muscle TG content | C57Bl/6J mice | *Ad libitum* | 6 days |
| 3 | [cPP$^{1-7}$, NPY$^{19-23}$, Ala$^{31}$, Aib$^{32}$, Gln$^{34}$]hPP | Cabrele *et al.*, 2000 | 0.2 & 2.0 nmol | i.c.v. | ↑ Feeding | Rats | *Ad libitum* | 24 h |
| | | Lecklin *et al.*, 2003 | 0.9 & 3.6 nmol | i.c.v. | ↑ Feeding | Dunkin-Hartley guinea pigs | *Ad-libitum* | 4 h |
| | | Kakui and Kitamura, 2007 | 100 nmol | i.c.v. | ↑ Plasma ACTH & CORT | Fisher rats | *Ad-libitum* | 30, 60 min |

## Table 4

*Continued.*

| | Antagonist | Reference | Dose | Route | Significant effects | Organism | Condition | Duration |
|---|---|---|---|---|---|---|---|---|
| 1 | CGP 71683A | Criscione *et al.*, 1998 | 3 & 10 mg·kg$^{-1}$ | i.p. | ↓ Feeding | Sprague-Dawley rats | NPY-induced | 8 h |
| | | | 10 & 100 mg·kg$^{-1}$ | i.p. | ↓ Feeding | Sprague-Dawley rats | *Ad libitum* | 24 h |
| | | | 1, 10, 100 mg·kg$^{-1}$ | i.p. | ↓ Feeding | Sprague-Dawley rats | Fasting-induced | 24 h |
| | | | 10 & 100 mg·kg$^{-1}$ | i.p. | ↓ Feeding | Streptozotocin rats | *Ad libitum* | 24 h |
| | | | 10 & 100 mg·kg$^{-1}$·day$^{-1}$ | i.p. | Transient ↓ feeding, ↓ BW & plasma TG | Sprague-Dawley rats | *Ad libitum* | 28 days |
| | | Della Zuana *et al.*, 2001 | 15, 30, 100 nmol·kg$^{-1}$ | i.c.v. | ↓ Feeding | Wistar rats | NPY-induced | 2 h |
| | | | 15 nmol·kg$^{-1}$ | i.c.v. | ↓ Feeding | Zucker *fa/fa* rats | NPY-induced | 2 h |
| | | | 300 nmol·kg$^{-1}$·day$^{-1}$ | i.c.v. | Transient ↓ feeding, ↓ BW | Zucker *fa/fa* rats | *Ad libitum* | 17 days |
| 2 | NPY5R-972 | Turnbull *et al.*, 2002 | 3 & 10 mg·kg$^{-1}$ | p.o. | ↓ Feeding | Wistar rats | Agonist 3-induced | 1, 2, 4 h |
| | | | 3 & 10 mg·kg$^{-1}$ | p.o. | ↔ Feeding | Wistar rats | NPY-induced | 4 h |
| | | | 1, 3 & 10 mg·kg$^{-1}$ | p.o. | ↔ Feeding | Wistar rats | Fasting-induced | 24 h |
| | | | 10 mg·kg$^{-1}$ × 2·day$^{-1}$ | p.o. | ↔ Feeding & BW | Wistar rats | *Ad libitum* | 9 days |
| | | | 10 mg·kg$^{-1}$ × 2·day$^{-1}$ | p.o. | ↔ Feeding & BW | Wistar rats | *Ad libitum* HF | 12 days |
| 3 | GW438014A | Daniels *et al.*, 2002 | 3 mg·kg$^{-1}$ | i.p. | ↓ Feeding | Sprague-Dawley rats | NPY-induced | 3 h |
| | | | 10 mg·kg$^{-1}$ | i.p. | ↓ Feeding | Sprague-Dawley rats | Fasting-induced | 3 h |
| | | | 10 mg·kg$^{-1}$ | i.p. | ↓ Feeding | *ob/ob* mice | Fasting-induced | 24 h |
| | | | 10 mg·kg$^{-1}$ | i.p. | ↓ Feeding | Sprague-Dawley rats | *Ad libitum* | 16 h |
| | | | 25 mg·kg$^{-1}$ | p.o. | ↔ Feeding | Sprague-Dawley rats | *Ad libitum* | 16 h |
| | | | 10 mg·kg$^{-1}$·day$^{-1}$ | i.p. | ↓ Feeding, BW, body fat | Zucker *Fa/Fa* | *Ad libitum* | 6 days |
| | | | 10 mg·kg$^{-1}$·day$^{-1}$ | i.p. | ↓ Feeding, BW, body fat | Zucker *fa/fa* | *Ad libitum* | 6 days |
| 4 | L-152,804 | Kanatani *et al.*, 2000a | 30 μg | i.c.v. | ↔ Feeding | Sprague-Dawley rats | NPY-induced | 2 h |
| | | | 10 mg·kg$^{-1}$ | p.o. | ↔ Feeding | Sprague-Dawley rats | NPY-induced | 2 h |
| | | | 30 μg | i.c.v. | ↓ Feeding | Sprague-Dawley rats | PP-induced | 2 h |
| | | | 10 mg·kg$^{-1}$ | p.o. | ↓ Feeding | Sprague-Dawley rats | PP-induced | 2 h |
| | | Ishihara *et al.*, 2006 | 30, 100 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↓ BW, feeding<br>↓ Fat pad, adipocyte size<br>↓ Plasma insulin, ↔ plasma glucose | C57Bl/6J mice | DIO, *ad libitum* MHF | 13 days |
| | | | 100 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↓ BW | C57Bl/6J mice | DIO, *ad libitum* MHF | 19 & 14 days |
| | | | 30, 100 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↔ BW | C57Bl/6J mice | *Ad libitum* | 14 days |
| | | | 30, 100 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↔ BW | *db/db* mice | *Ad libitum* | 16 days |
| | | | 30, 100 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↔ BW | *db/db* mice | *Ad libitum* MHF | 16 days |
| | | Mashiko *et al.*, 2007 | 30, 100 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↔ BW | Zucker *fa/fa* rats | *Ad libitum* | 14 days |

## Table 4

*Continued.*

| | Antagonist | Reference | Dose | Route | Significant effects | Organism | Condition | Duration |
|---|---|---|---|---|---|---|---|---|
| | | Mashiko *et al.*, 2007 | 100 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↓ BW, feeding, ↓ fat pad<br>↓ Plasma leptin, insulin<br>↓ Total cholesterol, non HDL-cholesterol<br>↓ Rectal temperature, plasma T4<br>↓BAT UCP1, UCP3<br>↓ WAT UCP3, B3AR, SREBP-1c<br>↓ Liver SREBP-1c, Dio1 | C57Bl/6J mice | DIO, *ad libitum* MHF | 30 days |
| | | Sato *et al.*, 2008 | 150 mg·kg$^{-1}$ | p.o. | ↔ Psychomotor activities, motor activities, muscle tone, CNS excitation, autonomic responses, reflexes | Sprague-Dawley rats | *Ad libitum* | Not mentioned |
| | | | 10, 30 mg·kg$^{-1}$ | p.o. | d.d. ↓ feeding | Sprague-Dawley rats | Agonist 2-induced | 2 h |
| 5 | FMS586 | Kakui *et al.*, 2006 | 25, 50, 100 mg·kg$^{-1}$ | p.o. | d.d. ↓ feeding | Wistar rats | NPY-induced | 1, 2, 4 h |
| | | | 25, 50, 100 mg·kg$^{-1}$ | p.o. | ↓ Feeding | Wistar rats | PP-induced | 1, 2, 4 h |
| | | | 50, 100 mg·kg$^{-1}$ | p.o. | Transient ↓ feeding | Wistar rats | Fasting-induced | 1, 2, 4 h |
| | | | 100 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↓ Feeding, BW | Wistar rats | *Ad libitum* | 4 days |
| | | Kakui and Kitamura, 2007 | 25 mg·kg$^{-1}$ | p.o. | ↓ Plasma ACTH & CORT | Fisher rats | Agonist 3-induced | 2 h |
| 6 | Spironolactone Y5 antagonist | Takahashi *et al.*, 2009 | 3, 10 mg·kg$^{-1}$ | p.o. | d.d. ↓ feeding | Sprague-Dawley rats | PP-induced | 2 h |
| | | | 10 mg·kg$^{-1}$ | p.o. | ↔ Feeding | Sprague-Dawley rats | NPY-induced | 2 h |
| | | Mashiko *et al.*, 2008 | 10, 30 mg·kg$^{-1}$·day$^{-1}$ | p.o. | d.d. ↓ BW | C57Bl/6J mice | DIO, *ad libitum* MHF | 35 days |
| | | | 30 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↓ BW, ↓ fat mass,<br>↑ Insulin responsiveness ↓ plasma insulin | C57Bl/6J mice | DIO, *ad libitum* HF | 42 days |
| | | | 30 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↓↓ BW, ↓ fat mass | C57Bl/6J mice | DIO, MHF + food restriction | 49 days |
| | | | 30 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↓↓ BW, fat mass, transient ↓ feeding | C57Bl/6J mice | DIO, *ad libitum* MHF + Sibutramine | 56 days |
| | | Moriya *et al.*, 2009 | 10 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↓ BW | C57Bl/6J mice | Agonist 2-induced | 7 days |
| | | | 10 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↓ Feeding | C57Bl/6J mice | Agonist 2-induced | 14 days |
| | | | 10 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↔ BW, feeding | C57Bl/6J mice | *Ad libitum* | 7 & 14 days |
| | | | 10 mg·kg$^{-1}$ | p.o. | ↓ Feeding | C57Bl/6J mice | *Ad libitum* + i.p. PYY3-36 | 2, 4 h |
| | | | 10 mg·kg$^{-1}$ | p.o. | ↓ Feeding | C57Bl/6J mice | DIO, *ad libitum* MHF + i.p. PYY3-36 | 4 h |
| | | | 10 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↓↓ BW, feeding, fat mass,<br>↓ Plasma insulin | C57Bl/6J mice | DIO, *ad libitum* MHF + i.p. PYY3-36 | 15 days |

## Table 4

*Continued.*

| | Antagonist | Reference | Dose | Route | Significant effects | Organism | Condition | Duration |
|---|---|---|---|---|---|---|---|---|
| | | Mashiko *et al.*, 2009 | 30 mg·kg$^{-1}$ | p.o. | ↓↓ BW, feeding | C57Bl/6J mice | DIO, *ad libitum* HF + i.p. J-115814 | 24 h |
| | | | 30 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↓↓ BW | Y1$^{-/-}$ mice (C57Bl/6J) | DIO, *Ad libitum* HF + i.p. J-115814 | 21 days |
| 7 | MK-0557 | Erondu *et al.*, 2006 | 30 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↓ BW, fat pad, feeding, plasma leptin | C57Bl/6J mice | *Ad libitum* MHF | 35 days |
| | | | 1, 3, 10 mg·kg$^{-1}$·day$^{-1}$ | p.o. | d.d. ↓ BW, feeding | C57Bl/6J mice | Agonist 2-induced | 7 days |
| | | | 0.2, 1, 5, 25 mg·day$^{-1}$ | p.o. | ↓ BW (–0.6, –1.3, –1.9, –1.7 & –2 kg) | Human | Overweight & obese + 6 weeks diet & exercise | 12 weeks |
| | | | 1 mg·day$^{-1}$ | p.o. | ↓ BW (MK-0557 = –3.4 kg vs. placebo = –1.8) | Human | Overweight & obese + 2 weeks diet & exercise | 52 weeks |
| | | Erondu *et al.*, 2007 | 1 mg·day$^{-1}$ | | ↓ BW regain following 6 weeks of VLCD (MK-0557 = +1.5 kg vs. placebo = +3.1) | Human | Overweight & obese + hypocaloric diet | 52 weeks |
| 8 | S-2367 (Velneperit) | Yukioka *et al.*, 2006 | 12.5, 25, 50 mg·kg$^{-1}$ | p.o. | ↓ feeding | Rats | NPY-induced | 1 h |
| | | | 3.1, 6.3, 12.5, 25, 50 mg·kg$^{-1}$ | p.o. | d.d. ↓ feeding | Rats | Agonist 3-induced DIO, *Ad libitum* | 4 h |
| | | | 25 mg·kg$^{-1}$ × 2·day$^{-1}$ | p.o. | ↓ BW, WATr, WATm<br>↓ Liver triglyceride | C57Bl/6J | DIO, *ad libitum* | 56 days |
| | | | 100 mg·kg$^{-1}$ × 2·day$^{-1}$ | p.o. | ↓ BW, WATe, WATr, WATm<br>↓ Liver triglyceride, serum leptin<br>↑ $O_2$ consumption | C57Bl/6J | DIO, *ad libitum* | 56 days |
| | | | 25 mg·kg$^{-1}$ × 2·day$^{-1}$ | p.o. | ↓ BW, FFA,<br>↑ Glucose tolerance, ↑ O2 consumption | *ob/ob* mice | DIO, *ad libitum* | 28 days |
| | | | 100 mg·kg$^{-1}$ × 2·day$^{-1}$ | p.o. | ↓ BW, FFA, liver TG<br>↓ Plasma glucose, FFA, TG,<br>↓ Feeding, ↑ O2 consumption | *ob/ob* mice | DIO, *ad libitum* | 28 days |
| | | Shimazaki *et al.*, 2007 | 50 mg·kg$^{-1}$ × 2·day$^{-1}$ | p.o. | ↓ BW | C57Bl/6J mice | DIO, 85% restriction | 35 days |
| | | | 100 mg·kg$^{-1}$ | p.o. | ↓ Feeding | C57Bl/6J mice | Agonist 3-induced | 4 h |
| | | | | | ↑ $O_2$ consumption | | | 5 h |
| | | | | | ↑ BAT UCP-1 | | | 8 h |
| | | Heshka *et al.*, 2006 | 400 mg·day$^{-1}$ | p.o. | ↔ BW | Human | Obese + 500 kcal·day$^{-1}$ caloric restriction | 12 weeks |
| | | | 1600 mg·day$^{-1}$ | p.o. | ↓ BW<br>(S-2367 = –3.6 kg vs. placebo = –2.4 kg) | Human | Obese + 500 kcal·day$^{-1}$ caloric restriction | 12 weeks |
| | | | 400 mg·day$^{-1}$ | p.o. | ↓ BW, waist circumference<br>(exact figures not disclosed in poster) | Human | Obese + 900–950 kcal·day$^{-1}$ for 4 weeks before treatment | 16 weeks |
| | | | 1600 mg·day$^{-1}$ | p.o. | ↓ BW, waist circumference<br>(S-2367 = –5.9 kg vs. placebo = –2.5 kg) | Human | Obese + 900–950 kcal·day$^{-1}$ for 4 weeks before treatment | 16 weeks |

## Table 4

*Continued.*

| | Antagonist | Reference | Dose | Route | Significant effects | Organism | Condition | Duration |
|---|---|---|---|---|---|---|---|---|
| | | Puopolo *et al.*, 2009 | 800 mg·day$^{-1}$ | p.o. | ↓ BW, waist & hip circumference (S-2367 = –4.60 kg vs. placebo = –1.19 kg) | Human | Obese + 800 kcal·day$^{-1}$ caloric restriction | 54 weeks |
| | | | 1600 mg·day$^{-1}$ | p.o. | ↓ BW, waist & hip circumference (S-2367 = –3.87 kg vs. placebo = –1.19 kg) | Human | Obese + 800 kcal·day$^{-1}$ caloric restriction | 54 weeks |
| | | Smith *et al.*, 2009 | 1600 mg·day$^{-1}$ | p.o. | ↓ BW, waist & hip circumference (S-2367 = –9.0 kg vs. placebo = –4.9 kg) | Human | Obese + 900–950 kcal·day$^{-1}$ for 6 weeks before treatment | 60 weeks |
| | | | 1600 mg·day$^{-1}$ | p.o. | ↓ BW (S-2367 = –6.9 kg vs. placebo = –1.19 kg) | Human | Obese + 900–950 kcal·day$^{-1}$ for 6 weeks at commencement of treatment | 60 weeks |
| 9 | Lu AA33810 | Walker *et al.*, 2009 | 3, 10, 30 mg·kg$^{-1}$ | p.o. | d.d. ↓ feeding | Sprague-Dawley rats | Agonist 3-induced | 6 h |
| | | | 30 mg·kg$^{-1}$ | p.o. | ↓ Plasma ACTH, CORT | Sprague-Dawley rats | Agonist 3-induced | 1 h |
| | | | 30 mg·kg$^{-1}$ | p.o. | ↔ Feeding | Sprague-Dawley rats | NPY-induced | 1 h |
| 10 | Arylpyrazole Y5 antagonist | Sato *et al.*, 2003 | 100 µg | i.c.v. | ↓ Feeding | Sprague-Dawley rats | PP-induced | 2 h |
| | | | 10, 30 mg·kg$^{-1}$ | p.o. | d.d. ↓ feeding | Sprague-Dawley rats | PP-induced | 2 h |
| | | | 100 µg | i.c.v. | ↔ Feeding | Sprague-Dawley rats | NPY-induced | 2 h |
| 11 | Imidazoline Y5 antagonist (2a) | Sato *et al.*, 2009 | 0.1, 0.3, 1 mg·kg$^{-1}$ | p.o. | d.d. ↓ feeding | Sprague-Dawley rats | Agonist 2-induced | 2 h |
| | | | 3, 10 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↓ BW | C57Bl/6N mice | DIO, *ad libitum* MHF | 20 days |
| 12 | Spiroindoline urea Y5 | Sakamoto *et al.*, 2009a, 2009b | 10, 30 mg·kg$^{-1}$ | p.o. | ↓ feeding | Sprague-Dawley rats | PP-induced | 2 h |
| | | | 3, 10 mg·kg$^{-1}$ | p.o. | ↓ feeding | Sprague-Dawley rats | PP-induced | 2 h |
| | | | 30 mg·kg$^{-1}$ | p.o. | ↓ BW, feeding | C-57Bl/6J mice | DIO, *Ad libitum* | 13 days |
| 13 | Spirocarbamate Y5 antagonist (23p & 23u) | Leslie *et al.*, 2010 | >3 mg·kg$^{-1}$ | p.o. | d.d. ↓ feeding | Rats | Agonist 3-induced | Not mentioned |
| 14 | Ureido Y5 antagonist (21) | Li *et al.*, 2008 | Not mentioned | p.o. | ↓ Feeding | Sprague-Dawley rats | Agonist 2-induced | Not mentioned |
| | | | 10 mg·kg$^{-1}$·day$^{-1}$ | p.o. | ↓ Feeding | Sprague-Dawley rats | *Ad libitum* HF | 28 days |
| | | | 1, 3, 10 mg·kg$^{-1}$·day$^{-1}$ | p.o. | d.d. ↓ BW | Sprague-Dawley rats | *Ad libitum* HF | 28 days |
| | | | 3, 10 mg·kg$^{-1}$·day$^{-1}$ | p.o. | d.d. ↓ fat mass | Sprague-Dawley rats | *Ad libitum* HF | 28 days |
| 15 | Benzimidazole Y5 antagonist (9b) | Pizzi *et al.*, 2010 | >3.0 mg·kg$^{-1}$ | p.o. | d.d. ↓ feeding | Rats | Agonist 3-induced | Not mentioned |
| 16 | Carboxamide Y5 antagonist (21j) | Haga *et al.*, 2009 | 1, 3, 10 mg·kg$^{-1}$ | p.o. | d.d. ↓ feeding | Sprague-Dawley rats | Agonist 2-induced | Not mentioned |
| | | | 10 mg·kg$^{-1}$ | p.o. | 22% ↓ feeding (N.S.) | Sprague-Dawley rats | NPY-induced | Not mentioned |

ACTH, adrenocorticotropic hormone; BW, body weight; CORT, corticosterone; d.d., dose-dependent; DIO, diet-induced obese; FFA, free fatty acid; HDL, high-density lipoprotein; HF, high-fat diet; i.c.v., intracerebroventricular; i.p., intraperitoneal; MHF, moderately high fat; NPY, neuropeptide Y; p.o., per oral; TG, triglyceride.

*et al.*, 2000). Intracerebroventricular administration of this compound with rats led to a greater increase in feeding relative to a similar dose of NPY, and – unlike the shorter-lived effect of NPY – the effect lasted for up to 24 h (Cabrele *et al.*, 2000). Evidence suggests that [cPP$^{1-7}$, NPY$^{19-23}$, Ala$^{31}$, Aib$^{32}$, Gln$^{34}$]hPP is specific for Y5, as it does not bind to human embryonic kidney (HEK)293 cells that had been transfected with human Y1, Y2 and Y4 receptor genes. Competitive binding assays in rat brain sections showed high level specific binding of [cPP$^{1-7}$, NPY$^{19-23}$, Ala$^{31}$, Aib$^{32}$, Gln$^{34}$]hPP in the lateral septum and area postrema (Dumont *et al.*, 2004), areas which had previously been reported to express Y5 receptors (Dumont *et al.*, 1998). The function of this particular NPY/PP chimeric analogue was also evaluated in guinea pig (Lecklin *et al.*, 2003). Competitive binding assay showed that [cPP$^{1-7}$, NPY$^{19-23}$, Ala$^{31}$, Aib$^{32}$, Gln$^{34}$]hPP binds to Y5 receptors in preference to Y1 and Y2 receptors, while its potency towards guinea pig Y4 receptors was not evaluated. Central administration of [cPP$^{1-7}$, NPY$^{19-23}$, Ala$^{31}$, Aib$^{32}$, Gln$^{34}$]hPP to guinea pigs stimulated feeding for up to 4 h post-injection, demonstrating a twofold potency relative to that of NPY (Lecklin *et al.*, 2003). Taken together, these reports demonstrate that [D-Trp$^{34}$]NPY] and [cPP$^{1-7}$, NPY$^{19-23}$, Ala$^{31}$, Aib$^{32}$, Gln$^{34}$]hPP are selective and potent ligands for Y5 receptors and are a useful tool for characterizing the pharmacology and functional role of the Y5 receptors. It must be noted, however, that the efficacy of these two compounds was in part established based on competitive binding assay or functional testing against CGP 71683A (Parker *et al.*, 2000; Dumont *et al.*, 2004), a compound that had previously been thought to target Y5 receptors (Criscione *et al.*, 1998) but which has since been demonstrated to act on other receptors relevant in the pathophysiology of obesity as described below (Della Zuana *et al.*, 2001).

## *Antagonists (Table 4)*

*CGP 71683A.* Criscione and colleagues described one of the first potent non-peptidic Y5 receptor antagonists, CGP 71683A (Criscione *et al.*, 1998). *In vitro* assays showed that this compound dose-dependently inhibited the effect of NPY on intracellular calcium concentrations, with the effect of NPY being completely abolished at a concentration of 50 nM CGP 71683A (Criscione *et al.*, 1998). Consistent with *in vitro* results, food intake in both satiated and food-deprived lean rats as well as in streptozotocin-induced diabetic rats was attenuated for up to 24 h following intraperitoneal administration of CG71683A (Criscione *et al.*, 1998). Additionally, acute and chronic central administration of this compound attenuated NPY-induced feeding in Wistar rats and ameliorated the hyperphagia of genetically obese Zucker *fa/fa* rats respectively (Della Zuana *et al.*, 2001). Daily administration of 10 mg·kg$^{-1}$ of CGP 71683A for 28 days produced a marked reduction in plasma triglyceride concentrations and a pronounced reduction in body weight in the absence of sustained inhibition of food intake (Criscione *et al.*, 1998), suggesting that this non-peptidic compound influences energy balance via mechanisms other than the regulation of appetite. It must be noted, however, that while CGP 71683A demonstrated a higher affinity for Y5 receptors ($K_i$ = 1.4 nM) than for Y1, Y2 and Y4 receptors, it displayed comparable affinity for muscarinic receptors ($K_i$ = 2.7 nM) and the serotonin (5HT) re-uptake recognition site ($K_i$ = 6.2 nM) and a low but significant affinity for the adrenergic α2 receptor ($K_i$ = 15.0 nM) in rat brain, implicating the interaction of this compound with cholinergic, serotoniergic and adrenergic pathways (Della Zuana *et al.*, 2001). Lastly, investigation of CGP 71683A-treated brains showed dose-related inflammation and necrosis, providing evidence of toxicity (Della Zuana *et al.*, 2001) that may lead to the production of pro-inflammatory chemokines, some of which are potent feeding-inhibitors (reviewed by Callewaere *et al.*, 2007). Taken together, these findings suggest that CGP 71683A may not be a suitable lead agent for the clinical management of obesity, and its utility in validating potent Y5-selective compounds may need to be reassessed (Parker *et al.*, 2000; Dumont *et al.*, 2004).

*NPY5R-972.* NPY5R-972 is an orally available Y5 receptor antagonist with negligible affinity for Y1, Y2 and Y4 receptors and that can access the central nervous system (Block *et al.*, 2002; Turnbull *et al.*, 2002). NPY5R-972 antagonized NPY-induced inhibition of forskolin-stimulated cAMP production in HEK293 cells stably expressing the rat Y5 receptor (Turnbull *et al.*, 2002). Whereas acute oral administration of the compound abolished feeding induced by the Y5-selective agonist, [cPP$^{1-7}$, NPY$^{19-23}$, Ala$^{31}$, Aib$^{32}$, Gln$^{34}$]hPP (Cabrele *et al.*, 2000), it was ineffective at inhibiting NPY- and fasting-induced hyperphagia in lean Wistar rats and at inhibiting feeding in satiated obese *fa/fa* rats monitored for up to 24 h. Additionally, chronic administration of NPY5R-972 did not enhance the anti-obesity effect of sibutramine on Wistar rats maintained on a standard or a palatable high-fat diet (Turnbull *et al.*, 2002), revealing limited use of this compound as an experimental or therapeutic lead.

*GW438014A.* In contrast to NPY5R-972, GW438014A significantly inhibited spontaneous as well as overnight fasting-induced and NPY-induced feeding in rats and in food-deprived genetically obese *ob/ob* mice when administrated intraperitoneally. Unfortunately, the anorexigenic property of GW438014A was abolished when administered orally, indicating its poor oral bioavailability (Daniels *et al.*, 2002). Chronic intraperitoneal administration of GW438014A produced marked reductions in body weight and body weight gain in obese (*fa/fa*) and lean (*Fa/Fa* or *Fa/fa*) Zucker rats, and these changes persisted for up to two days after cessation of the drug treatment (Daniels *et al.*, 2002). Relative to CGP 71673A and several other compounds described in this section, GW438014A showed reduced affinity not only for Y1, Y2 and Y4 receptors, but also for Y5 receptors. Despite this, cAMP assays in human endometrial cancer (HEC-1B) stably expressing Y5 revealed that GW438014A is a potent antagonist of Y5 receptor signalling (Daniels *et al.*, 2002).

*L-152,804.* L-152,804 is a xanthen Y5 receptor antagonist (Sato *et al.*, 2008) that has been demonstrated to readily displace 125I-PYY-specific binding to human and rat Y5 receptors *in vitro*, with $K_i$ values of 26 and 31 nM, respectively, and to dose-dependently inhibit NPY-induced increases in intracellular calcium levels in LMtk cells expressing human Y5 receptors, confirming the antagonistic action of L-152,804 at

Y5 receptors (Kanatani *et al.*, 2000a). Oral administration of L-152,804 to rats led to time- and dose-dependant increases in Y5 receptor occupancy in the brain, indicating that L-152,804 is an orally available brain permeable compound (Ishihara *et al.*, 2006). Additionally, oral administration of 10–30 μg·kg$^{-1}$ L-152,804 led to dose-dependant inhibition of [D-Trp34]NPY-induced hyperphagia in Sprague-Dawley rats (Sato *et al.*, 2008), further implicating antagonism of Y5 receptors. Interestingly, centrally (30 μg) and orally (10 μg) administered L-152,804 was able to inhibit PP- but not NPY-stimulated feeding in satiated rats (Kanatani *et al.*, 2000a), suggestive of distinctive binding or activating sites for NPY and [D-Trp34]NPY on the Y5 receptors.

In mice, the effect of L-152,804 on body weight was specifically mediated by Y5 receptors because repeated oral administration significantly reduced body weight of wild-type but not of Y5$^{-/-}$ mice maintained under a moderately-high fat diet (Ishihara *et al.*, 2006). Interestingly, L-152,804 suppressed body weight and adipose tissue gain in a dose-dependant manner in diet-induced obese mice in association with reduced caloric intake of up to 10%, but had no effect on the body weight of lean and genetically obese *db/db* mice and *fa/fa* rats (Ishihara *et al.*, 2006; Mashiko *et al.*, 2007; Sato *et al.*, 2008), demonstrating that this compound is not universally effective at reducing energy balance under all circumstances.

Evidence suggests that the observed anti-obesogenic effects of L-152,804 is achieved through modulation of both appetite and energy expenditure (Mashiko *et al.*, 2007). For instance, mice maintained on 90% of their normal daily food intake by pair-feeding to the reduced food intake of L-152,804-treated mice showed less body weight loss and lower body temperatures relative to L-152,804-administered animals (Mashiko *et al.*, 2007), revealing effects of this compound to reduce energy efficiency and possibly also to stimulate thermogenesis. In keeping with this, L-152,804-treated mice showed up-regulation of uncoupling protein-1 and uncoupling protein-3 expression in brown adipose tissue, a key regulator in thermogenesis, and no effect of the compound on body temperature was reported in that study (Mashiko *et al.*, 2007). These results suggest that the negative energy balance induced by Y5 antagonism with L152,804 was not compensated for by a suppression in thermogenesis as is observed in pair-fed control mice, and that energy expenditure may be elevated in response to L152,804 administration. These results are in line with the opposite effects of Y5 receptor agonism, which has been shown to reduce energy expenditure (Hwa *et al.*, 1999; Mashiko *et al.*, 2003).

*FMS586.* FMS586 is an orally active and brain-permeable Y5 receptor antagonist (Kakui *et al.*, 2006). At 25 and 50 mg·kg$^{-1}$ doses, maximal drug concentrations were detected in plasma and brain at 1 h and remained stable for up to 4 h following administration (Kakui *et al.*, 2006). Selectivity for Y5 was demonstrated by a significantly higher preference for the human Y5 receptor relative to Y1 and Y2 receptors, while affinity for the Y4 receptor was not evaluated (Kakui *et al.*, 2006). FMS586 significantly inhibited forskolin-induced cAMP accumulation and dose-dependently reversed NPY-induced cAMP accumulation *in vitro* (Kakui *et al.*, 2006). The specificity of FMS586 for the Y5 receptor was further demonstrated by immunohistochemistry, where application of FMS586 significantly inhibited PP-induced neuronal activation in Y5-positive neurons, as indicated by reduced c-Fos immunostaining when compared with vehicle-treated controls (Kakui *et al.*, 2006). *In vivo*, acute oral administration of FMS586 to rats potently suppressed NPY-, hPP- and fasting-induced food intake for up to 4 h. Whereas a maximum dose of 100 mg·kg$^{-1}$ FMS586 has no substantial effect on changes in feeding induced by subcutaneous injection of norepinephrine, galanin or the GABA receptor agonist muscimol, repeated daily administration of FMS586 for 4 days dose-dependently inhibited feeding and body weight in rats maintained on an *ad libitum* chow diet (Kakui *et al.*, 2006). This finding further highlights the specificity of FMS586 in regulating feeding via effects on the NPY-ergic system.

*Spironolactone Y5 antagonist.* Given the inconsistent anti-obesogenic effects of Y5 receptor antagonists in several models of rodent obesity as described above (Kanatani *et al.*, 2000a; Turnbull *et al.*, 2002; Ishihara *et al.*, 2006; Mashiko *et al.*, 2007), the effect of a structurally distinct Y5 antagonist was evaluated (Mashiko *et al.*, 2008; 2009; Moriya *et al.*, 2009; Takahashi *et al.*, 2009). The spironolactone Y5 antagonist showed specific binding to human and mouse Y5 receptors in preference to human Y1, Y2 and Y4 receptors (Mashiko *et al.*, 2008). The compound also dose-dependently inhibited NPY-induced increases in intracellular calcium concentrations in Y5-expressing cells, indicating antagonistic properties at Y5 receptors (Mashiko *et al.*, 2008; Takahashi *et al.*, 2009). Daily oral administration of the spironolactone Y5 antagonist to diet-induced obese wild-type C57Bl/6J mice for 1 month led to a significant reduction in body weight, mainly attributable to reduced fat mass, but this effect was not seen in diet-induced obese Y5$^{-/-}$ mice, demonstrating specific action of the compound through Y5 receptors (Mashiko *et al.*, 2008). In contrast to L-152,804, FMS586, NPY5R-972 and GW438014A, the anti-obesity effect of chronic spironolactone Y5 antagonist administration is apparently not mediated through suppression of appetite in diet-induced obese mice (Mashiko *et al.*, 2008).

Further evidence of metabolic effects of this compound is that fact that 10% feeding restriction alone led to a significant reduction in body weight and adiposity when compared with vehicle-treated DIO control groups, but not to the same extend as the compound-treated group (Mashiko *et al.*, 2008). In addition to its effects on body weight and adiposity, the spironolactone Y5 antagonist ameliorated the elevated fasting plasma insulin and impaired responsiveness to intraperiotoneally administered of diet-induced obese mice, effects that were not achieved through feeding restriction alone (Mashiko *et al.*, 2008). Moreover, the anti-obesity effect of the spironolactone Y5 antagonist was enhanced by co-administration of sibutramine, a centrally acting anorexinogen (Luque and Rey, 2002), or in combination with food restriction (Mashiko *et al.*, 2008), raising the possibility that combination of this type of Y5 receptor antagonism with other pharmacological and lifestyle interventions could offer significant benefits for the treatment of obesity.

Simultaneous agonism and antagonism of Y2 and Y5 receptors via intraperitoneal and oral administration of PYY3-36 and the spironolactone Y5 antagonist, respectively,

led to a greater reduction in body weight and fat loss in association with significant reductions in food intake in diet-induced obese mice when compared with the effect of either compound administered individually (Moriya *et al.*, 2009). Because intraperitoneal administration of PYY3-36 alone has been shown to attenuate food intake and promote fat metabolism without affecting overall energy expenditure (Adams *et al.*, 2006; van den Hoek *et al.*, 2007; Parkinson *et al.*, 2008), it was proposed that Y5 antagonism with the spironolactone compound promoted long-term weight loss through distinct modulations of energy intake and expenditures (Moriya *et al.*, 2009).

Similarly to the promising effects of simultaneous Y2 and Y5 receptor modulation, the simultaneous antagonism of Y1 and Y5 receptors using a combination of the Y1 receptor antagonist, J-115814 and the spironolactone Y5 antagonist enhanced the effect of the latter to attenuate food intake and reduce body weight after acute oral administration to diet-induced obese mice (Mashiko *et al.*, 2009). Additionally, oral administration of the spironolactone Y5 antagonist to $Y1^{-/-}$ mice led to a greater reduction in body weight than that seen in vehicle-treated wild-type mice or mice with genetic ablation or pharmacological blockade of either the Y1 or Y5 receptor alone (Mashiko *et al.*, 2009). Taken together, these findings highlight the coordinate role of Y1 and Y5 receptors in the regulation of energy homeostasis (Marsh *et al.*, 1998; Kanatani *et al.*, 2000b), and demonstrate the potential of combination therapy in achieving greater anti-obesity effects (Mashiko *et al.*, 2009).

*MK-0557.* MK-0557, an orally available Y5 antagonist, was demonstrated to suppress high fat diet-induced and Y5 agonist-induced obesity in mice after chronic oral administration (Erondu *et al.*, 2006). This reduction in body weight gain and adiposity in response to a daily 30 $mg \cdot kg^{-1}$ dose of orally administered MK-0577 for 7 days was accompanied by a reduction in feeding behaviour. Plasma leptin levels were significantly lower in treated animals when compared with vehicle-administered control mice (Erondu *et al.*, 2006), highlighting the effect of this Y5 antagonist to reduce adiposity. These results, along with the widely demonstrated anti-obesity potential of Y5 receptor antagonism shown in other studies, prompted clinical investigation of MK-0557 in humans (Erondu *et al.*, 2006). Disappointingly, however, in a cohort of 1661 overweight and obese individuals, a daily dose of 1 mg of MK-0557 over 52 weeks did not produce clinically meaningful weight loss (Erondu *et al.*, 2006). Similarly, MK-0557 treatment in combination with a very low energy diet (300 $kcal \cdot day^{-1}$) in non-diabetic obese men and women failed to produce any meaningful effect on weight loss over and above the effects of energy restriction *per se* (Erondu *et al.*, 2007).

*S-2367 (Velneperit).* S-2367 (Velneperit) is a cyclohexanecarboxamide-derived Y5 receptor antagonist discovered by Shionogi & Co in Osaka, Japan. S-2367 displayed a significant preference for the Y5 receptor ($K_i$ = 4.84 nM) over Y1 ($K_i$ > 12 000 nM), Y2 ($K_i$ > 10 700 nM), Y4 ($K_i$ > 21 200 nM) and 79 other receptors and 28 enzymes assayed (Yukioka *et al.*, 2006). *In vitro*, cAMP assay in cells stably expressing human Y5 receptors showed S-2367 to be a strong antagonist of NPY with an EC50 value of 10.6 nM (Yukioka *et al.*, 2006).

*In vivo*, oral administration of a single dose of S-2367 to rats acutely inhibited NPY- and Y5 agonist-induced hyperphagia at minimum doses of 12.5 and 3.1 $mg \cdot kg^{-1}$ respectively (Yukioka *et al.*, 2006). At a higher dose of 100 $mg \cdot kg^{-1}$, S-2367 administered orally or i.p. did not produce a decrease in saccharin preference, indicating that the anorectic effect of this compound is not due to taste aversion (Yukioka *et al.*, 2006).

Chronic administration of S-2367 to high fat-fed wild-type mice for 8 weeks and to *ob/ob* mice for 4 weeks produced significant dose-dependent attenuations in body weight gain (Yukioka *et al.*, 2006). Diet-induced obese wild-type mice treated with S-2367 showed a pronounced reduction in adiposity and serum leptin level, while liver triglyceride content was significantly lowered in mice given the higher dose of 100 $mg \cdot kg^{-1}$ S-2367 twice daily (Yukioka *et al.*, 2006). Similarly, this dose of S-2367 significantly reduced plasma glucose and free fatty acid levels, and reduced plasma and liver triglyceride levels in diet-induced obese and *ob/ob* mice (Yukioka *et al.*, 2006). Chronically treating diet-induced obese and *ob/ob* mice with a twice-daily dose of 25 $mg \cdot kg^{-1}$ S-2367 also led to significant improvements in glucose tolerance (Yukioka *et al.*, 2006). In a separate study, treating wild-type mice on a high fat diet with S-2367 for 35 days, in combination with 85% caloric restriction, significantly suppressed body weight gain during the treatment period compared with vehicle-treated controls (Shimazaki *et al.*, 2007), demonstrating the potential for sustained effectiveness of S-2367 on body weight.

While S-2367 demonstrated a potent and acute appetite-suppressant property (Yukioka *et al.*, 2006), the anti-obesity effects of S-2367 in these chronic settings appeared to be primarily mediated through enhancement of metabolic rate. Indeed, chronic administration of S-2367 led to a significant elevation in oxygen consumption in both wild-type and *ob/ob* mice (Yukioka *et al.*, 2006; Shimazaki *et al.*, 2007), but a reduction in energy intake was only reported in the S-2367-treated *ob/ob* mice (Yukioka *et al.*, 2006). In keeping with likely mediation of effects on body weight via altered metabolism, the 100 $mg \cdot kg^{-1}$ dose of S-2367 significantly suppressed Y5 agonist-induced feeding in wild-type mice for 4 h, but did not completely inhibit it (Shimazaki *et al.*, 2007). On the other hand, the same dose of S-2367 restored Y5 agonist-induced reduction in oxygen consumption and UCP-1 gene expression in the brown adipose tissue to basal values comparable to that of vehicle-treated controls (Shimazaki *et al.*, 2007). The effects of S-2367 in promoting energy expenditure are in line with the effects of previously described Y5 antagonists, L152,804 (Mashiko *et al.*, 2007).

It is interesting to note that *ob/ob* mice fed a high fat diet showed a greater inhibition in body weight gain during 4 weeks of S-2367 administration when compared with similar doses and duration of treatment in diet-induced obese wild-type mice (Yukioka *et al.*, 2006). While this difference could be due to a greater predisposition to body weight gain in the genetically obese mice relative to wild-type animals, it is also likely that the efficacy of S-2367 may be enhanced in conditions of elevated hypothalamic NPY expression levels, as in *ob/ob* mice (Schwartz *et al.*, 1996).

The promising outcomes of these animal trials prompted investigation into the efficacy and safety of S-2367 in a clinical setting. Firstly, S-2367 appeared to be well tolerated with no significant safety issue in three separate clinical studies involving medically stable and healthy obese subjects (Heshka *et al.*, 2006; Puopolo *et al.*, 2009; Smith *et al.*, 2009). Despite this, two studies reported a small and statistically insignificant reduction in the levels of erythrocyte count, haemoglobin and haematocrit in a total sample size of 656 and 771 subjects respectively (Puopolo *et al.*, 2009; Smith *et al.*, 2009). While no patients shifted from normal to low ranges of these haematology parameters, this finding may prompt concern for potential risk of anaemia and may require further investigation.

Administration of 1600 but not 400 mg·day$^{-1}$ S-2367 to obese people for 12 weeks, in combination with 500 kcal·day$^{-1}$ energy deficit, resulted in a small but statistically significant reduction in body weight when compared with the placebo-treated control group (Heshka *et al.*, 2006). Similarly, 800 and 1600 mg·kg$^{-1}$ daily doses of S-2367, in combination with an 800 kcal·day$^{-1}$ energy deficit for 54 weeks led to a significantly greater reduction in body weight, waist and hip circumference when compared with placebo-treated controls (Puopolo *et al.*, 2009). Interestingly, the 800 and 1600 mg·day$^{-1}$ doses produced comparable weight loss results, with a trend for greater weight loss in subjects treated with the lower dose, indicating that 800 mg·day$^{-1}$ may be the maximum effective dose for this compound. In addition, whereas the 400 mg·day$^{-1}$ dose was ineffective at reducing body weight when administration was commenced at the same time as caloric restriction, the same dose given to patients who underwent an initial 4 weeks on a low calorie diet before commencing 12 weeks on S-2367 treatment led to a significantly greater body weight loss and reduced waist circumference when compared with the placebo-treated control group. This effect of S-2367 to significantly promote ongoing weight loss in people who had already undergone a period of energy restriction appeared to be dose-dependent, because the 1600 mg·day$^{-1}$ dose led to a further reduction in body weight when compared with the lower dose (Heshka *et al.*, 2006).

The results from preclinical and clinical studies demonstrated that the effect of S-2367 is enhanced in animal models and in human where NPY level is expected to be elevated, such as in *ob/ob* mice and following and/or during caloric restriction. However, the average body weight loss in subjects receiving S-2367 after an initial 4 week period on the low calorie diet (5.9 kg) was greater than the weight loss of subjects receiving a similar dose of S-2367 but who continued to follow a diet with a 500 kcal·day$^{-1}$ (3.6 kg) (Heshka *et al.*, 2006) or an 800 kcal·day$^{-1}$ deficit (3.87 kg) (Puopolo *et al.*, 2009). This finding indicates a greater weight-reducing effect of S-2367 when administered after, but not in combination with, caloric restriction. Indeed, a separate study demonstrated that while S-2367 treatment, whether given during or following a low calorie diet, led to significantly greater weight losses than those seen in placebo-treated controls, a significantly greater weight reduction was observed in subjects receiving S-2367 for 54 weeks post-caloric restriction than in those who received S-2367 during and following the initial caloric restriction phase (Smith *et al.*, 2009).

Collectively, these results demonstrate a significant benefit of S-2367 in achieving and maintaining weight loss in obese individuals, particularly when administered in the post-restrictive phase of weight loss, and results of further trials are highly anticipated.

*Lu AA33810.* Lu AA33810 demonstrated high affinity to human and rat Y5 receptors relative to human Y1, Y2 and Y4 receptors (Walker *et al.*, 2009). Lu AA33810 dose-dependently antagonized NPY-induced calcium and cAMP mobilization in COS-7 and HEK-293 cells expressing human Y5 receptors (Walker *et al.*, 2009). An oral dose of 3–30 mg·kg$^{-1}$ of Lu AA33810 in rats inhibited feeding induced by 0.6 nmol of the Y5 agonist [cPP$^{1-7}$, NPY$^{19-23}$, Ala$^{31}$, Aib$^{32}$, Gln$^{34}$]hPP, but had no effect on NPY-induced feeding, further suggesting specificity for Y5-mediated hyperphagic effects.

Other effects of Y5 receptor antagonism with Lu AA33810 were evaluated in the context of stress and anxiety, where acute intragastric administration to rats attenuated the increases in plasma concentrations of adrenocorticotropic hormone and corticosterone evoked by Y5 agonism with [cPP$^{1-7}$, NPY$^{19-23}$, Ala$^{31}$, Aib$^{32}$, Gln$^{34}$]hPP. Moreover, acute and chronic administration of Lu AA33810 to Sprague-Dawley and Fischer rats increased social interaction and abolished the stress-induced decrease in sucrose intake in rats undergoing mild chronic stress (Walker *et al.*, 2009). Collectively, these results suggest that Lu AA33810 may be suited for the treatment of obesity, particularly behavioural aspects of obesity related to stress.

*Arylpyrazole, xanthen and imidazoline-derived Y5 antagonists.* Sato and colleagues generated an impressive chemical library of Y5-selective antagonists still undergoing structural modifications, including arylpyrazole (Sato *et al.*, 2003) and xanthen derivaties (Sato *et al.*, 2008) and most recently a series of imidazoline derivaties (Sato *et al.*, 2009). On account of its high brain penetrability and access to plasma after oral administration, compound 2a was selected from a series of imidazoline-derived Y5 antagonists (Sato *et al.*, 2009). Acute oral administration of 2a at a dose of 1 mg·kg$^{-1}$ in Sprague-Dawley rats induced an impressive 93% reduction in the food intake induced by the Y5 agonist D-[Trp34]NPY (Sato *et al.*, 2009). Moreover, repeated four times daily for 5 days followed by twice daily for 15 days oral administration of up to 10 mg·kg$^{-1}$ of 2a to weight stable diet-induced obese mice led to a significant reduction in body weight (3 g on average) in wild-type but not in Y5$^{-/-}$ mice, despite comparable plasma levels of the compound in both genotypes. However, compound 2a appeared to have significant cardiovascular effects, as indicated by the elevation in arterial and left ventricular systolic pressure, prompting further structural modification of this compound (Sato *et al.*, 2009). Further work will clarify the potential utility of compound 2a or its derivatives as anti obesity drugs.

*Other novel Y5 antagonists.* In addition to the above-investigated antagonists, several compounds have been identified from ongoing studies in the development of ureido (Li *et al.*, 2008), spiropiperidine (Takahashi *et al.*, 2009), spiroindoline urea (Sakamoto *et al.*, 2009a; 2009b) and

spirocarbamate-derived Y5 receptor antagonists (Leslie *et al.*, 2010). A number of these compounds showed positive results in early preclinical investigations in that they attenuated diet-induced obesity or Y5 agonist-induced hyperphagia in rodents (Li *et al.*, 2008; Takahashi *et al.*, 2009; Sakamoto *et al.*, 2009a; 2009b; Leslie *et al.*, 2010). In particular, repeated oral administration of 21, an ureido Y5 antagonist, led to dose-dependent attenuation of food intake and body weight gain in rats maintained on a high-fat diet (Li *et al.*, 2008). Furthermore, an overall reduction in fat mass was observed in rats given daily doses of 3 and 10 $mg \cdot kg^{-1}$ of compound 21, with no effect on lean body mass (Li *et al.*, 2008). These compounds await further comprehensive animal studies. Similarly, Biagetti *et al.*, (2010), Haga *et al.* (2009) and Pizzi *et al.* (2010) have reported newly synthesized orally active Y5-receptor antagonists with favourable pharmacokinetic profiles and good brain permeability that warrant further testing *in vivo*. A series of insurmountable Y5 receptor antagonists have also recently been synthesized (Mullins *et al.*, 2008) and these await further testing. However, in light of the obesity phenotype of $Y5^{-/-}$ mice (Marsh *et al.*, 1998; Higuchi *et al.*, 2008) absolute and irreversible antagonism of Y5 receptors may not be a suitable avenue for the therapeutic intervention of obesity.

These reports confirm that Y5 receptors play a significant role in modulating appetite and energy homeostasis. However, whereas some Y5-antagonistic compounds are effective at reducing diet-induced obesity (Criscione *et al.*, 1998; Della Zuana *et al.*, 2001; Daniels *et al.*, 2002; Ishihara *et al.*, 2006; Li *et al.*, 2008; Mashiko *et al.*, 2008), others had no such effect (Turnbull *et al.*, 2002). These differences could potentially be attributed to the distinct binding sites of the compounds arising from their structural diversity or distinct receptor binding sites. Additionally, the phenomenon of receptor dimerization, specifically of Y1 and Y5 receptors into Y1-Y5 receptor dimers (Gomes *et al.*, 2000; 2004; Gehlert *et al.*, 2007), may present alternative binding affinities and capacities that were previously unexplored for these receptors. This is particularly important given than much of the testing for novel Y receptor agonists and antagonists has been done with cell lines driven to express only one Y receptor type. Administration of Y5 receptor antagonists in combination with a Y2 receptor agonist, Y1 receptor antagonists or sibutramine led to enhanced anti-obesity effects relative to the effects of these individual agents, suggesting that co-therapies involving transient rather than irreversible or prolonged Y5 receptor antagonism may be an effective strategy for the medicinal management of obesity. As much as providing potential new approaches for anti-obesity therapy, the development and investigation of the Y receptor agonists and antagonists described in this review have and will continue to contribute towards the understanding of Y receptor signalling mechanisms and effects.

## Concluding remarks and future directions

In summary, investigations into the function of the NPY system using both transgenic animal models and pharmacological interventions with naturally occurring and synthetic compounds has revealed NPY and its Y-receptors as important regulators of feeding behaviour and energy homeostasis, making them attractive therapeutic targets for the development of anti-obesity drugs. A number of promising chemical leads that show potent anti-obesity effects have been developed. However, a lack of selectivity, low oral bioavailability, poor brain penetrability or interaction with other receptors, toxicity or lack of long-term effects still hamper the progress of some of these compounds into a clinical setting. Importantly, the major effort so far has been to target central Y-receptors with the aim of decreasing food intake or increasing satiety. However, the redundancy of the systems that controls feeding might compromise the success in long-term treatment. Interestingly, the emerging role of the NPY system in controlling also other aspects of energy homeostasis besides food intake, such as energy expenditure and oxidative fuel selection, may provide the opportunity to target Y receptors that modulate both sides of the energy balance equation for more efficient treatment. Furthermore, the role of peripheral Y-receptors in regulating energy homeostasis and lipid metabolism has not been fully explored. Targeting only peripheral Y-receptors with compounds specifically designed not to cross the blood-brain barrier might also have the advantage of reducing energy balance without modulating behaviour, anxiety and other functions controlled within the central nervous system. It has also become clear that simultaneously targeting more then one Y-receptor may have synergistic effects in combating obesity. However, because potential anti obesity treatments are likely to require long-term administration, caution must be taken such that other important regulatory roles of the NPY system, such as the modulation of bone mass or reproductive functions, do not potentially outweigh any anti-obesity benefits.

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## Conflict of interest

None.

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