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The neuropeptide Y system: Pathophysiological and therapeutic implications in obesity and cancer

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ABSTRACT

The neuropeptide Y (NPY) system – comprising of neuropeptide Y, peptide YY, pancreatic polypeptide and the corresponding Y receptors through which they act (Y1, Y2, Y4, Y5 and y6) – is well known for its role in the regulation of energy homeostasis and associated processes. Dysfunctions of the system have been implicated in human diseases such as obesity and cancer, raising the possibility that correction of the system may provide therapeutic benefits for these diseases. In addition to the regulation of appetite and satiety that has attracted most attention during the past years, insight has also been gained into the critical role of NPY in the control of energy expenditure, oxidative fuel selection and bone metabolism. Studies using conditional knockout models further shed light on the central versus peripheral, and hypothalamic versus extra-hypothalamic mechanisms of these regulatory effects of NPY. Moreover, a role of NPY family peptides and Y receptors in modulating the growth of tumours has emerged. These findings provide the basis for novel NPY system-targeted strategies to treat obesity as well as cancer. Such strategies include modifying both sides of the energy balance equation – energy intake versus energy expenditure – to achieve a greater weight/fat loss by particularly modulating peripheral Y receptor(s) to ameliorate metabolic conditions without interfering with central functions of Y receptors. In addition, targeting multiple Y receptors and/or multiple systems involved in the regulation of energy balance will have greater beneficial effects. However, long-term interference with the NPY system to target obesity or cancer related aspects needs to consider potential side effects on bone health.

Contents

1. Introduction

Neuropeptide Y (NPY), a 36 amino acid peptide and a potent orexigenic agent, is highly evolutionarily conserved and shares high amino acid sequence homology with the previously discovered polypeptide YY (PYY) (70%) and pancreatic peptide (PP) (50%) (Dumont et al., 1992; Cerda-Reverter & Larhammar, 2000). Similarities in primary structure of these peptides correspond to a tertiary structural homology, i.e. a common U-shaped structure consisting of an extended polyproline helix and an α helix connected by a β turn

Abbreviations: AP, area postrema; ARC, arcuate nucleus; BAT, brown adipose tissue; CNS, central nervous system; CPT-1, carnitine palmitoyltransferase-1; ESFT, Ewing's sarcoma family of tumours; ICV, intracerebroventricular; HPT, hypothalamo-pituitary-thyrotropic; HFD, high fat diet; i.p., intraperitoneal; i.v., intravenous; LCD, low calorie diet; MSH, melanocyte-stimulating hormone; MHF, moderately high-fat diet; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; PP, pancreatic peptide; PVN, paraventricular nucleus; PWS, Prader–Willi syndrome; PYY, polypeptide YY; RCD, reduced calorie diet; RER, respiratory exchange ratio; TRH, thyrotropin-releasing hormone.

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(Dumont et al., 1992; Larhammar, 1996; Michel et al., 1998; Cerda-Reverter & Larhammar, 2000). Moreover, evolutionary analysis suggests that NPY and PYY have evolved by duplication of a common ancestral gene in an early vertebrate ancestor, whereas PP gene probably arose by duplication of the PYY gene (Hort et al., 1995; Larhammar, 1996). Based on these sequential, tertiary structural and evolutionary criteria, NPY, PYY and PP have been classified into a family of homologous peptides. NPY is one of the most widely distributed peptides in the central and peripheral nervous system. In the periphery, NPY is found in the sympathetic nervous system, co-stored and co-released with norepinephrine during nerve stimulation (Ekblad et al., 1984). Centrally, NPY exhibits a complex distribution, with the highest expression level within hypothalamic nuclei (Bai et al., 1985). In contrast to NPY's neuronal expression, PYY and PP are gut-derived peptides, with PYY being mainly produced by the endocrine L cells of the colon, rectum, small intestine, stomach and pancreas (Ekblad & Sundler, 2002), and PP being primarily produced in F type cells of the pancreas (Adrian, 1978).

NPY, PYY and PP bind to a family of G protein-coupled Y receptors. At least five receptors have been cloned and classified as Y1, Y2, Y4, Y5 and y6 on the basis of their molecular and pharmacological properties (Blomqvist & Herzog, 1997; Michel et al., 1998; Berglund et al., 2003). In addition, there is evidence suggesting the existence of further unidentified Y receptors (Dumont et al., 2005; Lin et al., 2005). The distribution of Y receptors has been studied extensively at protein and mRNA levels in different species during the last 10 years (Parker et al., 2002; Berglund et al., 2003). Generally all Y receptors are expressed in higher organisms including man, except the y6 receptor that is absent in the rat and only present in a truncated form in the human and primate genome (Blomqvist & Herzog, 1997). All Y receptors are expressed in the brain and in the periphery. In the brain, all Y receptors have been demonstrated in high concentration in regions involved in energy intake and energy expenditure, such as hypothalamus (Fetissov et al., 2004). Furthermore, Y2 receptors are more often found pre-synaptically/pre-junctionally and their activation usually suppresses neurotransmitter release, whereas other Y receptors have mainly post-synaptic/post-junctional expression (Wahlestedt et al., 1986; King et al., 2000; Smith-White et al., 2001). Pharmacologically, NPY and PYY share similar Y receptor binding profiles with greatest affinity for the Y2 receptors, followed by Y1, Y5 and least affinity for Y4 receptors (Blomqvist & Herzog, 1997; Michel et al., 1998). In contrast, PP prefers the Y4 receptor with much lower affinity to all other Y receptors (Blomqvist & Herzog, 1997). Both NPY and PYY can be further processed by a specific protease — dipeptididyl peptidase-IV (DPPIV), which removes the first 2 amino acids from the N-terminus of full length NPY or PYY and produces a shorter form of the peptide, i.e. NPY3-36 or PYY3-36 (Mentlein, 1999; Unniappan et al., 2006). This post-translational modification leads to an altered pharmacological profile, with the affinity of the full-length form to the Y1 receptor being lost in the truncated form. It is important to note, however, that the affinity to Y2 receptors is unaltered by this modification. Thus, whereas resulting in a reduced activation on Y1 receptors, the conversion of NPY or PYY to the truncated forms NPY3-36 or PYY3-36 does not lead to an enhanced activation of Y2 receptors. Functionally, Y receptors have all been shown to mediate their response through pertussis toxin-sensitive G proteins, inhibiting the accumulation of cAMP (Herzog et al., 1992; Larhammar et al., 1992; Bard et al., 1995; Gerald et al., 1995; Lundell et al., 1995; Gerald et al., 1996; Mullins et al., 2000). It has also been shown that Y1, Y2 and Y4 receptors increase intracellular $Ca2^{+}$ concentration (Herzog et al., 1992; Bard et al., 1995; Gerald et al., 1995). Several intracellular signal transduction pathways of Y receptors downstream of their second messages have been reported. For example, a mitogen-activated protein kinase pathway is involved in the Y1 receptor signalling in gut epithelial cells (Mannon & Mele, 2000), whereas a protein kinase C-dependent pathway is involved in Y1, Y2, Y4 and Y5 receptor signalling in Chinese hamster ovary cells (Mullins et al., 2002). Moreover, results from in vivo studies suggest that activation of cAMP responding element binding protein is an important event in the Y receptor signalling cascade that ultimately results in NPY-induced feeding (Sheriff et al., 1997; Chance et al., 2000).

Through their actions on Y receptors, NPY family peptides regulate important physiological processes involved in energy homeostasis including appetite and satiety, energy expenditure, lipid metabolism and insulin secretion (Herzog, 2003; Renshaw & Batterham, 2005; Huda et al., 2006). Due to the initial lack of pharmacological tools, the functions of different Y receptors in vivo have been studied in knockout and transgenic mouse models (Herzog, 2003). Results from germline knockout models however, need to be interpreted with caution, since the NPY and Y receptor systems are known for their complexity and plasticity, and complete ablation of the ligand or Y receptors may cause compensatory changes (Trivedi et al., 2001; Lin et al., 2005; Wittmann et al., 2005). To overcome this, conditional knockout mouse models have been used in recent research and provided important insights into the physiology of the NPY system (Lee et al., 2010b; Shi et al., 2010; Zhang et al., 2010a). Moreover, over recent times various Y receptor selective agonists and antagonists have been developed and tested. Their therapeutic potential in treating obesity and other energy homeostasis-related disorders such as cancer-related anorexia is discussed in this review. Associated with this, in recent studies a role of NPY family peptides and Y receptors in modulating the growth of tumours has emerged. The implications of NPY and Y receptors in tumour biology and the potential of pharmacological modulation of the NPY system as anti-cancer treatment are discussed.

2. Neuropeptide Y system in obesity

2.1. Neuropeptide Y in obesity

Homeostasis of food intake and body weight is regulated by a large number of neurotransmitters and hormones, coordinated by complex neuronal pathways. Dysfunctions in this process will result in disrupted energy balance and eventually lead to the development of obesity or the other extreme, anorexia. The major regions in the central nervous system (CNS) where the regulation of energy homeostasis takes place are the hypothalamus and certain brainstem nuclei. NPY is most abundantly produced in the arcuate nucleus (ARC) that virtually innervates the entire hypothalamus, including the paraventricular nucleus (PVN) and dorsomedial nuclei (Fetissov et al., 2004). NPY neurons in ARC are sensitive to blood-borne signals from the periphery carrying information relevant to energy balance, since it lies in an area with a semi-permeable blood brain barrier (Broadwell & Brightman, 1976) and expresses high concentrations of binding sites for various peripheral signals such as insulin (Marks et al., 1990), ghrelin (Willesen et al., 1999) and leptin (Elmquist et al., 1998). Peripheral signals that are increased during positive energy balance states such as insulin (Schwartz et al., 1992) and leptin (Ahima et al., 1996) inhibit hypothalamic NPY expression, whereas ghrelin (Kamegai et al., 2000; Asakawa et al., 2001; Nakazato et al., 2001; Shintani et al., 2001), growth hormone and glucocorticoids stimulate it (Akabayashi et al., 1994b; Chan et al., 1996). Furthermore, NPY release from the hypothalamus is temporally correlated with food intake (Kalra et al., 1991), with an increase in rodents at the beginning of the dark phase when the majority of daily food is ingested (Akabayashi et al., 1994a), highlighting the physiological importance of NPY in the regulation of energy balance.

Changes in NPY-ergic tone have potent effects on energy metabolism. An increase in hypothalamic NPY-ergic tone elicits robust hyperphagia (Clark et al., 1984; Stanley et al., 1986; Leibowitz et al., 1988; Zarjevski et al., 1993; Pierroz et al., 1996; Sainsbury et al., 1997a; Small et al., 1997; Fekete et al., 2001; Raposinho et al., 2001; Sainsbury &

Herzog, 2001; Lin et al., 2006), decreases energy expenditure in association with decreased body temperature and suppressed thermogenic capacity of brown adipose tissue (BAT) (Billington et al., 1991, 1994; Szreder et al., 1994; Currie & Coscina, 1995; Kotz et al., 1998), and induces a range of neuroendocrine and metabolic changes that favour energy storage. Such changes include hyperinsulinemia (Moltz & McDonald, 1985; Dunbar et al., 1992), insulin resistance in skeletal muscle, insulin hyper-responsiveness and increased de novo lipogenesis in white adipose tissue (Billington et al., 1991, 1994; Zarjevski et al., 1993, 1994; Sainsbury et al., 1997b), activation of the hypothalamo-pituitary-adrenal axis (Wahlestedt et al., 1987; Leibowitz et al., 1988; Small et al., 1997), and decreased activity of the hypothalamo-pituitary-thyrotropic (HPT) (Harfstrand et al., 1987), -somatotropic, and -gonadotropic axes (Catzeflis et al., 1993; Pierroz et al., 1996). These effects ultimately lead to excessive fat and weight gain. Interestingly, the excessive fat gain persists, when NPY-induced hyperphagia is prevented by pair-feeding, i.e. providing treated animals daily with the amount of food eaten by matched ad libitum control animals (Zarjevski et al., 1993; Pierroz et al., 1996; Sainsbury et al., 1997a; Fekete et al., 2001; Raposinho et al., 2001; Sainsbury & Herzog, 2001). This suggests that the decreased energy expenditure and metabolic and hormonal changes induced by central elevation of NPY play an important role in mediating NPY's obesogenic effects.

Chronically elevated NPY-ergic tone has been shown to be associated with an obese status. Obese rodents fed with a 22-week high fat diet (HFD) (Huang et al., 2003) or gene mutation resulting in defective leptin signalling (Bchini-Hooft van Huijsduijnen et al., 1993; Stephens et al., 1995) show increased hypothalamic NPY mRNA expression compared to lean controls. Examining hypothalamic NPY expression in humans is more difficult. However, circulating NPY levels were found to be elevated in obese women in the absence (Baranowska et al., 2005) or presence of hypertension and/or type 2 diabetes (Milewicz et al., 2000; Baranowska et al., 2003) compared to lean healthy subjects. Some evidence suggests that the increased NPY-ergic activity may be causal in some cases of obesity. For instance, the increase in hypothalamic NPY expression in obese fa/fa rats occurs early after weaning corresponding with the time when their syndrome first becomes apparent (Bchini-Hooft van Huijsduijnen et al., 1993). Although single gene mutations that lead to obesity and metabolic syndrome in humans are rare, polymorphisms in various genes are commonly associated with these conditions. One variant causing an amino acid change from leucine to proline at codon 7 in the signal peptide of NPY is associated with increased body mass index (Ding et al., 2005), impaired glucose tolerance and type 2 diabetes (Nordman et al., 2005; Ukkola & Kesaniemi, 2007), higher serum levels of total and low-density lipoprotein cholesterol (Karvonen et al., 1998) and predicts myocardial infarction and stroke in hypertensive patients (Wallerstedt et al., 2004). Furthermore, a recent study shows that the Leu7/Pro polymorphism of the NPY gene alters the packaging and secretion of NPY, leading to an increased peptide synthesis and release in endocrine cells and CNS neurons (Mitchell et al., 2008). This is consistent with the elevated plasma levels of NPY observed in subjects carrying the NPY Leu7/Pro polymorphism during exercise and elevated sympathetic activity (Kallio et al., 2001). Taken together, an increased NPY-ergic tone leading to an enhanced NPY production may be an etiological factor in obesity, at least in some obese patients. Thus, dampening this tone via pharmacological interventions in this population may be an effective approach for weight loss and improving metabolic conditions.

For the majority of obesity cases, an elevated NPY-ergic tone is likely due to a central resistance to peripheral signals of energy excess such as leptin, which increases in response to a prolonged positive energy balance (Lin et al., 2000; Huang et al., 2003; Enriori et al., 2007; Fam et al., 2007). In fact, rodent studies show that during the early stage of high fat feeding, hypothalamic NPY expression is decreased (Lin et al., 2000), which is associated with an increased energy expenditure and physical activity (Fam et al., 2007), consistent with an adaptive homeostatic mechanism in response to excess energy intake. With prolonged feeding periods, however, the regulatory systems break down, central leptin resistance develops and more rapid weight gain occurs (Lin et al., 2000). Interestingly, rodents resistant to diet-induced obesity show significantly reduced mRNA expression levels of NPY, Y1, Y2 and Y5 receptors in the hypothalamus compared to obese animals on the same diet (Huang et al., 2003; Wang et al., 2007). This suggests a significant role of an increased activity of hypothalamic NPY neurons in the establishment of obese status during the chronic consumption of an obesogenic diet. Thus dampening the increase in NPY-ergic tone may provide a stronger defence mechanism against weight gain and achieve weight loss in obesity.

Furthermore, energy deficit, a necessity for weight loss, is known to increase the activity of NPY neurons, which plays an important role in triggering adaptive responses to prevent negative energy balance and weight loss (Sainsbury & Zhang, 2009). Such adaptive responses include increases in food intake or appetite, and reductions in resting or total daily energy expenditure (Sainsbury & Zhang, 2009). Therefore, combining measures that reduce the NPY-ergic tone with other weight loss means such as diet and exercise may lead to more effective results. More importantly, both animal and human studies show that weight loss-induced increase in appetite and decrease in energy expenditure persists during weight maintenance (Sainsbury & Zhang, 2009). Although some but not all studies have shown that the adaptive mechanisms induced in response to energy deficit in overweight or obese humans are transient, they are significant predictors for weight regain and likely to explain the high attrition in weight loss attempts (Dulloo & Girardier, 1990; Pasman et al., 1999; Goran, 2000; MacLean et al., 2004). A recent study shows a persistent elevation of hypothalamic NPY mRNA expression during the weight maintenance period in post-obese mice (Yu et al., 2009), suggesting that a continuously elevated NPY-ergic tone after weight loss may play an important role in keeping adaptive mechanisms active during the weight maintenance period, and thus dampening this tone may help to keep weight off permanently.

2.2. Y1 receptors and obesity

The Y1 receptor was the first Y receptor to be cloned and characterised (Herzog et al., 1993). Of all Y receptors, it has the widest distribution in the periphery as well as in the CNS particularly in the PVN of the hypothalamus (Goumain et al., 1998; Parker & Herzog, 2000; Serradeil-Le Gal et al., 2000; Berglund et al., 2003; Yang et al., 2008). From early on the Y1 receptor has been one of the focuses in obesity research due to its initial recognition as a “feeding receptor” to mediate NPY-induced hyperphagia (Haynes et al., 1998; Kanatani et al., 1999; Kanatani et al., 2001; Mullins et al., 2001; Henry et al., 2005; Antal-Zimanyi et al., 2008). Later studies using Y1 receptor ligands (Henry et al., 2005) and germline and conditional knockout models (Kushi et al., 1998; Pedrazzini et al., 1998; Sainsbury et al., 2006; Baldock et al., 2007; Cox et al., 2010; Zhang et al., 2010a) reveal that, in addition to feeding, the Y1 receptor plays important roles in mediating other metabolic effects of NPY related to energy homeostasis, such as energy expenditure, physical activity, oxidative fuel selection, pancreatic insulin secretion and mucosal secretion in the gut. Moreover, recent studies attest to an emerging role of the Y1 receptor as an important player in regulating bone homeostasis in coordination with body weight (Baldock et al., 2007; Lundberg et al., 2007; Lee et al., 2010a, 2010b), adding another dimension to the potential of the Y1 receptor as an anti-obesity treatment target.

2.2.1. Animal models

Several laboratories have generated germline Y1 receptor knockout mouse models using different gene deletion strategies (Kushi et al., 1998; Pedrazzini et al., 1998; Howell et al., 2003). Surprisingly,

all different knockout lines do not show major abnormalities regarding to spontaneous food intake (Kushi et al., 1998; Pedrazzini et al., 1998; Baldock et al., 2007). However some subtle changes in feeding exist in these germline Y1 receptor knockout models. For instance, Pedrazzini et al. (1998) reported a slightly diminished daily food intake and markedly reduced fasting-induced refeeding in Y1 receptor-deficient mice compared to wild type controls (Pedrazzini et al., 1998). Furthermore, hyperphagia in ob/ob mice was significantly reduced by Y1 receptor deletion (Pralong et al., 2002). These results show that the effect of Y1 receptor deletion on food intake is particularly obvious under conditions of an elevated NPY-ergic activity such as fasting and leptin deficiency, and argue in favour of a role of the Y1 receptor in mediating NPY-induced feeding. In keeping with this, the feeding response induced by intracerebroventricular (ICV) NPY administration was diminished in Y1 receptor knockout mice compared to that in wild type mice (Pedrazzini et al., 1998; Kanatani et al., 2000b, 2001).

Despite a decrease in stimulated-food intake, germline Y1 receptor knockout mice develop late-onset obesity that is more pronounced in females than in males (Kushi et al., 1998; Pedrazzini et al., 1998; Baldock et al., 2007; Zhang et al., 2010a). This increase in adiposity by germline Y1 receptor deletion was associated with hyperinsulinemia (Kushi et al., 1998; Baldock et al., 2007; Zhang et al., 2010a) and increased glucose uptake and glycogen synthesis in adipose tissue (Burcelin et al., 2001). Since insulin is known to be adipogenic (Cusin et al., 1990), it is suggested that increased circulating insulin levels in Y1 receptor knockout mouse promote glucose storage in adipose tissue and thus contribute to the development of obesity in these animals (Kushi et al., 1998). Studies have shown that NPY has a central action to promote insulin release (Sainsbury et al., 1997b; Gao et al., 2004) and a local action on the pancreas to inhibit it (Waeber et al., 1993; Wang et al., 1994; Morgan et al., 1998), and both effects have been reported to involve Y1 receptors (Morgan et al., 1998; Gao et al., 2004). Thus, the hyperinsulinemia in Y1 receptor germline deletion suggests that the direct local inhibition of insulin release may override the central stimulation of pancreatic insulin release. This may be an important factor to consider when developing Y1 receptor-targeted anti-obesity therapeutics.

Contradicting data on physical activity in germline Y1 receptor knockout mice were reported. Whereas Pedrazzini et al. (1998) showed a decrease in physical activity (Pedrazzini, et al., 1998), two other studies have demonstrated an increase in physical activity (Karl et al., 2006; Zhang et al., 2010a). Whereas the reason for these discrepancies is unclear possibly due to the testing environment and age/gender of the mice, all reports show a decrease in metabolic rate with germline Y1 receptor deletion (Pedrazzini et al., 1998; Zhang et al., 2010a). In the later study (Zhang et al., 2010a), an unaltered overall energy expenditure in Y1 receptor knockout mice was associated with a significant increase in activity levels, suggesting a decreased resting/basal metabolic rate. The decrease in metabolic rate in Y1 receptor knockout mice may explain the late-onset obesity in these animals (Kushi et al., 1998; Pedrazzini et al., 1998; Baldock et al., 2007; Zhang et al., 2010a) and is in keeping with the exacerbated obese phenotype of Y1 receptor knockout mice on a HFD (Sainsbury et al., 2006). Interestingly, ob/ob mice with a Y1 receptor deletion displayed a modest but significant reduction in body weight compared to their obese controls (Pralong et al., 2002). Whilst this reduced body weight may be primarily due to a reduced energy intake (Pralong et al., 2002), it also provides evidence that Y1 receptor antagonism may ameliorate obesity in severe cases. Moreover, ICV administration of the Y1 receptor agonist ([Phe^{7},Pro^{34}]pNPY) inhibits the HPT axis in rodents (Fekete et al., 2002), suggesting that Y1 receptor antagonism at least acutely is able to increase energy expenditure via actions that increase the activity of the HPT axis. The finding also suggests that the decrease in metabolic rate in germline Y1 receptor knockouts may arise from compensations by other regulatory pathways involved in energy metabolism or other Y receptors such as Y5 receptors. Therefore, Y1 receptor antagonism-based anti-obesity drugs may have greater therapeutic potential when combined with treatments targeting other energy balance-regulatory pathways and/or administered short-term or intermittently.

Germline Y1 receptor deletion has been recently shown to result in a decrease in respiratory exchange ratio (RER), indicative of increased lipid oxidation and/or reduced lipogenesis (Zhang et al., 2010a). Importantly, this is observed in Y1 receptor knockout mice at young age before their obese phenotype and associated increases in circulating insulin and free fatty acid levels occur, showing a primary role of the Y1 receptor to control oxidative fuel preference (Zhang et al., 2010a). Moreover, peripheral Y1 receptors appear to play an important role in mediating this effect. Crossing mice harbouring the Y1 receptor gene flanked by recombinase recognition (LoxP) sites (Y1$^{lox/lox}$) onto transgenic mice expressing Cre-recombinase under the control of interferon-responsive Mx1 promoter, the Y1 receptor is conditionally knocked down in peripheral tissues only due to the poor Mx1-activated Cre-mediated gene deletion in the brain (Kuhn et al., 1995; Zhang et al., 2010a). Mice with conditional Y1 receptor knockdown in the periphery show a reduction in RER similar to that observed in germline Y1 receptor knockout mice (Zhang et al., 2010a). The mechanisms underlying the Y1 receptor deletion-induced increase in lipid oxidation may involve an increased mitochondrial oxidative capacity in muscle and liver as evidenced by the significant increase in muscle and liver protein levels of carnitine palmitoyltransferase-1 (CPT-1) in germline and conditional Y1 receptor knockout models (Zhang et al., 2010a). CPT-1 is a key enzyme controlling the entry of fatty acid into mitochondria (McGarry et al., 1983), and an increase in CPT-1 expression itself has been shown to enhance lipid oxidation (Bruce et al., 2009). In keeping with a direct action of peripheral Y1 receptor signalling on lipid metabolism, blocking Y1 receptors expressed on adipocytes antagonises the anti-lipolytic effects of NPY and enhances lipolysis (Valet et al., 1990; Castan et al., 1994; Serradeil-Le Gal et al., 2000; Bowers et al., 2004; Bradley et al., 2005; Kuo et al., 2007; Yang et al., 2008), which increases fatty acid supply and promotes lipid oxidation (Turner et al., 2007).

Importantly, in contrast to the exacerbated diet-induced obesity in germline Y1 receptor knockout mice (Sainsbury et al., 2006), mice with conditional Y1 receptor knockdown in the periphery are protected against diet-induced obesity, as evidenced by a significant reduction in weight gain and fat gain compared to control mice on the same diet (Zhang et al., 2010a). This resistance to diet-induced obesity in conditional peripheral Y1 receptor knockdown mice is associated with increased energy expenditure and lipid oxidation compared to HFD-fed control mice (Zhang et al., 2010a). These findings revealed an important role of the Y1 receptor in the regulation of energy metabolism, and suggest that peripheral Y1 receptor antagonism (except in the pancreas, see previous discussion) may have great therapeutic potential to treat obesity via increasing fat oxidation and energy expenditure. Furthermore, targeting peripheral as opposed to central Y1 receptors would have the additional benefit that other important central Y1 receptor-mediated functions, such as control of anxiety and emotionality, would not be at risk of being affected. Thus it will be of considerable interest to develop strategies to antagonise Y1 signalling in tissues that increase lipid oxidation without inducing hyperinsulinemia or late onset obesity.

Studies on germline and conditional Y1 receptor knockout models have also revealed an important role of Y1 receptor signalling in the regulation of bone metabolism. Germline Y1 receptor deletion leads to a generalised increase in osteoblast activity on both the cancellous and cortical surface with consistent changes in femoral, tibial and vertebral bones (Baldock et al., 2007). These effects are most likely mediated by non-hypothalamic Y1 receptors and possibly involve a peripheral and direct action of the Y1 receptor on bone, since specific deletion of Y1 receptors in the hypothalamus had no effects on bone homeostasis, and since Y1 receptor expression has been found on

osteoblastic and bone marrow stromal cells (Baldock et al., 2007; Lee et al., 2010a). Interestingly, Lee et al. (2010a, 2010b) reported that osteoblast-specific Y1 receptor deletion resulted in a similar bone phenotype as that seen in germline Y1 deletion, however in the absence of obesity, hyperinsulinemia or other metabolic changes reported in germline Y1 receptor knockout mice (Lee et al., 2010b). These findings highlight that antagonising Y1 receptors in peripheral tissues may not only have a benefit as anti-obesity treatment but could also have an additional benefit on bone mass.

2.2.2. Ligand studies in animals

The first Y1 receptor selective agonist [Leu^{31},Pro^{34}]NPY was developed by Schwartz and co-workers who demonstrated that this NPY analogue had Y1 selectivity, relative to the Y2 receptor, mobilised intracellular calcium in SK–N–MC cells bearing Y1 receptors, and exhibited hypertensive activity in rats in vivo with a potency comparable to that of intact NPY during intravenous (i.v.) administration (Fuhlendorff et al., 1990). However, following the cloning and characterization of other Y receptors, it was found that [Leu^{31},Pro^{34}]NPY also has affinity to Y4 and Y5 receptors (Gehlert et al., 1996; Balasubramaniam, 1997). Subsequent efforts led to the development of some highly selective Y1 receptor ligands, that have served as useful tools in studying the physiology of Y1 receptors and revealed a spectrum of important roles of the Y1 receptor in the regulation of food intake and energy metabolism.

Activation of central Y1 receptors using Y1 receptor agonists causes a reliable increase in food intake. For instance, ICV administration of the selective Y1 agonists [$_D$-Arg^{25}]NPY, [$_D$-His^{26}]-NPY or Des-AA$^{11\text{-}18}$[Cys^{7,21},$_D$-Lys^9(Ac),$_D$-His^{26},Por^{34}]-NPY in rats stimulated food intake dose-dependently, and these effects lasted for at least 4 h after the infusion (Mullins et al., 2001). Furthermore, prolonged activation of Y1 receptors by the agonists [Phe^7,Pro^{34}]pNPY (Soll et al., 2001; Fekete et al., 2002) or [$_D$-Arg^{25}]NPY (Mullins et al., 2001; Henry et al., 2005) leads to significant body weight gain and fat gain. Interestingly, whereas pair-feeding abolished weight gain, it had little effect on Y1 receptor agonist-induced increase in fat mass (Fekete et al., 2002; Henry et al., 2005), suggesting that Y1 receptor agonism regulates energy metabolism and adiposity independently of its effects on feeding and body weight. Indeed, chronic central infusion of the Y1 receptor agonist [$_D$-Arg^{25}]NPY for 6 days via an osmotic minipump in rats increased RER (Henry et al., 2005), which points to increased carbohydrate oxidation and/or increased lipogenesis and is consistent with the increased lipid oxidation in Y1 receptor knockout mice (Zhang et al., 2010a). Energy expenditure was not changed in animals chronically treated with a Y1 receptor agonist compared to control animals (Henry et al., 2005). However, considering that feeding itself increases energy expenditure (Roberts et al., 1990; Leibel et al., 1995; Levine et al., 1999; Rosenbaum et al., 2000), the unaltered energy expenditure in hyperphagic Y1 receptor agonist-treated animals indicates that Y1 receptor agonism per se. may decrease the metabolic rate. In keeping with a down-regulation of metabolic rate by Y1 receptor agonism, continuous administration of the Y1 receptor agonist [Phe^7,Pro^{34}]pNPY into the cerebrospinal fluid over 3 days in ad libitum-fed or pair-fed rats led to a significant suppression of pro-thyrotropin-releasing hormone mRNA in the PVN of the hypothalamus, which was associated with decreased circulating levels of thyroid hormones (T3 and T4), showing a Y1 receptor agonism-induced inhibition of the HPT axis (Fekete et al., 2002). Moreover, in line with a lipogenic effect of Y1 receptor agonism, acute ICV administration of the Y1 receptor agonist [$_D$-Arg^{25}]NPY led to a significant increase in circulating insulin levels in rats, that was not fully dependent on the agonist-induced feeding response (Gao et al., 2004). Collectively, the pharmacological studies using Y1 receptor agonists show that the Y1 receptor is an important player in mediating NPY's obesogenic effects via actions to increase food intake, induce hyperinsulinemia, and decrease energy expenditure and lipid oxidation. These findings have led to the development of a panel of Y1 receptor antagonists with the view that Y1 receptor antagonism may have effects opposite to those seen with an agonist, thus leading to weight loss and fat loss.

The first non-peptide Y1 receptor antagonist BIBP3226 was developed by Doods and colleagues (Rudolf et al., 1994). Whereas this compound bound to Y1 receptors with a low nanomolar affinity and exhibited little or no activity at other Y receptors (Rudolf et al., 1994; Doods et al., 1995; Wieland, et al., 1995), its low aqueous solubility and toxicity have hampered the use of this antagonist, especially in probing CNS functions (Redrobe et al., 1999). To overcome the shortcomings of BIBP3226, Doods and co-workers modified BIBP3226, which led to the development of BIBO3304 (Wieland et al., 1998). BIBO3304 exhibits 100-fold greater affinity than BIBP3226 to the Y1 receptor, and is essentially inactive at Y2, Y4 and Y5 receptors (Wieland et al., 1998). Injection of BIBO3304 directly into the PVN inhibited hyperphagia induced by fasting, central administration of NPY or [Leu^{31},Pro^{34}]NPY, but not feeding induced by galanin or norepinephrine (Wieland et al., 1998). Moreover, the S-enantiomer of BIBO3304, BIBO3457, is basically inactive and had no effect on feeding as demonstrated with BIBO3304 (Wieland et al., 1998). These results show that the Y1 receptor has a physiological role in mediating NPY's effects on feeding, and that central Y1 receptor antagonism using selective Y1 receptor antagonists is able to dampen this effect.

The inhibition by Y1 receptor antagonism of food intake seen with BIBO3304 is supported by studies using another selective and potent peptide Y1 receptor antagonist, 1229U91 (Daniels et al., 1995). This compound has an about 7000-fold greater selectivity for Y1 than for Y2 receptors (Kanatani et al., 1996). ICV injection of 1229U91 dose-dependently inhibited hyperphagia induced by ICV NPY administration or fasting in Sprague Dawley rats (Kanatani et al., 1996), as well as the spontaneous food intake in lean and obese Zucker rats, with greater effects in obese animals (Ishihara et al., 1998). In keeping with a specific inhibition of NPY-induced feeding, 1229U91 at the dose effective to suppress NPY-induced food intake had no effect on galanin-induced feeding in rats (Ishihara et al., 1998). It has been reported that 1229U91 has appreciable agonist activity at human Y4 receptors, raising the possibility that the agonist effects at Y4 receptors may contribute to 1229U91-induced inhibition of feeding (Parker et al., 1998). However, autoradiography studies using radio-labelled 1229U91 showed no specific binding on brain sections of Y1 receptor knockout mouse, whereas the same ligand produced a strong specific binding on wild type mouse brain sections (unpublished observation from our laboratory), demonstrating that there is no Y4 receptor cross-reactivity with this ligand in the mouse brain. In addition to the effects on food intake, ICV administration of 1229U91 in rats amplified insulin-induced hypoglycaemia that is known to be associated with elevated NPY-ergic activity (Akabayashi et al., 1993; Sergeyev et al., 2000; Nedungadi & Briski, 2010). Moreover, a single ICV injection of NPY increased hepatic triglyceride production in the form of very low-density lipoprotein in the absence of food intake, and ICV administration of 1229U91 decreased the elevated triglyceride secretion by 50% compared to vehicle-injected fasted rats (Stafford et al., 2008). These findings suggest that in addition to inhibiting feeding, Y1 receptor antagonism has broader actions to antagonise NPY-induced abnormalities such as dyslipidemia and hyperglycaemia.

Several other selective and potent Y1 receptor antagonists have been developed. Studies using these antagonists are in general agreement with those using BIBO3304 and 1229U91, and support the therapeutic potential of Y1 receptor antagonism as anti-obesity treatment (Serradeil-Le Gal et al., 1995; Hipskind et al., 1997; Zimmerman et al., 1998; Murakami et al., 1999). However, their poor bioavailability via oral or other means of peripheral administration (Hipskind et al., 1997; Zimmerman et al., 1998; Kanatani et al., 2001) has limited them to be used mainly as pharmacological tools.

The first orally-active Y1 receptor antagonist SR120819A was reported by Serradeil-Le Gal et al. (1995). This compound exhibited lower nanomolar activity at the Y1 receptor and did not interact with Y2, Y4 and Y5 receptors. Orally administered SR120819A (5 mg/kg) antagonised [Leu^{31},Pro^{34}]NPY (5 μg/kg i.v.)-induced hypertensive activity in guinea pigs with a duration of action longer than 4 h, however with no feeding-related data reported (Serradeil-Le Gal et al., 1995). The first orally available Y1 receptor antagonist with effects on food intake (J-104870) was reported by Kanatani et al. (1999). J-104870 binds to the Y1 receptor with high affinity and inhibits the NPY-induced increase in intracellular calcium levels in cells expressing human Y1 receptors, whereas it has very low affinity to other Y receptors (Kanatani et al., 1999). The ability of J-104870 administered orally or ICV to suppress ICV NPY-induced food intake or spontaneous food intake in Zucker fatty rats suggests that the compound is brain penetrable (Kanatani et al., 1999). A 2-week oral treatment with J-104870 in Zucker fatty rats produced a significant reduction in weight gain, adipose cell size and liver lipid accumulation (Ishihara et al., 2002). Interestingly, food intake showed a transient reduction during the 2-week oral treatment with J-104870, an effect that was most prominent on the 4th day and then gradually disappeared towards the end of the treatment (Ishihara et al., 2002). Similar results were reported in a recent study using another Y1 receptor antagonist, BMS-193885 (Antal-Zimanyi et al., 2008). This compound has good affinity for the Y1 receptor but no appreciable affinity to other Y receptors (Antal-Zimanyi et al., 2008). Although it lacks oral bioavailability, intraperitoneal (i.p.) injection of BMS-193885 reduced ICV NPY- and fasting-induced food intake (Antal-Zimanyi et al., 2008). Chronic treatment of rats with BMS-193885 via i.p. injection once daily for 44 days caused a transient reduction in daily food intake that was associated with a sustained and significant reduction in weight gain (Antal-Zimanyi et al., 2008). Collectively, these studies support the hypothesis that long-term Y1 receptor antagonism has therapeutic value to treat obesity and associated metabolic syndromes. Moreover, the anti-obesity benefit conferred by peripherally administered Y1 receptor antagonists may not only come from reduced food intake but also from alterations in other aspects of energy homeostasis via central and/or peripheral mechanisms. However, the long-term effects of Y1 receptor antagonism on aspects of energy metabolism other than food intake have not been studied in detail, and currently there are no human studies reported.

2.3. Y2 receptors and obesity

The Y2 receptor is the most prominent Y receptor expressed in the CNS representing approximately 2/3 of the total binding capacity of NPY (Lin et al., 2005). Particular high levels are found in the ARC of the hypothalamus and the area postreama (AP) in the brainstem — both areas known to have a semi-permeable blood brain barrier (Broadwell & Brightman, 1976). This makes the Y2 receptor accessible to circulating NPY, PYY or their shortened versions, i.e. NPY3-36 and PYY3-36, respectively. Activation of Y2 receptors has been linked to the induction of satiety thus leading to the generation of great interest in evaluating the anti-obesity potential of Y2 receptor agonism using Y2 receptor agonists such as PYY3-36. Interestingly, evidence emerging from Y2 receptor knockout mouse models suggests that Y2 receptor antagonism may have the potential to treat obesity with additional benefits on bone health. As such, future studies may need to look more closely into the effects of Y2 receptor antagonists alone on energy metabolism and in various obese animal models.

2.3.1. Animal models

2.3.1.1. Germline and conditional Y2 receptor knockout mice models. The first study on germline Y2 receptor knockout mice reported increased food intake, fat mass and body weight accompanied with an attenuated response to leptin in female mice (Naveilhan et al., 1999). Another study by Sainsbury et al. (2002a, 2002b, 2002c, 2002d) showed that female germline Y2 receptor knockout mice also had increased food intake, however with reduced body weight, whereas male Y2 receptor knockout mice had transiently reduced food intake and a sustained decrease in body weight gain associated with decreased adiposity at 16 weeks of age (Sainsbury et al., 2002b; 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 from different strategies targeting the Y2 receptor gene, hence affecting the completeness of the Y2 receptor deletion (Herzog, 2003). Importantly, evidence emerging from more recent studies on conditional Y2 receptor knockout models suggests that the interpretation of changes in body weight and body composition observed in different Y2 receptor knockout models needs to consider the possibility of differential central versus peripheral effects, and/or hypothalamic versus non-hypothalamic effects of this Y receptor. Thus, adult-onset hypothalamic-specific Y2 receptor deletion 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). Since the majority of the hypothalamic Y2 receptors are expressed on NPY-containing neurons (Broberger et al., 1999), the authors also used a strategy to specifically delete Y2 receptors expressed only by NPY neurons in adult mice (Shi et al., 2010). Mice with this conditional NPY neuron-specific Y2 receptor deletion showed significantly increased NPY mRNA expression and decreased pro-opiomelanocortin (POMC) mRNA expression in ARC (Shi et al., 2010), providing direct evidence that Y2 receptors on NPY-ergic neurons act as auto-receptors to regulate NPY expression, and directly or indirectly influence neighbouring POMC neurons in the ARC. Moreover, female mice with conditional Y2 receptor deletion in hypothalamic NPY neurons exhibited increased adipose mass, hepatic steatosis and greater muscle protein expression of phosphorylated acetyl-CoA carboxylase — a key enzyme in fatty acid synthesis demonstrating the obesogenic effect of selective blockade of Y2 receptor signalling in NPY neurons (Shi et al., 2010). Collectively, these findings from conditional Y2 receptor knockout models are consistent with the notion that PYY3-36 or a Y2 receptor agonist can act as a satiety and anti-obesogenic factor by interacting with hypothalamic Y2 receptors, and lack of hypothalamic Y2 receptor 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 a germline Y2 receptor knockout model (Sainsbury et al., 2002c; Sainsbury et al., 2003; Zhang et al., 2010b). The hypothesis that agonism at hypothalamic Y2 receptors contributes to weight loss, whereas agonism at peripheral Y2 receptors has the opposite effect, would reconcile observations that both a Y2 receptor agonist and germline Y2 receptor knockout can reduce body weight and fat mass.

Interestingly, crossing Y2 receptor knockout mice onto the ob/ob background attenuated the increased adiposity, hyperinsulinemia, and hyperglycaemia typical for ob/ob mice, without affecting food intake or body weight gain (Naveilhan et al., 2002; Sainsbury et al., 2002c). Y2 receptor deletion has also been shown to confer protection against diet-induced obesity and associated hyperinsulinemia in the absence of changes in food intake (Sainsbury et al., 2006). Furthermore, increased body weight and adiposity in female mice due to ovariectomy, a model mimicking the situation during menopause in women, were normalised by global Y2 receptor ablation but not hypothalamic-specific Y2 receptor deletion (Allison et al., 2006). Finally, Y2 receptors have been implicated in the obesity syndrome induced by chronic corticosterone administration, since corticosterone treatment-caused increases in adiposity, circulating leptin and insulin levels were ablated in germline Y2 receptor knockout mice

(Sainsbury et al., 2002b). Taken together, these studies suggest 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 receptor agonists in the hypothalamus. As such, Y2 receptor antagonism may overall be more beneficial for treating obesity.

The mechanisms underlying the anti-obesity effects of Y2 receptor antagonism are not fully understood. Y2 receptor knockout mice do not show significant changes in overall physical activity, energy expenditure or daily food intake compared to wild type mice (Zhang et al., 2010b). Interestingly, there are reports on some subtle changes in these parameters in Y2 receptor knockout mice such as altered locomotor activity under stress condition (Painsipp et al., 2008), shifts in circadian rhythm in food intake and water intake (Edelsbrunner et al., 2009), suggesting that the Y2 receptor may be involved in the fine control of locomotor and ingestion behaviours. Importantly, a recent study shows that Y2 receptor knockout mice exhibit a small but significant decrease in RER during the light phase, indicating an increased lipid oxidation and/or decreased lipogenesis (Zhang et al., 2010b). The shift in oxidative fuel preference to lipid in Y2 receptor knockout mice is associated with a significant increase in muscle protein levels of CPT-1 (Zhang et al., 2010b). This increased CPT-1 expression and potential subsequent increase in lipid oxidation caused by Y2 receptor deletion are likely to be mediated by a peripheral mechanism, since deletion of Y2 receptors in hypothalamic NPY-ergic neurons results in decreased rather than increased muscle protein levels of CPT-1 (Shi et al., 2010). In line with a peripheral action of Y2 receptors in regulating fat metabolism, NPY released from nerve terminals stimulates fat angiogenesis and adipogenesis in pre-adipocytes primed for differentiation, and promotes adipocyte proliferation via direct actions on Y2 receptors expressed by adipocytes and endothelial cells (Kuo et al., 2007). Collectively, these findings suggest that the anti-obesity effect induced by Y2 receptor deficiency or antagonism results primarily from an increase in substrate utilisation. This action leads to greater use of fat as a fuel source and is most likely mediated by peripheral mechanisms.

Studies on Y2 receptor knockout models have also revealed a significant role of Y2 receptors in the regulation of bone metabolism. Germline Y2 receptor knockout mice have a two-fold 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. The increased bone mass in germline Y2 receptor knockout mice coincides with the reduced fat mass in these animals, suggesting that Y2 receptor deletion may cause energy partitioning changes between fat and lean tissue mass. In support of this hypothesis, Y2 receptor deletion abolishes fasting-induced reduction in the 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., 2002c), suggesting a role of Y2 receptor signalling in regulating the 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, since Y2 receptors have been shown to co-localise with growth hormone-releasing hormone neurons in the ARC and ventromedial hypothalamic nuclei, and since hypothalamus-specific Y2 receptor deletion prevented fasting-induced inhibition of hypothalamic growth hormone-releasing hormone mRNA expression (Lin et al., 2007). Interestingly, hypothalamus-specific Y2 receptor deletion recapitulated the bone phenotype observed in germline Y2 receptor knockout mice (Baldock et al., 2002), demonstrating a key role of hypothalamic Y2 receptors in regulating bone metabolism. 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 gonadectomised 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 gonadectomised wild type animals (Allison et al., 2006). These studies suggest that Y2 receptor agonists targeting the hypothalamus such as PYY3-36, which may be beneficial in treating obesity, are likely to have detrimental effects on bone mass in the long-term. Thus, pharmacological agents that antagonise Y2 receptors in the hypothalamus as well as non-hypothalamic sites (e.g. in peripheral tissues) could confer dual anti-obesity and anti-osteoporotic activities.

2.3.1.2. Polypeptide YY knockout mice and polypeptide YY transgenic mice. Following studies demonstrating PYY3-36 as a satiety factor with therapeutic potential for obesity (see discussion in the next section), PYY knockout and transgenic mice models were generated to study the long-term role of PYY in the regulation of energy homeostasis. It is important to note that genetic modification of the PYY gene alters both the full-length PYY as well as PYY3-36, thus these genetically modified models may not be directly comparable with models based on PYY3-36 administration. 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 strain that has been shown to be obesity-resistant when fed a high-fat diet or when carrying a transgene that normally leads to obesity on a C57Bl/6 strain (Ludwig et al., 2001; Chen et al., 2005; Schonhoff et al., 2005). Another study by Batterham et al. (2006) showed that both male and female homozygous PYY deficient mice were hyperphagic and developed marked obesity, suggesting PYY ablation may cause obesity through increased food intake. A third PYY knockout model by Boey et al. (2006b) demonstrated late-onset obesity in female mice on a chow diet and an exacerbated diet-induced obese phenotype in male and female mice without changes in either basal or fasting-induced food intake. 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 that hyperinsulinemia plays a role in the development of increased adiposity associated with PYY deletion. The fourth PYY knockout model generated by Wortley et al. (2007) showed no difference from wild type mice in food intake or body weight on a standard chow diet, but exhibited significant weight gain and fat gain on a HFD compared to wild types on the same diet. The exacerbated diet-induced obesity in PYY knockout mice was not associated with any significant changes in food intake, energy expenditure, RER, locomotor activity or serum insulin levels, relative to control mice, leaving the cause for the greater diet-induced weight and fat gain in this PYY knockout model unexplained (Wortley et al., 2007). In summary, 3 out of 4 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 PYY deficiency, PYY over-expression in PYY 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). PYY transgenic ob/ob mice exhibited increased body temperature, enhanced hypothalamic expression of thyrotropin-releasing hormone (TRH) mRNA and decreased BAT depot weight, suggesting a PYY-induced activation of the HPT axis and increased thermogenic activity (Boey et al., 2008). Thus, PYY may have a long-term benefit 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).

2.3.2. Y2 receptor ligand studies in animals

The notion of Y2 receptor agonism as anti-obesity treatment is supported by the 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 nonobese rats and mice (Adams et al., 2004; Challis et al., 2004; Martin et al., 2004; Pittner et al., 2004). Moreover, the anorectic effect of PYY3-36 was abolished in mice deficient of Y2 receptors (Batterham et al., 2002), or in wild type rodents when 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 the PYY3-36-mediated anorectic effect. Although several Y2 receptor 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 via peripheral administration in vivo in the context of food intake and energy metabolism (Hwa et al., 1999; Batterham et al., 2002). Nevertheless, 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 nonpeptidic moiety (2-mercaptonicotinic acid) at the peptide N terminus – reduced food intake in 18-hour fasted lean rodents. This effect was abolished by pre-treatment with BIIE0246 (Ortiz et al., 2007), lending support to the therapeutic potential of the peripheral administration of a Y2 receptor agonist to reduce energy intake and potentially treat obesity. Importantly, i.p. injection of PYY3-36 to diet-induced obese mice resulted in a reduced food intake up to 8 h compared with saline-injected control mice, suggesting that the sensitivity to the acute anorectic effect of exogenous PYY3-36 is maintained in the obese state (le Roux et al., 2006). The mechanism underlying the anorectic effect of peripherally administered PYY3-36 and Y2 receptor agonists remains to be fully elucidated. However, it has been proposed that PYY3-36 acts on Y2 auto-receptors on NPY neurons in ARC to reduce an orexigenic drive (Batterham et al., 2002; Acuna-Goycolea & van den Pol, 2005). In keeping with this, many studies of the inhibitory effect of PYY3-36 on food intake have used fasting as part of the experimental paradigm that enhances hypothalamic NPY expression and orexigenic activity. Recent evidence suggests that the AP, subfornical organ and vagus nerve may also be involved in mediating the anorectic effect of PYY3-36 (Baraboi et al., 2010).

Long-term effects of PYY3-36 or Y2 receptor agonists on food intake and weight gain have been investigated in lean and various obese rodent models. In lean rodents, reductions in cumulative food intake and weight gain with i.p. administration of PYY3-36 over a 7-day period were observed in one study (Batterham et al., 2002), but not the other (Challis et al., 2004). However, 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 obese Zucker fa/fa rats (Pittner et al., 2004), have produced consistent results. These studies show that PYY3-36 (Pittner et al., 2004; Adams et al., 2006; Vrang et al., 2006) or the Y2 receptor PEGalated peptide agonist (Ortiz et al., 2007) administered over 7 to 56 days via repeated daily subcutaneous injections (Ortiz et al., 2007) or continuous infusion by a subcutaneously implanted osmotic minipump (Pittner et al., 2004; Adams et al., 2006; Vrang et al., 2006), leads to dose-dependent reductions in body weight and/or adiposity in obese animals. The anorectic effect of PYY3-36 or the Y2 receptor agonist seems to be transient, because the reduction in daily food intake or cumulative food intake is apparent only 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 to continuous or intermittent administrations of PYY3-36 or the Y2 receptor agonist. Importantly, the sustained reduction in body weight and/or adiposity in contrast to the transient feeding response during the chronic PYY3-36 or Y2 agonist treatment suggests that alterations in components of energy metabolism other than pure changes in energy intake occur. Indeed, acute administration of PYY3-36 in lean or diet-induced obese rodents, whilst having little effect on total energy expenditure, resulted in a significant reduction in RER, which could not be accounted for by the reduction in food intake and persisted during prolonged treatment (Adams et al., 2006; van den Hoek et al., 2007). Thus, an enhanced lipid oxidation induced by PYY3-36 may contribute to its effects in reducing body weight and adiposity. The mechanism underlying the effect of PYY3-36 in enhancing lipid oxidation is unclear. An indirect action rather than direct action on adipocytes is likely to be involved, since PYY3-36 did not have an effect on ex vivo lipolysis in murine fat pad preparations (Adams et al., 2006). The Y2 receptor-mediated reduction in hypothalamic NPY expression by PYY3-36 would be consistent with an indirect action of PYY3-36 to increase lipid oxidation, since NPY acts to promote carbohydrate oxidation and inhibit lipid oxidation (Henry et al., 2005). In keeping with a central Y2 receptor-mediated mechanism, chronic ICV administration of the Y2 receptor preferring agonist N-acetyl-[Leu^{28}, Leu^{31}] NPY(24–36) to mice via an osmotic minipump caused transient but significant reductions in food intake and body weight gain, and resulted in a lower RER compared to pair-fed vehicle-infused control mice (Henry et al., 2005).

In contrast to the extensive research effort in studying Y2 receptor agonism as a potential obesity treatment, effects of Y2 receptor antagonism on energy homeostasis have received little attention. Amongst the few Y2 receptor antagonists that were developed so far (T4-$[NPY(33–36)]_4$, BIIE0246 and JNJ-5207787 to name a few) (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 mainly focused on the analysis of Y2 receptor agonist actions rather than on their direct effects on energy homeostasis.

2.3.3. Human studies

Recently, it was shown that polymorphisms in the PYY and Y2 receptor genes are associated with severe obesity in Pima Indian men, a population with a high prevalence of hyperinsulinemia, obesity and type 2 diabetes (Ma et al., 2005). Similarly it was demonstrated that variants in the 5′ region of the Y2 receptor gene are associated with obesity in Caucasian Danish subjects (Torekov et al., 2006) and French white subjects (Siddiq et al., 2007). A genetic case control association study found that obese men had lower allele and homozygosity frequencies for a common Y2 allele, suggesting that this Y2 receptor variant is protective against obesity in Swedish men (Lavebratt et al., 2006). These data suggest that variations in the functionality of the PYY and Y2 receptor genes are involved in the aetiology of obese phenotypes. In keeping with a role of PYY and Y2 receptors in the development of obesity in humans, healthy people with a strong genetic propensity to obesity and type 2 diabetes have significantly lower fasting serum PYY levels than controls without any family history (Boey et al., 2006a).

Studies investigating the role of PYY3-36 in humans have provided fairly consistent results. A few discrepancies found in these studies are

mainly due to the current inability to precisely measure the exact levels of PYY1-36 and PYY3-36, and perhaps more importantly the inability to distinguish these active peptides from their inactive products PYY3-35 and PYY3-34 due to proteolytic degradation (Abid et al., 2009). Most studies show that, compared to normal weight subjects, obese adults (Alvarez Bartolome et al., 2002; Batterham et al., 2003a; le Roux et al., 2006) and children (Roth et al., 2005) have lower fasting total PYY peptide levels, which are negatively correlated with body mass index (van den Hoek et al., 2004) and various indices of adiposity as well as resting metabolic rate (Guo et al., 2006). In obese children who underwent a 1-year weight loss programme, circulating PYY levels increased significantly in those children showing most effective weight loss, but decreased in the subgroup of children who showed weight gain (Roth et al., 2005). Furthermore, postprandial PYY levels in the circulation are lower in obese subjects compared to lean controls (Alvarez Bartolome et al., 2002; Batterham et al., 2003a; Stock et al., 2005; le Roux et al., 2006), suggesting a blunted PYY release in response to meal ingestion in obese subjects, which may explain at least in part the lack of meal-induced satiety in obese subjects (le Roux et al., 2006; Maier et al., 2008). A recent study shows that PYY release is controlled by the cholinergic system that is markedly impaired in obese subjects, and suggests that cholinergic system dysregulation is likely to play a role in the disturbed PYY release at basal state and in response to meal ingestion in obesity (Maier et al., 2008).

The anorectic effects of PYY3-36 observed in animal models have been reproduced in many human studies (Batterham et al., 2002; Batterham et al., 2003a; Degen et al., 2005; Ballantyne, 2006; Sloth et al., 2007a, 2007b; Field et al., 2010). For instance, in normal weight subjects, a 90-minute i.v. infusion of PYY3-36 at a dose producing PYY plasma levels within the postprandial range, leads to a decrease in appetite and food intake for up to 12 h and a 33% decrease in cumulative caloric consumption in the 24-hour period after the infusion (Batterham et al., 2002). More importantly, obese people remain responsive to the anorectic effect of i.v. infused PYY3-36 (Batterham et al., 2003a; Sloth et al., 2007a, 2007b; Field et al., 2010). Moreover, this anorectic effect of PYY3-36 can be additive with other anorexic agents such as the glucagon-like peptide receptor agonist oxyntomodulin (Field et al., 2010). In contrast to the focus on the effect of PYY3-36 in inducing satiety, few studies have examined other actions of exogenous PYY3-36 in humans relating to 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 RER, suggesting an enhanced lipid oxidation (Sloth et al., 2007b). The increased thermogenesis by PYY3-36 administration in humans (Sloth et al., 2007b) is in accordance with the increased core body temperature in PYY transgenic mice (Boey et al., 2008). Whereas the evidence points to a therapeutic value of exogenous PYY3-36 in the treatment of obesity, nausea and vomiting appear to be the common side effects associated with exogenous PYY3-36 i.v. infusion, particularly at higher doses (Degen et al., 2005; Gantz et al., 2007; Sloth et al., 2007b; le Roux et al., 2008). A study shows that supra-physiological doses of i.v. PYY3-36 cause nausea without an additional reduction in food intake, and the authors suggest that two PYY thresholds may exist, — a lower threshold for reducing food intake without nausea; and a higher threshold for causing nausea without a further reduction in food intake (le Roux et al., 2008). Interestingly, there is also a study showing that PYY3-36 administered subcutaneously at a well tolerated dose did not reduce food intake but enhanced circulating free fatty acids levels (Sloth et al., 2007a). Thus it would be interesting to examine whether the effect of PYY3-36 to increase energy expenditure and fat oxidation occurs at a lower dose than that required to induce an anorectic effect.

Currently, studies on chronic effects of peripheral administration of PYY3-36 in humans are lacking. Thus it remains to be determined whether the anti-obesity therapeutic benefits of 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 receptors in the regulation of bone metabolism, and since weight loss itself is often 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 clinic use as an anti-obesity drug, and thus a modified version of the peptide with greater stability and longer-lasting effects will need to be developed to avoid highly frequent administration.

2.4. Y4 receptors and obesity

The Y4 receptor is the least studied Y receptor in any aspect of physiology. The preferred high affinity ligand for the Y4 receptor, PP, has been suggested to have an anti-obesity potential due to the anorectic effects when injected peripherally in rodents and humans. Y4 receptors have been found to be expressed in brain regions involved in the regulation of appetite and energy metabolism including the AP, nucleus tractus solitarius and dorsal vagal complex in the brainstem, as well as ARC and PVN of the hypothalamus (Parker & Herzog, 1999). It is important to note that PP, whereas having high affinity to Y4 receptors, is only found to be expressed in F cells of the pancreas and not in the brain itself. Thus some of the Y4 receptor-mediated effects in the CNS might be mediated by NPY, which also has considerable affinity to Y4 receptors particularly at the high expression levels reached during prolonged starvation.

2.4.1. Animal models

2.4.1.1. Y4 receptor knockout mice. Germline deletion of Y4 receptors leads to reduced body weight and fat mass with concomitant increases in lean mass (Sainsbury et al., 2003; Zhang et al., 2010b). The lean phenotype of Y4 receptor knockout mice occurs in the absence of significant changes in food intake, whilst energy expenditure and physical activity are increased and the RER is decreased, which is indicative of increased lipid oxidation and/or decreased lipogenesis (Zhang et al., 2010b). Consistent with an increased energy expenditure, mice lacking Y4 receptors have an increased expression of TRH mRNA in the PVN of the hypothalamus (Sainsbury et al., 2003), suggesting an increased thyroid function, an important determinate of metabolic rate (Bianco et al., 2005; Silva, 2006). Furthermore, Y4 receptor deletion eliminates fasting-induced inhibition of the gonadotropic axis (Lin et al., 2007), and restores the low testosterone levels and the fertility of ob/ob mice (Sainsbury et al., 2002d), which suggests that an increased energy expenditure towards reproductive functions could contribute to the leanness of Y4 knockout mice. Taken together, results from the Y4 receptor knockout model show that long-term Y4 receptor antagonism may lead to reduction in body weight and adiposity via effects on metabolic rate, oxidative fuel selection and energy distribution. These implications highlight the therapeutical potential of Y4 receptor antagonists as anti-obesity treatment.

It is of considerable interest that, whereas either Y2 or Y4 receptor knockout results in significant reductions in adiposity and attenuation of various obesity syndromes (Sainsbury et al., 2002b, 2002c, 2002d; 2003, 2006; Zhang et al., 2010b), double knockout of both Y2 and Y4 receptors results in even greater (synergistic) reduction of adiposity (Sainsbury et al., 2003; Zhang et al., 2010b) and greater (synergistic) protection against genetic (Lee et al., 2008) and diet-induced (Sainsbury et al., 2006) obesity. The synergistic action of dual deletion of Y2 and Y4 receptors to reduce adiposity is associated with a significant increase in lipid oxidation and synergistic increases in energy expenditure and physical activity (Zhang et al., 2010b).

Furthermore, whereas bone volume was increased approximately 2-fold in Y2 receptor knockout mice and not at all in Y4 receptor knockouts, Y2 and Y4 receptor double knockout mice showed a 3-fold increase in bone volume associated with enhanced osteoblastic activity, showing a synergistic action of these two pathways to regulate bone formation (Sainsbury et al., 2003). It is possible that the increased energy demand due to a greater growth of lean tissues such as bone and stronger activation of the reproductive axes may collectively contribute to the synergistic reduction in adiposity observed in Y2 and Y4 receptor double knockout animals. In summary, whereas Y2 receptor antagonism may be a possible pathway for novel anti-obesity and anti-osteoporotic treatments, findings from Y2 and Y4 receptor double knockout mice suggest that dual antagonism of Y2 and Y4 receptors may provide even greater benefits for weight loss and bone health.

2.4.1.2. Pancreatic peptide transgenic mice. Long-term effects of PP have been studied in mice with pancreatic PP over-expression (Ueno et al., 1999), which exhibit reduced adiposity that is accompanied by reduced food intake and gastric emptying (Ueno et al., 1999). These effects are reversible by i.p. injection of PP antiserum, demonstrating that they are specific to PP over-expression (Ueno et al., 1999). Moreover, the mRNA expression of ghrelin in the stomach and of orexin in the hypothalamus of fasted PP-over-expressing mice is significantly reduced compared to that of littermate controls (Asakawa et al., 2003). In contrast to the increased energy expenditure observed with acute PP administration in wild type rodents, mice with PP over-expression show no change in oxygen consumption or locomotor activity (Ueno et al., 1999), suggesting that PP may not have a long-term effect on energy expenditure or physical activity. Moreover, PP over-expression had no significant effect on bone mass or aspects of bone physiology such as osteoblast or osteoclast functions (Sainsbury et al., 2003). Taken together, these results demonstrate a role of PP as a postprandial satiety factor and point to a therapeutic value of exogenous PP and Y4 receptor agonists to suppress appetite and potentially induce weight loss and fat loss. However, chronic treatment with PP or Y4 receptor agonists is unlikely to enhance energy expenditure or have beneficial effects on bone metabolism.

2.4.2. Y4 receptor ligand studies in animals

Currently, there are very few ligands selective for Y4 receptors, and thus most ligand studies have been focusing on the endogenous Y4 receptor ligand — PP. PP is primarily produced in F type cells of the pancreas (Adrian, 1978; Katsuura et al., 2002), and released following ingestion of food in proportion to the caloric intake (Sive, et al., 1979; Track et al., 1980; Inui, et al., 1993) and in response to other signals such as hypoglycaemia (Havel et al., 1993). In fasting individuals circulating PP concentrations show a circadian rhythm with lowest levels at 02:00 and a peak at 21:00 h (Track et al., 1980). Both the circadian and postprandial PP releases appear to be regulated by the vagal tone (Schwartz et al., 1978; Taylor et al., 1978). The physiological effects of PP include inhibition of pancreatic exocrine function, gall bladder contraction and stimulation of gastrointestinal motility and gastric acid secretion (McTigue & Rogers, 1995a, 1995b; Kojima et al., 2007).

Increasing evidence suggests that PP plays a role in the regulation of food intake and energy metabolism. Acute peripheral administration of PP at physiological doses induces a rapid and persistent reduction in food intake in fasted normal weight mice (Asakawa et al., 1999, 2003; Balasubramaniam et al., 2006; Liu et al., 2008; Neary et al., 2008; Lin et al., 2009), and this effect of PP is abolished in Y4 receptor knockout mice (Balasubramaniam et al., 2006), demonstrating that Y4 receptors are required for the PP-induced anorectic response. Responsiveness to exogenous PP appears to be maintained in the obese state. I.p. injection of PP to genetically obese ob/ob and fatty liver Shionogi-ob/ob mice induced a significant decrease in food intake (Taylor & Garcia, 1985; Asakawa et al., 2003). Moreover, repeated PP administration to ob/ob obese mice resulted in reduced food intake along with a decreased body weight gain (Malaisse-Lagae et al., 1977; Asakawa et al., 2003) and ameliorated insulin responsiveness (Asakawa et al., 2003). Whilst these results demonstrate that endogenous PP is a potent satiety and an anti-obesity factor, its short half-life of ~6 min in plasma may limit its use as an anti-obesity treatment (Adrian, 1978). To address this problem, Balasubramaniam et al. developed a series of Y4 receptor agonists based on NPY(32–36) (Balasubramaniam et al., 2006). Injection of one of these selective Y4 receptor agonists, BVD-74D, the one exhibiting the greatest affinity to Y4 receptors, potently inhibited food intake in fasted wild type mice (Balasubramaniam et al., 2006), diet-induce obese mice and fatty liver Shionogi-ob/ob mice (Li et al., 2010). Moreover, the anorectic effect of the Y4 receptor agonist in wild type mice was absent in Y4 receptor knockout mice, demonstrating the selective action of this compound (Balasubramaniam et al., 2006).

In addition to its anorectic effect, peripheral administration of PP has also been shown to influence energy expenditure at least at acute settings. I.v. infusion of PP to rats increases efferent activity of sympathetic nerves innervating white adipose tissue, indicating an increase in sympathetic outflow that plays an important role in the regulation of energy expenditure (Asakawa et al., 2003). In support of this hypothesis, i.p. injection of PP to ob/ob mice enhances the activity of sympathetic nerves innervating the adrenal medulla and BAT, and increases oxygen consumption during the first 2 h after PP administration (Asakawa et al., 2003). A recent study shows that PP injected i.p. immediately before the onset of the dark phase increases spontaneous activity in wild type mice (Liu et al., 2008), suggesting that increased physical activity may contribute to the increased energy expenditure with acute administration of PP. Interestingly, the PP-induced increase in activity only occurred with the lower dose range of PP that showed no or much less pronounced effect on food intake, whereas at higher doses PP had no effect on activity but markedly reduced food intake (Liu et al., 2008). This differential effect of dose on activity and food intake suggests that PP may exert these effects through different neuronal circuits (Liu et al., 2008). Whether such a dissociation in the dose response to PP exists between activity and energy expenditure is unknown. Furthermore, whether the increase in energy expenditure and physical activity in response to acute administration of PP persists with chronic administration of PP remains also to be determined.

Y4 receptor agonism with PP is thought to mediate effects on appetite and energy balance by actions within the brainstem to modulate digestive processes such as gastric secretion, motility and emptying via modulating vagal cholinergic pathways (McTigue & Rogers, 1995a, 1995b; McTigue et al., 1997). In keeping with vagally-mediated alterations in gut function, the ability of peripheral PP administration to decrease both efferent activities of the gastric vagal nerve as well as food intake was abolished in vagotomised rodents (Asakawa et al., 2003). In addition, increases in efferent activity of sympathetic nerves innervating white adipose tissue, BAT and adrenal medulla in response to PP i.v. infusion were eliminated by hepatic vagotomy in rats (Asakawa et al., 2003), in line with a role of an involvement of the vagal nerve in PP's regulation on energy metabolism. Whilst Y4 receptor agonism with PP in the brainstem is implicated in PP's function, emerging evidence suggests that the hypothalamus is also involved. Peripheral injection of PP decreases the hypothalamic expression of NPY and orexin, whilst increasing the hypothalamic expression of anorexigenic urocortin (Asakawa et al., 2003). A recent study tracked changes in the expression of the neuronal activation marker, c-Fos, in response to i.p. PP injection, and demonstrated that the ARC of the hypothalamus was activated as early as 30 min after the PP injection (Lin et al., 2009). The time course of activation in the ARC was similar to that in the nucleus tractus

solitarius and the AP, suggesting that PP can activate the ARC directly rather than through a secondary relay from the brainstem (Lin et al., 2009). The POMC/α-melanocyte-stimulating hormone (MSH) signalling pathway in the ARC appears to play a key role in PP's action, since POMC mRNA expression and α-MSH immunoactivity showed significant co-localization with c-Fos in the ARC, and since POMC mRNA levels were significantly increased in response to PP administration (Lin et al., 2009). In keeping with a direct action of PP on the ARC, conditional deletion of Y4 receptors in the ARC in adult mice abolished PP-induced activation of c-Fos and induction of POMC expression in the ARC (Lin et al., 2009). The important role of this Y4 receptor-mediated POMC/α-MSH pathway in PP-induced anorexia was evidenced by the demonstration that the inhibitory action of PP on feeding (as seen in wild type mice) was abolished in mice lacking the melanocortin 4 receptor (Lin et al., 2009), the receptor via which α-MSH induces its anorexigenic effects (Ellacott & Cone, 2004). In addition to the ARC, other regions of the hypothalamus, namely PVN, dorsomedial part of the ventromedial nucleus of the hypothalamus and the lateral hypothalamic area also showed marked c-Fos activation in response to peripheral PP, and these responses were heightened with time and longer lasting than those in ARC (Lin et al., 2009). Considering the important roles of these hypothalamic regions in the regulation of energy balance (Sainsbury et al., 2002a), activation of these regions may contribute to the ability of PP not only to reduce food intake but also to induce other physiological effects such as sympathetically-mediated thermogenesis and energy expenditure.

2.4.3. Human studies

Despite consistent reports on PP regulation of appetite and energy homeostasis, the role of PP in the pathophysiology of obesity and anorexia in humans is unclear due to conflicting data. Patients with Prader–Willi Syndrome (PWS) characterised by childhood-onset hyperphagia and morbid obesity consistently exhibit reduced circulating levels of basal and meal-stimulated PP (Zipf et al., 1981, 1983; Tomita et al., 1989). In non-PWS obese subjects, however, lower circulating PP levels were observed in some (Lassmann et al., 1980; Holst et al., 1983; Lieverse et al., 1994) but not all studies (Pieramico et al., 1990; Wisen et al., 1992). Higher basal PP levels have been reported in patients with advanced malignant disease, suggesting that elevated PP concentrations may contribute to the general lack of appetite in these patients (Hjalmarsen et al., 2004). In subjects with anorexia nervosa, some studies demonstrated an increased diet-induced PP release (Alderdice et al., 1985; Uhe et al., 1992), whereas other studies failed to observe this (Abell et al., 1987). A study investigating the role of PP in weight regulation in Pima Indians demonstrated that an increase in post-prandial PP levels was associated with decreased weight gain (Koska et al., 2004). Surprisingly high fasting PP levels, however, were associated with increased weight gain (Koska et al., 2004).

The role of PP as a postprandial satiety signal demonstrated in animal models is also observed in humans. Administration of PP i.v. at 10 pmol/kg/min to healthy men reduced energy intake by 22% at a buffet lunch provided 2 hours after the end of the infusion, and decreased the 24-hour cumulative energy intake by 25%, indicating sustained inhibition of food intake (Batterham et al., 2003b). Administration of a lower dose of PP (5 pmol/kg/min, i.v.) to fasted lean subjects reduced energy intake at a buffet lunch served 1 h after the end of PP infusion by 11% (Jesudason et al., 2007). Moreover, subjects with PSW had reduced food intake in response to i.v. infusion of PP (Berntson et al., 1993). Whether part of PP's anorectic effect is mediated by reduced gastric emptying is unclear, with one group reporting no effect on gastric emptying (Adrian et al., 1981) whilst another reported an inhibition of gastric emptying (Schmidt et al., 2005). Taken together, human studies on PP support Y4 receptor agonism to be a promising new avenue to treat obesity. However, more work is required to generate such ligands or prolong PP's in vivo half-life and make PP or its analogues a suitable drug candidate.

2.5. Y5 receptors and obesity

The Y5 receptor exhibits highest identity (35%) to the Y1 receptor (Blomqvist & Herzog, 1997). It is expressed in many regions of the brain, with high levels of mRNA expression reported in the hypothalamus (Gerald et al., 1996; Nichol et al., 1999; Parker & Herzog, 1999; Durkin et al., 2000). Interestingly, Y5 receptor mRNA appears to be consistently co-localised with Y1 receptor mRNA although Y1 receptors have a much broader distribution and are expressed in many areas without the presence of Y5 receptors (Naveilhan et al., 1998; Parker & Herzog, 1999). This close expression pattern of Y1 and Y5 receptors is in accordance with the demonstration that these two genes are transcribed in opposite directions from a common promoter region on chromosome 4q31.3-32, suggesting a co-ordinated regulation (Herzog et al., 1997). The Y5 receptor shares the "feeding receptor" reputation with the Y1 receptor, and its role in mediating NPY-induced feeding and other effects on energy balance such as energy expenditure has been shown by studies involving knockout animal models and Y5 receptor selective agonists (Gerald et al., 1996; Marsh et al., 1998; Wyss et al., 1998; Hwa et al., 1999; Cabrele et al., 2000; McCrea et al., 2000). A panel of Y5 receptor antagonists has been developed and their anti-obesity potential has been evaluated in rodent and human studies. Moreover, the co-ordinated regulation of gene transcription and similar roles of Y1 and Y5 receptors in NPY's function led to the hypothesis that dual antagonism of Y1 and Y5 receptors may promise greater therapeutic potential to treat obesity. Evidence from recent animal studies has given support to this hypothesis.

2.5.1. Animal models

A Y5 receptor deficient mouse model was first reported by Marsh et al. (1998). In contrast to the pharmacological identification of the Y5 receptor as a mediator of the feeding response to NPY (Gerald et al., 1996), mice with Y5 receptor germline deletion exhibited normal growth and leptin sensitivity as well as similar spontaneous and fasting-induced feeding compared to wild type mice at younger age (Marsh et al., 1998). With increasing age, Y5 receptor knockout mice developed mild obesity characterised by increased body weight, adiposity and food intake (Marsh et al., 1998). Moreover, Y5 null mice on the leptin deficient ob/ob background were indistinguishable from ob/ob mice with regard to body weight, fat mass, body temperature or food intake (Marsh et al., 1998), in contrast to the ameliorated obesity and metabolic syndrome by Y1 (Pralong et al., 2002) or Y2 receptor deletion (Sainsbury et al., 2002c) on the ob/ob background. These results negate a role of the Y5 receptor in mediating NPY's feeding and obesogenic effects, and/or suggest the existence of compensations (and over-compensations at older age) from other Y receptors or other pathways involved in the regulation of energy homeostasis for the loss of Y5 receptors. Nevertheless, food intake induced by ICV NPY at a higher dose (5 μg) but not at a lower dose (1 μg) was significantly reduced in Y5-deficient mice (Marsh et al., 1998), supporting a role of the Y5 receptor in mediating NPY-induced food intake and suggesting an incomplete compensation by other Y receptors. Furthermore, ICV NPY-induced food intake was partially inhibited by the Y1 receptor antagonist 1229U91 in wild type mice but completely abolished in Y5 receptor-deficient mice (Marsh, et al., 1998), suggesting that Y1 together with Y5 receptors mediates the food intake response to centrally administered NPY. Recently, in seeking causes for the development of late-onset obesity by germline Y5 receptor deletion, Higuchi et al. (2008) reported that germline Y5 receptor knockouts display exacerbated fasting-induced increases in hypothalamic expression of the orexigenic acting peptides NPY and agouti related peptide, and exacerbated decreases in that of POMC and the

anorexigenic cocaine and amphetamine-related transcript (Higuchi et al., 2008). These results provide important insights into the paradoxical phenotype of Y5 receptor germline knockout mice and highlight the need of conditional Y5 receptor deletion models for future studies. In addition, the profound responses from multiple regulatory pathways to compensate the loss of Y5 receptors may point to the indispensable functions mediated by the Y5 receptor in energy homeostasis.

2.5.2. Ligand studies in animals

Animal studies using selective Y5 receptor agonists have produced consistent reports that Y5 receptor agonism stimulates feeding and energy conservation (Gerald et al., 1996; Hwa et al., 1999; Cabrele et al., 2000; McCrea et al., 2000; Parker et al., 2000; Mashiko et al., 2003; Gao et al., 2004; Henry et al., 2005). In the study leading to the initial identification and characterization of the Y5 receptor as a "feeding" receptor, [$_D$-Trp^{32}]hNPY was identified as a weak but selective Y5 receptor agonist (Gerald et al., 1996). ICV administration of this compound resulted in small but significant stimulatory effects on food intake during the 4-hour post-infusion period in rats (Gerald et al., 1996). A later study by Hwa et al. (1999) showed that ICV administration of [$_D$-Trp^{32}]NPY increased 2-hour food intake dose-dependently in rats, with a lower potency compared to that with NPY. Interestingly, administration of this compound into the lateral ventricle of rats led to significant reductions in BAT temperature and energy expenditure during the 1-hour post-infusion period without significant effects on RER (Hwa et al., 1999), demonstrating a role of the Y5 receptor in the regulation of metabolic rate. Screening NPY analogues with $_D$-Trp or $_L$-Trp substitutions at various positions in the C-terminus of NPY led to the discovery of another Y5 receptor agonist [$_D$-Trp^{34}]NPY with 3-fold greater potency than [$_D$-Trp^{32}]NPY, and about 8-fold less potency than NPY in inhibiting forskolin-stimulated cAMP formation in cells expressing Y5 receptors (Parker et al., 2000). [$_D$-Trp^{34}]NPY has an affinity 25-fold more selective for Y5 over Y1, Y2, Y4 and y6 receptors (Mashiko et al., 2003). ICV administration of [$_D$-Trp^{34}]NPY dose-dependently stimulated food intake in rats with a potency greater than that of [$_D$-Trp^{32}]NPY but less than that of NPY, in line with their in vitro potency order (Parker et al., 2000). Chronic ICV administration of [$_D$-Trp^{34}]NPY to C57BL/6J mice for 7 days resulted in behavioural, metabolic and endocrine changes that essentially recapitulate those seen with chronic ICV NPY administration (Zarjevski et al., 1993; Pierroz et al., 1996; Sainsbury et al., 1997a; Fekete et al., 2001; Raposinho et al., 2001; Sainsbury & Herzog, 2001; Mashiko et al., 2003). Such changes include hyperphagia, significant fat and weight gain, hypercholesterolemia, hypertriglyceridemia, hyperinsulinemia, hyperleptinemia, increased lipoprotein lipase activity but decreased hormone-sensitive lipoprotein activities in white adipose tissue, — all changes that accelerate the accumulation of triglyceride in adipose tissue by stimulation of the uptake of plasma triglyceride and the suppression of lipolysis in triglyceride storage. Furthermore, a marked decrease in BAT uncoupling protein-1 mRNA expression was observed, consistent with the decreased energy expenditure seen after acute ICV administration of the Y5 receptor agonist [$_D$-Trp^{32}] (Hwa et al., 1999; Mashiko et al., 2003). Importantly, when [$_D$-Trp^{34}]NPY-mediated hyperphagia is prevented by pair-feeding, the increase in fat mass and changes in most metabolic parameters persist (Mashiko et al., 2003), which is similar to the effects observed with ICV NPY administration (Zarjevski et al., 1993; Pierroz et al., 1996; Sainsbury et al., 1997a; Fekete et al., 2001; Raposinho et al., 2001; Sainsbury & Herzog, 2001). These findings show that, in addition to being "feeding" receptors, Y5 receptors play important roles in mediating effects of NPY on other aspects of energy homeostasis. Similar results have been reported using another more potent and selective Y5 receptor agonist, [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP (Cabrele et al., 2000). This peptide agonist is 12- and 145-fold more selective than [$_D$-Trp^{34}]NPY and [$_D$-Trp^{32}]NPY, respectively (Parker et al., 2000), and has over 2000-fold greater affinity for the Y5 over the Y1 and Y2 receptor and over 200-fold greater affinity for the Y4 receptor, and is 3-fold more potent than NPY (Cabrele et al., 2000). Acute ICV administration of [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP to rats stimulated feeding more potently than NPY (Cabrele et al., 2000). Chronic central administration of this peptide via an osmotic minipump in mice for 6 days elicited hyperphagia, and significant fat and weight gain associated with decreased insulin sensitivity (Henry et al., 2005). Interestingly, mice under chronic [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}] hPP treatment exhibited a significantly increased RER (Henry et al., 2005), indicating a decreased carbohydrate oxidation and/or increased lipogenesis induced by central Y5 receptor agonism. Total energy expenditure showed a trend towards a decrease in mice infused with [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP compared to vehicle-infused animals under both ad libitum-fed and pair-fed conditions (Henry et al., 2005). In keeping with a Y5 receptor agonism-induced down-regulation of energy expenditure, a 3-day continuous administration of [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP into the cerebrospinal fluid in ad libitum-fed or pair-fed rats led to significant suppression of proTRH mRNA in the PVN of the hypothalamus. This was associated with decreased circulating levels of thyroid hormones (T3 and T4), attesting to a Y5 receptor agonism-induced inhibition of the HPT axis (Fekete et al., 2002). Collectively, studies using Y5 receptor agonists have produced fairly consistent results and support a role of Y5 receptors in mediating NPY-induced feeding and other obesogenic changes in energy metabolism. These results have also led to the hypothesis that Y5 receptor antagonism may confer anti-obesity benefits.

Considerable progress has been made during the past 15 years in the development of Y5 receptor antagonists with anti-obesity potential (Criscione et al., 1998; Block et al., 2002; Daniels et al., 2002; Turnbull et al., 2002; Della-Zuana et al., 2004; Gillman et al., 2006). Criscione et al. (1998) reported the first potent Y5 receptor antagonist, CGP71683A, with good affinity and selectivity for Y5 receptors. Acute i.p. administration of this compound dose-dependently attenuated food intake in a variety of animal models (i.e. ICV NPY administration, fasting and satiated lean and obese Zucker rats). Repeated i.p. injections of the compound into rats for 28 days reduced food intake, fat and weight gain (Criscione et al., 1998; Kask et al., 2001). However, a later study by Della Zuana et al. (2001) reported that CGP71683A reduced feeding in Y5 receptor knockout mice and its inhibition of food intake may involve interactions with other receptors or inflammatory mediators. Thus, CPG71683A had similarly high affinity for muscarinic acetylcholine receptors and for the serotonin re-uptake recognition site as for Y5 receptors in the rat brain (Della Zuana et al., 2001). Moreover, the fall in food intake by CPG71683A treatment was associated with brain inflammatory responses as revealed by anatomic analysis. A similar off-target action on feeding and body weight was reported for another potent Y5 receptor antagonist, S-25585, which not only potently inhibited ICV NPY- or Y5 receptor agonist-induced food intake in rats, but also reduced food intake and weight gain following chronic administration to Y5 receptor-deficient mice (Della-Zuana et al., 2004).

Other potent and selective Y5 receptor antagonists with a favourable in vivo pharmacokinetic profile were assessed for their Y5 receptor-based action by evaluating the activity of the compounds towards a panel of receptors/channels in vitro or by the ability of the compound to inhibit food intake induced by a range of NPY-unrelated orexigenic agents. The results from these studies are not uniform. However, a majority is in favour of a therapeutic potential of Y5 receptor antagonism as an anti-obesity treatment. Thus three Y5 receptor antagonists, GW438014A (Daniels et al., 2002), MK-0577 (Erondu et al., 2006) and FMS586 (Kakui et al., 2006), which had been assessed for their Y5 receptor selectivity in vitro (GW438014A, MK-0577) and in vivo (FMS586), suppressed feeding induced by ICV NPY or fasting in lean animals, and reduced weight gain in dietary or genetically obese rodents. It is of particular note that in vitro screening

of MK-0557 over 183 receptors, enzymes and ion channels revealed activity at only three proteins (Erondu et al., 2006). However subsequent in vitro studies failed to demonstrate a physiologically relevant activity of MK-0557 at these three receptors, suggesting that MK-0557 should not have significant off-target activity in vivo (Erondu et al., 2006). Oral administration of MK-0577 significantly suppressed weight gain in diet-induced obese mice, which was associated with pronounced reduction in the mass of intra-abdominal fat pads (Erondu et al., 2006). These promising anti-obesity effects of MK-0577 observed in rodents led to the subsequent clinical evaluation of this compound in humans (discussed in the next section).

Two Y5 receptor antagonists, L-152,804 (Ishihara et al., 2006) and 3-oxo-N-(5-phenylpyrazinyl) spiro (isobenzofuran-1(3H),4′-piperidine)-1′-carboxamide (termed spironalactone Y5 antagonist hereinafter) (Mashiko et al., 2008) have been examined in Y5 receptor knockout model for their Y5 receptor-based action. L-152,804 is orally active, binds to human and rat Y5 receptors, but shows little affinity to Y1, Y2 or Y4 receptors (Kanatani et al., 2000a). Oral administration of this compound for 2 weeks to mice, beginning 2 days before the switch to a moderately high-fat diet (MHF), reduced caloric intake, suppressed diet-induced increases in body weight, fat mass and circulating insulin levels (Ishihara et al., 2006). Importantly, the inhibition by L-152,804 of MHF-induced obesogenic effects was absent in Y5 receptor knockout mice, demonstrating the selectivity of this Y5 receptor antagonist (Ishihara et al., 2006). Furthermore, mice with established obesity by 10-month MHF feeding responded to a 30-day L-152,804 treatment with significant decreases in body weight, adiposity and hepatic triglyceride content, indicating the effectiveness of Y5 receptor antagonism to induce weight/fat loss and ameliorate obesity-associated metabolic conditions (Mashiko et al., 2007). Although L-152,804 reduced food intake in dietary obese mice, pair-fed animals lost body weight less efficiently in response to L-152,804 and exhibited no loss in fat mass (Mashiko et al., 2007). This suggests that changes in energy homeostasis other than food intake may also play important roles in conferring Y5 receptor antagonism-induced anti-obesity benefits (Mashiko et al., 2007). In line with a stimulatory action of Y5 receptor antagonism on metabolic rate, mice treated with L-152,804 exhibited significantly elevated uncoupling protein-1 mRNA expression in BAT compared to vehicle controls (Mashiko et al., 2007). Rectal temperature was not significantly altered by L-152,804 compared to vehicle controls, but showed a marked decrease in pair-fed animals (Mashiko et al., 2007). In this regard, Y5 receptor antagonism may prevent decreases in metabolic rate due to a deficit in energy intake.

Like L-152,804, the spironolactone Y5 receptor antagonist significantly reduced MHF-induced weight gain in wild type but not in Y5 receptor-deficient mice (Mashiko et al., 2008). Obesity established by consuming a diet with greater fat content than MHF was markedly attenuated by a 6-week oral treatment with the spironolactone Y5 receptor antagonist, whilst food intake was only mildly suppressed (Mashiko et al., 2008). These results show that Y5 receptor antagonism may be effective in treating more severe obesity and the benefits may primarily come from changes in energy metabolism rather than food intake. In keeping with an effect of Y5 receptor antagonism on energy expenditure, weight loss induced by food restriction, that is known to cause compensatory decrease in metabolic rate, was significantly enhanced by the addition of spironolactone Y5 receptor antagonist treatment in dietary obese mice (Mashiko et al., 2008). Moreover, effects of Y5 receptor antagonism on energy expenditure may have important implications beyond the weight loss phase, i.e. a period to maintain and stabilise the reduced body weight. Since weight loss-induced decrease in energy expenditure persist during this weight maintenance period and elevated NPY-ergic tone has been suggested to play an important role (Sainsbury & Zhang, 2009; Yu et al., 2009). Importantly, an increase in energy expenditure or prevention of weight loss-induced decrease in metabolic rate by Y5 receptor antagonism during the weight maintenance period may achieve a quicker weight stabilisation and better weight management. Recently, Mashiko et al. (2009) reported that acute dual antagonism of Y1 and Y5 receptors with the Y1 receptor antagonist J-115814 and the spironolactone Y5 receptor antagonist synergistically suppressed spontaneous food intake in diet-induced obese mice. Furthermore, chronic dual blockade of Y1 and Y5 receptors by Y1 deletion and administration of the spironolactone Y5 receptor antagonist led to a greater weight reduction in dietary obese mice than either Y receptor blockade alone (Mashiko et al., 2009). These findings provide evidence for an interaction between Y1 and Y5 receptors in the regulation of energy homeostasis, and suggest that double pharmacological antagonism of Y1 and Y5 receptors may have greater anti-obesity potential than antagonising either receptor alone.

2.5.3. Human studies

Completed phase II clinical trials assessing the anti-obesity effects of Y5 receptor antagonism have been reported for MK-0577 (Erondu et al., 2006, 2007) and velneperit (S-2367) (Shionogi & Co., 2009). In examining the effectiveness of MK-0577 to induce weight loss, obese subjects were assigned to a 500 kcal/day deficit diet for 2 weeks, followed by treatment with placebo (0 mg) or 1 mg of MK-0577 once daily in conjunction with the same 500 kcal/day deficit diet for 52 weeks (Erondu et al., 2006). After 52 weeks, patients treated with MK-0577 lost significantly more body weight (−3.4 kg from baseline) than patients on placebo (−1.8 kg from baseline). Moreover, 23.3% of the patients on MK-0577 achieved more than 5% reduction of their baseline body weight, whereas this ratio was significantly lower (17.5%) in patients on placebo (Erondu et al., 2006). There were no statistically significant benefits on waist circumference and plasma parameters observed with MK-0577 treatment (Erondu et al., 2006). Thus it was concluded that Y5 receptor antagonism does not induce a clinically meaningful weight loss in overweight and obese subjects (Erondu et al., 2006).

It was further investigated whether MK-0577 administered orally at 1 mg once daily could limit weight regain after a very-low-calorie diet induced weight loss (Erondu et al., 2007). In this study (Erondu et al., 2007), obese subjects were assigned to an 800 kcal/day liquid diet for 6 weeks. Patients who lost more than 6% of their initial body weight were randomised to 52 weeks of placebo or MK-0577 treatment and maintained on a hypocaloric diet, i.e. 300 kcal below weight maintenance requirements (Erondu et al., 2007). During the initial 6 weeks on the very-low-calorie diet, randomised patients lost an average of 9.1 kg which was taken as baseline. At the end of the 52-week weight maintenance period, the mean weight change from baseline was 3.1 and 1.5 kg for the MK-0577 and the placebo group, respectively. No significant benefits of MK-0577 treatment on waist circumference, blood pressure or plasma measurements were observed, suggesting that pharmacological Y5 antagonism with MK-0577 does not prevent weight regain with a clinically meaningful efficacy (Erondu et al., 2007).

The long-term effectiveness of velneperit in weight loss and weight maintenance was assessed under two distinct diet conditions, reduced calorie diet (RCD) and low calorie diet (LCD) (Shionogi & Co., 2009). In the RCD study, obese subjects were assigned to a 6-week 800 kcal/day deficit diet, followed by 54 weeks of oral placebo (0 mg), 800 mg velneperit or 1600 mg velneperit once daily whilst being maintained on the same 800 kcal/day deficit diet. The 800 mg velneperit group represented the strongest performing group relative to placebo, with a weight loss from baseline taken after the 6-week RCD run-in period of 3.8 kg (3.9% of baseline value) and 0.8 kg (0.9% of baseline value) for the 800 mg velneperit and placebo groups, respectively. In the LCD study, obese subjects were placed on a fixed LCD of 950 kcal/day for 6 weeks, followed by 54 weeks of placebo (0 mg) or velneperit 1600 mg once daily in conjunction with an

800 kcal/day deficit diet. Obese subjects lost 7.1 kg (6.9% of baseline value) of their baseline body weight taken at the start of the fixed LCD schedule, versus 4.3 kg (4.4% of baseline value) in the placebo group. In contrast to MK-0577 which does not show a significant effect on the secondary endpoints (Erondu et al., 2006, 2007), velneperit was reported to have a statistically significant effect in decreasing waist circumference and improving serum lipid profile (Shionogi & Co., 2009).

Both MK-0577 and velneperit are apparently clinically well tolerated in humans (Erondu et al., 2006, 2007; Shionogi & Co., 2009). A combination with other anti-obesity agents, particularly with with compounds modulating other Y receptors such as Y1 receptor antagonists, as suggested by animal studies (Mashiko et al., 2009), would be of great interest.

2.5.4. Summary 1

Taken together, with the aid of knockout mouse models and selective Y receptor ligands, a significant amount of knowledge has been generated over recent years on the regulation of energy homeostasis by the NPY system, with particular insights into specific actions mediated by each Y receptor subtype. In addition to regulating food intake and appetite, NPY family peptides and Y receptors have been shown to play important roles in controlling other components of energy homeostasis such as energy expenditure and oxidative fuel selection. These findings attest to the potential of Y receptor-targeted strategies to treat obesity and associated metabolic conditions with actions not only to control appetite but also to modulate energy metabolism, and provide mechanism-based information as to which Y receptors to be targeted to obtain these benefits. Importantly, studies on conditional knockout mouse models revealed a role of peripherally expressed Y receptors in mediating some of these effects, suggesting that some anti-obesity benefits of Y receptor-based treatments may be achieved via peripheral mechanisms thus avoiding potential side effects often associated with centrally acting drugs. Finally, the NPY system has been identified as having an important coordination role in matching body weight to bone mass. Thus effects on bone health need to be considered when designing NPY system-based anti-obesity therapies. The potential effects on body weight and bone mass by modulating the activities of different Y receptor are summarised in Fig. 1.

3. Neuropeptide Y, obesity and anorexia in cancer

An increase in NPY-ergic activity may be implicated in cancer development via actions to promote weight gain and fat gain, since overweight and obesity are associated with an increased risk to develop cancers such as breast, colon/rectum and pancreas malignancies (Calle et al., 2003) and with an increased risk to die of malignancies such as prostate cancer (Ma et al., 2008). Although a causal association between obesity and cancer risk is currently a matter of investigation, weight loss has been reported in some but not all studies to reduce cancer incidence, particularly of breast and endometrial cancers (Renehan, 2009).

Imbalances in the normal regulation of food intake can not only lead to obesity but also to the other extreme, severe weight loss or anorexia. Severe anorexia and weight loss leading to cachexia are more common due to underlying disease processes such as cancer. Cancer-related anorexia is a debilitating condition and characterises the clinical journey of over 60% of terminally ill cancer patients (Maltoni et al., 1999; Pirovano et al., 1999). Evidence suggests that a decrease in NPY-ergic activity may be implicated in cancer-related anorexia. Tumour-bearing rats with anorexia exhibit a significantly reduced NPY-immunoreactive innervation of hypothalamic nuclei (Makarenko et al., 2003), which is normalised by tumour resection (Makarenko et al., 2005). Consistently, NPY protein concentrations in the hypothalamus of anorectic tumour-bearing rats were found to be decreased relative to sham-operated control rats, and this decrease in NPY levels could be reversed by tumour resection (Meguid et al., 2004). In keeping with reduced NPY-ergic activity, anorectic tumour-bearing rats were reported also to display significantly reduced Y1 receptor immunostaining in ARC and PVN (Chance et al., 2007). Whereas data on human hypothalamic NPY level and activity in cancer are lacking, plasma levels of NPY have been reported to be significantly lower in anorectic cancer patients compared to control subjects (Jatoi et al., 2001). These data suggest that pharmacological modifications of Y receptor activities to enhance NPY-mediated obesogenic and anabolic effects may have therapeutic value in treating cancer anorexia and ameliorating associated catabolic conditions. In support of this notion, megestrol acetate, an orexigenic drug used in the treatment of human anorectic cachexia, has been shown to increase hypothalamic NPY levels (McCarthy et al., 1994).

4. Neuropeptide Y and cancer

The NPY system has complex and important implications in cancer development via effects on energy homeostasis (as discussed in the previous section), immune function, as well as direct actions on tumour biology (Fig. 2). In addition, the NPY system has important interactions with the immune system as reviewed recently (Wheway et al., 2007a, 2007b), suggesting that peptides of the NPY family and Y receptors may

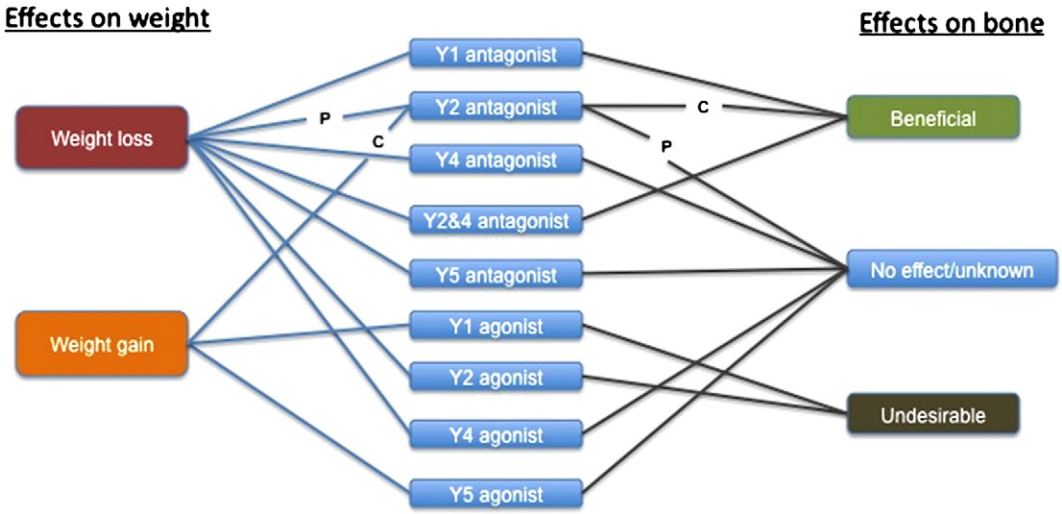

Fig. 1. Effects on body weight and bone mass by various Y receptor agonists and antagonists. "p" and "c" indicate peripherally acting and centrally acting mechanisms, respectively.

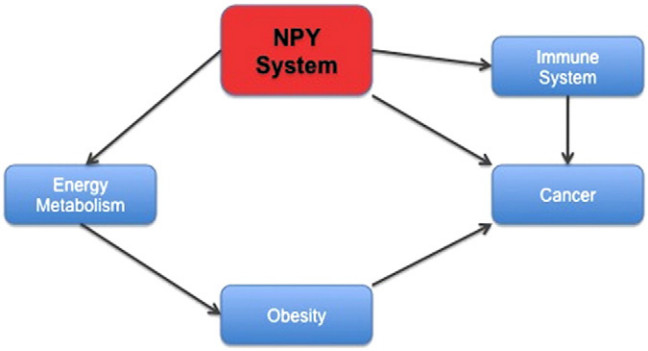

Fig. 2. The interaction of the NPY system with obesity and cancer. The NPY system plays a pivotal role in the regulation of energy homeostasis, interruption of which leads to obesity that is associated with an increased risk of many cancers. Disrupted energy metabolism by altered NPY-ergic activity may contribute to cancer-related anorexia that complicates metabolic conditions in cancer patients. In addition, the NPY system may influence cancer progression via interaction with the immune system, and/or directly with local actions on tumour biology.

influence cancer progression via modifying immune functions. Interestingly, recent research has identified a large number of tumours sensitive to NPY/PYY mediated Y-receptor signalling. The effect of signalling via Y receptors can vary from tumour growth promotion to tumour growth inhibition and induction of apoptosis. Below we discuss the potential effects and the therapeutic value of interfering with the signalling of Y receptors on a variety of different tumours.

4.1. Neuropeptide Y system in tumours derived from the autonomic system

High amounts of NPY are secreted from tumours of the autonomic nervous system. Such tumours include neuroblastoma and pheochromocytoma both derived from the sympathetic nervous system; and the Ewing's sarcoma family of tumours (ESFTs) derived from the parasympathetic nervous system (Kitlinska, 2007a).

4.1.1. Neuroblastoma

Amongst the autonomic system-derived tumours, neuroblastoma produces the highest amount of NPY, with serum NPY levels in neuroblastoma patients being 10 times higher than those in healthy controls (352 ± 99 pM versus 36 ± 4 pM) (Kogner et al., 1990). Moreover, the high levels of serum NPY in neuroblastoma patients have been linked to poor prognosis (Kogner et al., 1994). The majority of neuroblastomas expresses the Y2 and Y5 receptors and can often be distinguished from ESFTs that express the Y1, Y5 (Kitlinska et al., 2005) and Y4 receptors (Li & Ritter, 2005). NPY-mediated Y2 and Y5 signalling promotes neuroblastoma growth in vitro (Kitlinska et al., 2005; Kitlinska, 2007b) and in xenografts in vivo (Kitlinska, 2007b) via the activation of the p44/42 mitogen-activated protein kinase pathway (Kitlinska et al., 2005). Furthermore, NPY also promotes vascularisation by the activation of Y2 and Y5 receptors on endothelial cells (Ekstrand et al., 2003; Kitlinska et al., 2005), resulting in a continuous supply of nutrients to these fast growing tumours. Indeed, human neuroblastoma cells injected into immune compromised nude mice exhibited significantly reduced tumour growth and decreased tumour vascularisation (Lu et al., 2010) when receiving Y2 receptor antagonist treatment. Using Y2 (or Y5) receptor antagonists could therefore be a strategy to treat neuroblastomas and at the same time may have beneficial effects on cancer induced weight loss (Fig. 3).

4.1.2. Pheochromocytoma

Although not all patients with pheochromocytoma have elevated serum levels of NPY (Allen et al., 1987; Takahashi et al., 1987; deS Senanayake et al., 1995), malignant pheochromocytoma (Kitlinska, 2007a) has consistently been shown to be associated with high NPY serum levels. Pheochromocytomas have been reported to express Y1, Y2, and Y5 receptor mRNA (Kitlinska et al., 2005). However, only the Y1 receptor protein was found to be expressed in these tumours (Kitlinska et al., 2005). Surprisingly, addition of NPY in vitro to pheochromocytoma cell cultures did not seem to have any effect on tumour cell growth (Kitlinska et al., 2005). This is most likely due to the high level of NPY expression in these tumour cells (100 ng/10^6 cells) which causes saturated Y1 receptor binding/signalling. Indeed, attenuating Y1 receptor signalling by the addition of a Y1 receptor antagonist significantly increased viable cells, suggesting that NPY may act on Y1 receptors in an autocrine fashion to exert pheochromocytoma growth inhibition (Kitlinska et al., 2005; Movafagh et al., 2006). Thus, activation of Y1 receptor signalling in pheochromocytoma would be a potential therapy for pheochromocytoma (Fig. 3).

4.1.3. Ewing's sarcoma family of tumours

Importantly, NPY inhibits ESFT cell growth by up to 40% in vitro (Kitlinska et al., 2005). This inhibition is likely to be mediated via Y1 and Y5 receptors because a Y1 and Y5 receptor antagonist act synergistically in blocking NPY mediated growth inhibition. The increased cell survival caused by Y1 and Y5 receptor antagonists suggests that NPY secreted from ESFT cells acts in an autocrine fashion to exert growth inhibition (Kitlinska et al., 2005). However, since ESFTs show a high incidence and high density of Y1 receptor expression (Korner et al., 2008), and since NPY-mediated ESFT tumour cell growth is partly mediated by Y1 receptors, the use of synthetic Y1 receptor agonists in combination with PYY neutralisation in vivo could be a successful strategy (Fig. 3). In addition to the Y1 receptor, ESFTs also express the Y5 receptor (Kitlinska et al., 2005). Therefore, the use of a Y5 receptor agonist in combination with a Y1 receptor agonist could synergize to decrease tumour cell growth in vivo.

4.2. Neuropeptide Y system in pancreas, prostate and breast cancers

4.2.1. Pancreatic cancer

Using ^{3}H-thymidine incorporation as a readout, it was shown that NPY and, to a lesser extent, PYY were able to induce proliferation of exocrine pancreatic carcinoma cells in vitro (Ramo et al., 1990). However, these data have been challenged by others (McFadden and colleagues) showing that the Y2 receptor agonist BIM-43004-1 and, to a lesser extent, PYY were able to inhibit tumour cell growth in vitro and in xenografts in vivo using the same cell line (MiaPCa-2) (Liu, et al., 1995a, 1995b, 1996; Heisler et al., 2000). Moreover, a therapy combining PYY or the Y2 receptor agonist BIM-43004-1 with either vitamin E (Heisler et al., 2000) or 5-fluorouracil and leucovorin (Liu et al., 1996) caused synergistic inhibition in vitro of pancreatic tumour cell growth and reduced the expression of epidermal growth factor on the tumour cells. This approach, however, would not be beneficial for pancreatic cancer patients with anorexia because increased PYY levels or specific Y2 receptor agonism would cause increased satiety, potentially worsening anorexia in these patients (Fig. 3).

4.2.2. Prostate cancer

It has been known for some time that prostate cancers express Y receptors (Mack et al., 1997). McFadden and colleagues provided the first report on the effects of PYY on prostate tumour cell growth. Using the PC-3 tumour cell line they demonstrated that PYY inhibited tumour cell growth, and that this inhibition was augmented when PYY was combined with biologically active vitamin E alpha-tocopherol succinate (Yu et al., 2002). In contrast, Magni and Motta (2001) found that prolonged treatment with NPY promoted the growth of prostate cancer cells (PC-3) and this effect was accompanied by a

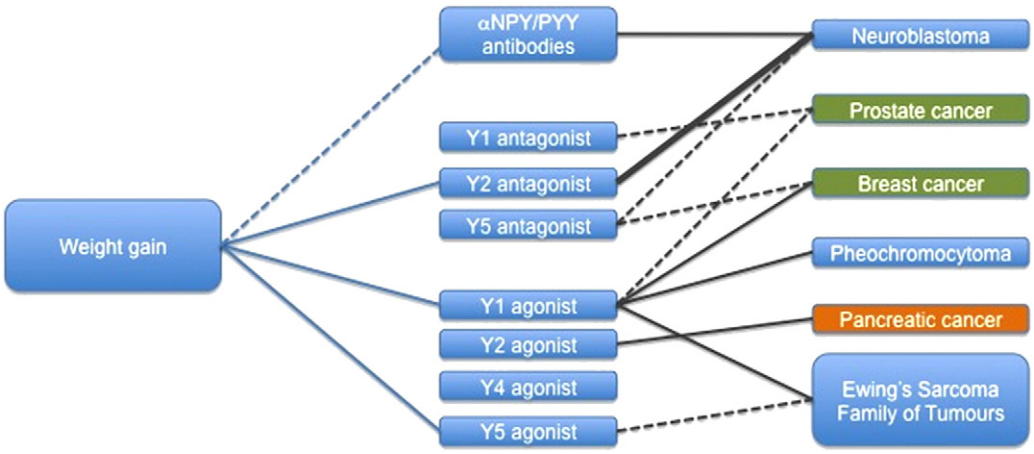

Fig. 3. Reducing cancer cell growth by various Y receptor agonists and antagonists. The solid thick line is a treatment strategy that has been confirmed in vivo. Solid thin lines are treatment strategies based on experimental in vitro data. Thin dashed lines are second best options or less likely alternative options based on either in vitro data or expression levels of Y receptors on tumours.

reduction of forskolin-induced cAMP accumulation and an increase of intracellular calcium concentration. Ruscica et al. (2006) confirmed this finding in PC-3 prostate cancer cells and further showed that in other prostate cancer cell lines, — LNCaP or DU145, NPY also had an inhibitory effect. The NPY-mediated cell growth inhibition could be blocked with a Y1 receptor antagonist, suggesting a critical role for the Y1 receptor in reducing cell growth in these two prostate cancer cell lines (Ruscica et al., 2006). The differences in the effects of NPY on different cell lines have been shown to depend on differences in mitogen-activated protein kinase signalling (Ruscica et al., 2007). This could be due to the fact that these cell lines are derived from different sources, such as brain (DU145), bone (PC-3) or lymph node (LNCaP) metastases instead of primary prostate cancer cells. Therefore it would be important to use primary prostate cancer cells to shed more light onto the effect of NPY/PYY signalling on prostate cancer cell growth and the potential therapeutic use of Y receptor agonists/antagonists in reducing tumour growth in vivo. As all three prostate cancer cell lines express the Y1 receptor (Ruscica et al., 2006), interference with Y1 mediated pathways seems to be a promising strategy for treating prostate cancers. A combination with neutralising antibodies towards PYY could be of further benefit to prostate cancer patients with anorexia.

4.2.3. Breast cancer

Another tumour class that is highly sensitive to NPY/PYY signalling is breast cancer. Using in vitro autoradiography, Reubi et al. (2001) showed that 85% of primary breast tumours were predominantly Y1 receptor positive, as were 100% of lymph node metastases derived from NPY-positive primary tumours. In contrast, non-neoplastic human breast cells expressed preferentially the Y2 receptor, suggesting that upon neoplastic transformation, breast cells switch from predominantly Y2 expression to predominantly Y1 expression (Reubi et al., 2001). In vitro studies showed that micromolar amounts of PYY were able to reduce tumour cell growth by 40% in the oestrogen-dependent breast cancer cell line MCF-7 (Grise et al., 1999). Addition of vitamin E synergized with PYY in inhibiting MCF-7 tumour cell growth in vitro (Heisler et al., 2000). PYY was also very effective in vivo in reducing the MCF-7 tumour weight by 50% and the tumour volume by a third (Grise et al., 1999). As MCF-7 cells express Y1 and Y5 but not Y2 receptors (Sheriff et al., 2010), PYY-mediated growth inhibition is most likely mediated via Y1 and/or Y5 receptor signalling. Strikingly, when the breast cancer BT-549 was used – expressing only the Y5 receptor – PYY-mediated Y5 receptor stimulation resulted in tumour cell growth, whilst a Y5 receptor antagonist (CGP71683A)-induced apoptosis in a time-dependent manner (Sheriff et al., 2010). Y1 receptor agonism might therefore be the best strategy to target (most) breast cancers in vivo, as almost all breast tumour cell lines (Sheriff et al., 2010), tumours and metastases (Reubi et al., 2001) express this Y receptor. As is true for prostate cancer, in vivo studies on breast cancer need to be performed in a standardised fashion, so that consistent and reliable results are obtained. Employing specific Y1 receptor agonists combined with measures to reduce circulating PYY levels could be a promising strategy to manage both breast cancer and any associated anorexia.

4.3. Summary 2

The use of molecular techniques and the generation of highly selective Y receptor ligands have enabled scientists to better characterise the expression levels of Y receptors in different tumours and to confirm that these receptors are biologically active. Furthermore, the use of NPY/PYY analogues has led to a better understanding of the downstream pathways of Y receptor activation and has elucidated the biological effects of Y receptor activation in different tumour cell lines. Interestingly, NPY/PYY mediated activation of Y receptors can have either growth inhibitory or growth stimulatory effects, depending on which tumour type is tested. Therefore agonists as well as antagonists for Y receptors could be used to target cancers cells. However, it will also be important to consider what consequences such treatments have on energy homeostasis and if this could actually be used to target cancer associated anorexia at the same time. More work is certainly needed in this area but the next decade will tell us if targeting the NPY ligands and/or Y receptors can provide effective treatment strategies for different cancers and cancer-associated anorexia.

5. Conclusions and future directions

The NPY 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 to treat disorders due to disrupted energy balance such as obesity and cancer anorexia. Strong emphasis has been put on identifying which Y receptor(s) would be the best targets to reduce food intake either via inhibiting appetite or increasing satiety. With the emerging role of the NPY system in controlling other aspects of energy homeostasis such as energy expenditure and oxidative fuel selection, greater anti-obesity benefits may be achieved by targeting Y receptors modulating both sides of the energy balance equation. Importantly, in light of the recent demonstration of the important role of peripheral Y receptors in the regulation of energy homeostasis and lipid metabolism,

selective Y receptor ligands without central penetration would be useful for in vivo studies to evaluate the anti-obesity potential of peripheral Y receptor-based therapeutics. In addition, since a sophisticated multi Y receptor system has evolved to mediate the various effects of NPY family peptides, combined therapies targeting multiple Y receptors and/or multiple systems involved in the regulation of energy balance may be required to more effectively achieve weight loss in obesity or weight gain and increase in appetite in anorectic patients. Furthermore, considering the relatively long period of time that is normally required to achieve weight loss, long-term effects of Y receptor-based anti-obesity therapies on bone health need to be evaluated in addition to their anti-obesity benefits. Moreover, whereas the effects of Y receptor-mediated signalling on tumour growth needs to be assessed in more specifically in vivo models, it opens up new avenues for treating cancer as well as cancer anorexia separately or at the same time.

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