Y2, Y4 receptors and obesity

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Abstract

The neuropeptide Y system - comprising neuropeptide Y, peptide YY, pancreatic polypeptide and the Y receptors through which they act (Y1, Y2, Y4, Y5 and y6) - has been at the center of attention with regards to regulation of feeding behavior and its possible involvement in obesity. In the past, research has focused mainly on the orexigenic and obesogenic action of this system, with Y1 and Y5 receptors being prime candidates as mediators of neuropeptide Y-induced hyperphagia and obesity. However, in recent years, the role of other members of the neuropeptide Y family, peptide YY, pancreatic polypeptide and the Y2 and Y4 receptors through which they predominantly act, have commanded increasing attention on account of their effects to mediate satiety and promote weight loss via actions in key brain structures, such as the arcuate nucleus of the hypothalamus and the brain stem. This review focuses on the role of peptide YY- and pancreatic polypeptide-like compounds as possible antiobesity drugs, taking into account their effects, not only on energy balance, but also in the regulation of bone formation, and highlights potential benefits of using Y2 and/or Y4 antagonists (as opposed to agonists such as peptide YY or pancreatic polypeptide) in the treatment of obesity.

Obesity, defined as a body mass index (BMI) of above 30 kg/m2 , is raising serious concerns as its accelerating prevalence in both developing and developed countries reaches epidemic levels [1]. Worldwide, approximately 1 billion people are obese or overweight and 10% of children are estimated to be above normal body weight, according to the WHO [2]. Obesity is a major contributor to morbidity and mortality, with fat deposition in the abdominal area being a particular risk factor for Type II diabetes and cardiovascular disease [1]. In the USA, obesity accounts for 280,000 deaths annually and is one of the primary causes of premature death [3].

Despite significant increases in our understanding of energy homeostasis, to date, there are few safe and effective pharmacological treatments for obesity [4]. The only three currently approved antiobesity drugs, sibutramine (Meridia® /Reductil® ; Knoll Pharmaceutical Co.), orlistat (Xenical® ; Roche) and rimonabant (Acomplia® ; Sanofi-Aventis) appear to be only modestly effective in promoting weight loss [5,6]. So far, the most effective treatment for morbid obesity (BMI: >40) is gastric bypass surgery, which acts in part by reducing appetite in association with alterations in circulating concentrations of gut hormones [7-9]. Circulating gut hormones secreted or suppressed after food consumption act in the brain as satiety or hunger signals. One potential therapeutic strategy is to develop drugs that can both mimic and enhance the body's own satiety signals, thereby harnessing the endogenous mechanisms that suppress appetite and caloric intake [10].

Neuropeptide Y & obesity

A large number of neurotransmitters and hormones, and a variety of different neural pathways, have been shown to play a role in the regulation of energy homeostasis and body weight [11]. The major regions in the CNS where the regulation of energy homeostasis takes place are the hypothalamus and certain brain stem nuclei. One of the most abundantly expressed neuropeptides in the hypothalamus is neuropeptide Y (NPY), a 36-amino acid peptide that is implicated in the etiology of obesity and insulin resistance owing to its orexigenic and metabolic effects [12-14]. The majority of the known hormones and peptides that regulate energy homeostasis exert an important component of their effects via actions on the NPY system [11], demonstrating the pivotal role of NPY in coordinating this important physiological process. Within the hypothalamus, NPY is produced most abundantly in the arcuate nucleus (ARC), which has connections to most other areas of the hypothalamus, including the paraventricular nucleus (PVN) and dorsomedial nuclei [15]. NPY neurons in the ARC are sensitive to peripheral signaling molecules since this nucleus lies in an area with a semipermeable blood-brain barrier [16]. NPY

levels in the hypothalamus are linked temporally with the onset of the daily feeding pattern [17]. Upregulation of NPY signaling in the ARC-PVN neural axis is responsible for hyperphagia evoked by conditions of negative energy balance, such as fasting and during lactation [18-20]. Intracerebroventricular (ICV) or direct hypothalamic administration of NPY to normal rodents leads to massive hyperphagia, accelerated body weight gain, hyperleptinemia, hypercorticosteronemia, hyperinsulinemia, decreased circulating concentrations of insulin-like growth factor (IGF)-1 and increases in white adipose tissue weight [14,21,22]. Similarly, a robust obesity syndrome is induced following viral vector-mediated chronic overexpression of NPY in the hypothalamus [23]. These data suggest that the observed increases in hypothalamic NPY content in genetically obese rodents, such as fa/fa and cp/cp rats, as well as ob/ob mice [24-26] are a causative factor in their obesity syndromes.

Circulating NPY levels were found to be elevated in obese hypertensive and diabetic women compared with healthy lean women [27]. Although single gene mutations that lead to obesity and Type 2 diabetes are rare, polymorphisms in various genes are commonly associated with these conditions. For example, linkage analysis has revealed an association between NPY and obesity in a population of Mexican Americans [28]. A recent study in two separate Swedish populations of normal and overweight individuals revealed that a leucine to proline variation in the signal peptide of NPY is associated with increased body mass [29].

NPY mediates its effects through the activation of at least five different G protein-coupled receptors: Y1, Y2, Y4, Y5 and, in mouse, also y6 [30], all acting in an inhibitory fashion, notably inhibition of cyclic adenosine monophosphate accumulation. Based on the appetite-stimulating and orexigenic effects of NPY, many pharmaceutical companies have moved ahead with the search for agonists and antagonists of Y receptors as antiobesity agents. Initial interests have been targeted towards developing antagonists of the Y1 and Y5 receptors, since analogues of NPY with high selectivity for these receptors strongly stimulated food intake [31,32]. However, attempts to suppress food intake and induce body weight loss via Y1 and Y5 receptor antagonism were not as effective as initially desired [31,33,34].

This review will concentrate on the Y2 and Y4 receptors that have been attracting increasing attention in recent years as emerging potential targets for antiobesity treatments following the observations that other members of the NPY family, the gut and pancreas-derived peptide YY (PYY) and its processed form (PYY3-36), as well as pancreatic polypeptide (PP), suppress appetite by stimulating Y2 and Y4 receptors, respectively [35,36].

Y2 receptor & obesity

Ligand studies in animals

Y2 receptors are distinguished from Y1 receptors by their higher affinity towards C-terminal fragments of NPY and PYY, which shares 70% identity with NPY. PYY and NPY share similar Y receptor-binding profiles, with greatest affinity for the Y2 receptors, followed by Y1, Y5, and least affinity for Y4 receptors [37]. By contrast, PP prefers the Y4 receptor to the rest of the Y receptors. PYY is a gut-derived hormone secreted from endocrine L cells of the gastrointestinal tract in proportion to caloric ingestion [38]. There are two forms of PYY: the full-length form, PYY1-36, and the truncated form, PYY3-36, which is produced by the cell-surface enzyme dipeptidyl peptidase (DPP)-IV. The full-length PYY binds to the Y1, Y2 and Y5 receptor, with lesser affinity for the Y4 receptor, whereas PYY3-36 predominantly binds to the Y2 and, to a lesser extent, also the Y5 receptor [39,40]. Thus, whereas Y2- and Y5-elicited effects remain, the transformation from full-length PYY to PYY3-36 removes any Y1 receptor-related effects.

In humans, PYY3-36 is the main form of PYY circulating after a meal, contributing to approximately 63% of circulating PYY in the fed state and only 37% in the fasted state [41]. PYY levels are lowest during fasting [42], but increase within 15 min of ingesting a meal, peaking at approximately 60 min after a meal and remaining elevated for up to 6 h [43]. The initial rise of PYY is thought to be due to an indirect neural/hormonal reflex involving cholinergic nerves, whereas the sustained release of PYY appears to be a direct effect of intraluminal contents acting on L cells [44]. PYY3-36 decreases gastric, intestinal and pancreatic exocrine secretion, and decreases gastric emptying [45].

Recent interest in the Y2 receptor as a potential drug target for obesity was supported by Batterham and colleagues' demonstration that PYY3-36 reduces food intake and/or body weight in animals and humans via hypothalamic Y2 receptor-mediated mechanisms [35]. Peripheral administration of PYY3-36 in the concentration range normally seen postprandially

inhibited feeding dose-dependently in 24-h fasted and freely feeding rats prior to the onset of the dark phase, suggesting a physiological role for PYY in the regulation of food intake [35]. To establish whether or not the suppressive effect on feeding by PYY3-36 was mediated via hypothalamic Y2 receptors, the authors injected PYY3-36 or the Y2 receptor-preferring agonist N-acetyl-[Leu28, Leu31]-NPY [24-36] directly into the ARC of fasted rats and demonstrated that both of these Y2 receptor ligands inhibited food intake dose dependently. Moreover, the anorectic effect of PYY3-36 was abolished in mice deficient of Y2 receptors, indicating the critical role of this receptor in the control of food intake [35]. More recently, it was shown that the anorectic effects of intravenously administered PYY3-36 on fasted rats were completely blocked by concomitant administration of the Y2 antagonist BIIE0246 [46]. However, the anorectic effect of PYY3-36 has raised significant controversy among different research groups, with some groups failing to replicate the same findings [47-49]. A recent review by Feletou and Levens provides further discussion concerning the Y2 receptor and body-weight regulation [50].

Compared with the many studies on the effects of acutely administered PYY, fewer studies have explored the long-term effects of PYY or Y2 agonists on food intake and weight gain. In one study, while PYY3-36 acutely suppressed fasting-induced food intake, 7 days of PYY3-36 administered via the intraperitoneal route twice daily to free feeding mice had no effect on cumulative food intake or body weight in mice [51]. A recent study showed that chronic ICV administration of the Y2-preferring agonist N-acetyl-[Leu28, Leu31]-NPY to mice via mini osmotic pumps produced a transient hypophagia and weight reduction, which returned to normal within 4 days [32]. However, percentage body fat was considerably lower in the Y2 agonist-infused mice following 6 days of drug administration [32]. Perhaps one of the most promising animal studies suggestive of a potential therapeutic application of PYY in the treatment of obesity is the investigation of the chronic effect of peripherally administered PYY3-36 infusion to various obese rodent models reported by Pittner and coworkers (2004) [52]. For the duration of the 4-week study, ob/ob mice exhibited a significant dose-dependent reduction in body weight independent of changes in food intake. In diet-induced obese C57/BL6 mice, chronic PYY3-36 infusion dose-dependently reduced food intake and body weight gain, as well as adiposity when compared with wild-type controls. Also, in fa/fa rats, chronic infusion of PYY3-36 for 8 weeks had a transient inhibitory effect on food intake, and body weight gain was significantly reduced. Two independent groups also demonstrated that PYY3-36 was effective in reducing food intake in both preobese and obese agouti A y mice [53,54]. These data show that the obese state is not associated with resistance to the actions of PYY3-36 and supports the therapeutic potential of PYY3-36, a Y2 receptor agonist, in the long-term regulation of food intake, body weight and adiposity.

It is known that NPY neurons in the ARC of the hypothalamus release an inhibitory transmitter, GABA, onto proopiomelanocortin (POMC)/cocaine and amphetamine-related transcript (CART) neurons, thereby inhibiting the anorexigenic and weight-reducing effects of the latter. It has been proposed that NPY neurons in the hypothalamus are inhibited by Y2 receptors expressed on these neurons, and this leads to reduced GABA transmission, thereby leading to activation of POMC/CART neurons and producing an anorectic response to central Y2 receptor agonism. After feeding, stimulation of the Y2 receptor on NPY neurons would be expected to have little effect on food intake, since both NPY and GABA release are at low levels and the prominent drive in this case would be α-melanocyte-stimulating hormone (MSH) release from POMC neurons. By contrast, in fasted animals, in which hypothalamic NPY expression is elevated, Y2 receptor activation would both directly inhibit NPY release in the hypothalamic paraventricular nucleus and remove the tonic inhibition of GABA on POMC/CART neurons, which would result in the release of α-MSH and decreased fasting-induced food intake. In keeping with this, many studies that have shown an inhibitory effect of PYY3-36 on food intake have used fasting as part of the experimental paradigm. The endogenous Y2 receptor ligand, PYY3-36, is thought to traverse the semipermeable blood-brain barrier in the region of the ARC to bind to Y2 receptors on arcuate NPY neurons [16]. Consistent with this hypothesis, it has been demonstrated that both PYY3-36 and a Y2 agonist inhibit NPY release and activate POMC neurons [35,55]. However, the current model remains incomplete as PYY3-36 was shown to inhibit food intake in both melanocortin-4 receptor knockout and POMC knockout mice [51,53,54], suggesting that additional mechanisms may be involved. A recent study also challenged the original model by demonstrating, in an electrophysiological slice preparation, that PYY3-36 potently and reversibly inhibits POMC neurons via postsynaptic Y2 receptors [56]. These data revealed a complex role for Y2 receptors in regulation of the NPY/POMC circuitry, as

they are present as inhibitory receptors on the orexigenic NPY neurons, as well as the anorexigenic POMC neurons. It was also demonstrated that the anorexigenic actions of PYY3-36 are not dependent on the vagus nerve, a common pathway of satiety signaling [57]. Rather, the authors proposed that peripherally administered PYY3-36 activates neurons in the area postrema and nucleus tractus solitarius, and causes conditioned taste aversion in mice, which may in part lead to an inhibition of food intake [57].

Animal models

Y2 knockout mice

The first study on germline Y2 receptor knockout reported increased food intake, fat mass and body weight accompanied with an attenuated response to leptin in female mice [58]. The phenotypic differences of male mice were not discussed in detail in this report. A later study by Sainsbury and coworkers showed that female germline Y2 knockout mice also had increased food intake; however, with reduced body weight [59]. Male Y2 knockout mice generated by Sainsbury and coworkers had transiently reduced food intake and a sustained decrease in body-weight gain associated with decreased adiposity at 16 weeks [59]. The discrepancies between the two germline knockout models may arise from the different background of the two mouse strains, as well as the different strategy targeting the Y2 gene, hence the completeness of Y2 receptor knockout. In addition, holding and test conditions (e.g., light cycles, single or group housing, number of mice per cage, food composition, time of day when tests are performed and way of sacrificing animals) can have significant influences on the outcomes of specific experiments [60]. Another important consideration in interpretation of changes in body weight observed in different Y2 receptor knockout models is the possibility of differential central and peripheral effects of this receptor. For instance, Sainsbury and coworkers showed that adult-onset hypothalamus-specific Y2 receptor deletion resulted initially in transiently increased food intake and transiently decreased body weight [59]. Further data from our laboratory on adult-onset hypothalamus-specific Y2 receptor knockout mice (this time using recombinant adenoassociated viral vector-mediated deletion of hypothalamic Y2 receptors) has shown a sustained increase in food intake accompanied by a steady and significant body-weight gain [61]. This is consistent with the notion that PYY3-36 acts as a satiety factor by interaction with hypothalamic Y2 receptors [35,42] and that lack of hypothalamic Y2 receptor signaling results in increased food intake and weight gain. These findings also suggest that lack of Y2 receptor signaling in nonhypothalamic tissues (i.e., in adipose tissue) could contribute to weight loss, as was observed in one germline Y2-receptor knockout model [59,62,63]. The hypothesis that agonism of hypothalamic Y2 receptors contributes to weight loss, whereas agonism of peripheral Y2 receptors has the opposite effect, would reconcile observations that both PYY3-36 - a Y2 receptor agonist - and germline Y2 receptor knockout have been shown to reduce body weight.

The effects of Y2 receptor ablation have also been studied in several obese rodent models. Crossing Y2-/- mice onto the ob/ob background attenuates the increased adiposity, hyperinsulinemia, hyperglycemia, increased hypothalamo-pituitary-adrenal axis activity and low circulating IGF-1 levels, and lean tissue mass of ob/ob mice, without affecting food intake or body-weight gain [64,65]. 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 [63]. Furthermore, increased body weight and adiposity in female mice owing to ovariectomy, a model mimicking the situation during menopause in women, was normalized by Y2 deficiency [66]. Finally, the Y2 receptor has been implicated in the obesity syndrome induced by chronic corticosterone administration since corticosterone-induced increases in adiposity, circulating leptin and insulin levels were ablated in Y2 knockout mice [59].

Collectively, these studies are consistent with a role of Y2 receptors in the hormonal and metabolic regulation of energy balance, and suggest that Y2 receptor antagonism may overall be beneficial for treating obesity. Since available data suggest that agonism of hypothalamic Y2 receptors (i.e., with PYY3-36 or PYY-like compounds) could also be beneficial in the treatment of obesity, the challenge for pharmaceutical companies developing antiobesity drugs may be to develop Y2 receptor agonists that target the hypothalamus and/or Y2 receptor antagonists that act in nonhypothalamic regions. However, in the design of antiobesity drugs, consideration must be given to possible side effects or added benefits. With this in mind it is particularly noteworthy that hypothalamus-specific Y2 receptor antagonism, while likely to promote long-term hyperphagia and weight gain in association with blockade of the satiety and weight-reducing

effects of PYY3-36, also promotes bone formation.

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 associated with a significantly enhanced bone formation rate [62,67]. In addition, hypothalamus-specific Y2 receptor deletion was shown to recapitulate this phenotype, demonstrating the importance of hypothalamic Y2 receptors in the regulation of bone physiology [67]. The potential of hypothalamic Y2 receptors as a target for novel anabolic treatments for osteoporosis was demonstrated in a study showing that hypothalamic Y2 receptor deletion in gonadectomized sex hormone-deficient adult male and female mice prevented ongoing bone loss, an effect attributable to activation of an anabolic osteoblastic bone formation response that counterbalances the persistent elevation of bone resorption seen in gonadectomized wild-type animals [66]. Interestingly, germline, but not hypothalamus-specific, deletion of Y2 receptors in this sex hormone-deficient model prevented the obesity syndrome that normally results from gonadectomy [67]. Taken together, these findings suggest that pharmaceutical agents that antagonize Y2 receptors in the hypothalamus, as well as in nonhypothalamic sites (e.g., in peripheral tissues), may confer dual antiobesity and antiosteoporotic qualities.

Peptide YY knockout mice & peptide YY transgenic mice

Following studies demonstrating PYY as a satiety factor with therapeutic potential for obesity, PYY knockout and transgenic mouse 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 studies administrating either PYY or PYY3-36. The first reported PYY knockout model exhibited normal growth, body weight, food intake, energy expenditure and responsiveness to PYY3-36 [68]. However, in this mouse model, the PP gene was also affected, suggesting that these mice are double knockout for both PYY and PP. Moreover, the founder colony used in this study was on 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 [68-70]. A later study by Batterham and coworkers showed that both male and female homozygous PYY-deficient mice were hyperphagic and developed marked obesity, suggesting that PYY ablation might cause obesity through increased food intake [10]. A third PYY knockout model by Boey and coworkers demonstrated late-onset obesity in female mice on a chow diet and an exacerbated diet-induced obese phenotype in male mice without changes in either basal- or fasting-induced food intake [71]. Interestingly, there was an increase in basal and glucose-induced serum insulin levels in both male and female PYY knockout mice in this study, raising a possible role for hyperinsulinemia in the development of increased adiposity associated with PYY deletion. In summary, two out of three 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 overexpression in PYY transgenic mice induces marked resistance to diet-induced obesity and significantly attenuates the obesity syndrome (including the increased adiposity and glucose intolerance) of genetically obese ob/ob mice in the absence of changes in body weight or basal and fasting-induced food intake [Boey et al. , submitted]. PYY transgenic ob/ob animals exhibited increased body temperature, enhanced hypothalamic expression of thyrotropin-releasing hormone mRNA and decreased brown adipose tissue depot weight, suggesting PYY-induced activation of the hypothalamo-pituitary-thyroid axis and increased thermogenic activity [Boey et al. , submitted]. These findings suggest that 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 recently shown to increase serum levels of thyrotropin (thyroid-stimulating hormone) in fasted rats [72].

While these data provide evidence for the possible use of PYY as an antiobesity agent, a caveat exists; transgenic overexpression of PYY significantly reduces bone mass [Boey et al. , submitted]. In addition, whereas PYY transgenic mice showed decreased bone mass in association with a significant decrease in circulating IGF-1 levels, PYY knockout mice showed an opposite phenotype of increased bone mass [Boey et al ., submitted] in association with increased circulating IGF-1 levels and enhanced hypothalamic expression of growth hormone-releasing hormone (GHRH) [71]. These findings suggest that PYY can inhibit bone formation via

centrally mediated inhibition of the somatotropic axis. This is likely to occur via Y2 receptors since our recent data showed colocalization of Y2 receptors on GHRH neurons in the ARC and ventromedial hypothalamic nuclei, and hypothalamus-specific Y2 receptor deletion prevented fasting-induced inhibition of hypothalamic GHRH expression [Lin et al. , submitted]. In addition to effects on bone via modulation of the somatotropic axis, PYY may also influence bone physiology via central Y2 receptor-mediated alterations in sympathetic output [73-75]. In summary, whereas PYY or PYY-like compounds that agonize central Y2 receptors may provide benefits in the treatment of obesity, long-term effects on bone health would need to be investigated carefully.

Human studies

Studies investigating the role of PYY3-36 in humans have provided fairly consistent messages. The few discrepancies found in these studies are mainly due to the current inability to distinguish levels of PYY from PYY3-36, which makes comparison between studies difficult. However, most studies show that both obese adults and children have significantly lower fasting and postprandial levels of circulating PYY when compared with lean controls, with fasting PYY levels being negatively correlated with BMI [8,42,76-78]. In obese children who underwent a 1-year weight-loss program, circulating PYY levels increased significantly in those children showing the most effective weight loss, but decreased in the subgroup of children who showed weight gain [76].

The acute anorectic effect of PYY3-36 has been demonstrated consistently in humans. A single 90-min infusion of PYY3-36 administered to nonobese people resulted in a decrease in appetite and food intake for up to 12 h postinfusion, resulting in a 33% decrease in cumulative caloric consumption in the 24-h period after the infusion [35]. In normal-weight volunteers, infusions of exogenous PYY3-36 increased fullness scores significantly and decreased food intake dose-dependently [78], providing strong evidence that PYY is an important factor influencing postprandial satiety. Similar efficacies of PYY3-36 on food intake suppression in obese subjects and lean subjects suggest that obesity does not induce peripheral resistance to PYY3-36 [42], consistent with animal studies [42,52].

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 [79]. Similarly, it was demonstrated that three common variants in the 5´ region of the Y2 receptor are associated with obesity in Caucasian Danish subjects [80]. A recent genetic case-control association study found that obese men had lower allele and homozygosity frequencies for a Y2 common allele, suggesting that this Y2 receptor variant is protective against obesity in Swedish men [81]. These data suggest that variations in functionality of PYY and Y2 receptor genes are involved in the etiology of obese phenotypes. In keeping with a role of PYY in the development of obesity, healthy people at risk of a subsequent development of obesity and Type 2 diabetes mellitus on account of a family history of diabetes demonstrate significantly lower fasting circulating PYY levels than control subjects [71].

Y4 receptor & obesity

Compared with other Y receptor subtypes, the involvement of Y4 receptors in obesity is the least studied. However, recent results point to a potential therapeutic action of Y4 receptor ligands in regulating food intake and energy homeostasis.

Ligand studies in animals

Currently, there are very few ligands selective for the Y4 receptor. The endogenous ligand for the Y4 receptor is pancreatic peptide (PP), which is secreted from F cells of the pancreatic islets following ingestion of food in proportion to the caloric intake [82] and in response to other signals such as hypoglycemia [83,84]. In addition to PP, NPY and PYY can bind the Y4 receptor, although with much lower affinity than PP. PP acts to inhibit pancreatic exocrine function, gall bladder contraction and stimulation of gastrointestinal motility and gastric acid secretion [85,86].

Recently, Asakawa and coworkers demonstrated that peripheral administration of PP at physiological doses to mice induced a rapid and persistent reduction in food intake in association with slower gastric emptying, and an increase in energy expenditure indicated by increased

sympathetic activity and oxygen consumption [36]. These effects of PP were associated with reduced hypothalamic expression of the orexigenic hormones, NPY, orexin and gastric ghrelin, along with upregulation of the anorexigenic peptide, urocortin, in the hypothalamus [36]. PP reduced leptin expression in white adipose tissue and blunted the hypothalamic expression of corticotropin-releasing hormone [36]. Importantly, responsiveness to exogenous PP was maintained in the obese state, with repeated administrations of PP significantly decreasing body weight gain and ameliorating insulin resistance and hyperlipidemia in both ob/ob and FLS-ob/ob obese mice [36]. While these results demonstrate that endogenous PP is a potent satiety and antiobesity factor, its short half-life of approximately 6 min in plasma may limit its use per se as a candidate for antiobesity treatment [87]. To address this problem, Balasubramaniam and coworkers dimerized Trp-Arg-Nva-Arg-Tyr-NH2 using various diaminodicarboxylic acids containing either di-, tri- or tetramethylene spacers to develop a series of Y4 receptor-selective agonists [88]. Injection of one of these Y4 receptor-selective agonists, the one that exhibits the greatest affinity for Y4 receptors, potently inhibited food intake in fasted wild-type mice [88]. Moreover, this compound inhibited food intake in wild-type, but not in Y4 receptor knockout, mice, confirming that the actions of this Y4 receptor agonist on food intake are specifically mediated by Y4 receptors [88].

Animal models

Y4 receptor knockout mice

Contrary to the expected increase in food intake and body weight gain based on the hypophagic and antiobesity effects of PP, germline deletion of the Y4 receptor leads to reduced food intake and significant reduced bodyweight in mice [89]. One explanation for this phenotype could be the compensatory strongly elevated plasma levels of PP observed in Y4 knockout mice, possibly owing to a lack of feedback inhibition of this pathway, and exertion of PP-mediated antiobesity effects via non-Y4 receptor pathways [89]. Another potential factor contributing to the leanness of Y4 receptor knockout animals is the increased energy expenditure on activities related to reproductive functions. Y4 receptor deletion lead to fat loss associated with increased gonadotropin-releasing hormone expression and improved fertility and lactation in ob/ob mice [89]. Furthermore, new data from our laboratory show a strong increase in motor activity in Y4 receptor knockout mice, potentially leading to increased energy expenditure and, thereby, contributing to the lean phenotype of these mice.

It is of considerable interest that whereas deletion of either Y2 or Y4 receptors results in significant reductions in adiposity and attenuation of various obesity syndromes [59,64-66,89], double knockout of both Y2 and Y4 receptors results in even greater (synergistic) reductions in adiposity and protection against genetic and diet-induced obesity [62,63]. Furthermore, whereas bone volume was increased approximately twofold in Y2 receptor knockout mice and not at all in Y4 receptor knockouts, Y2Y4 receptor double-knockout mice showed a threefold increase in bone volume associated with enhanced osteoblastic activity, suggesting a synergistic action of these two pathways to regulate bone formation. It is possible that the increased energy demand owing to greater growth of lean tissues, such as bone, and greater functionality of the reproductive axes may collectively contribute to the synergistic reduction in adiposity observed in Y2Y4 receptor double-knockout animals. In summary, whereas generalized Y2 receptor antagonism may be a possible pathway for novel antiobesity and antiosteoporotic treatments, our findings with Y2Y4 receptor double-knockout mice suggest that dual antagonism of Y2 and Y4 receptors may provide even more benefits for weight loss and bone health.

Pancreatic polypeptide transgenic mice

Overexpression of PP in mice leads to the development of a lean phenotype accompanied by reductions of food intake and fat mass, and a reduced rate of gastric emptying [90]. These effects are reversible by intraperitoneal injection of PP antiserum, demonstrating that they are specifically due to the overexpression of PP [90]. There are no significant effects on water intake or oxygen consumption in these mice. These results confirm the role of PP as a postprandial satiety signal, which most likely acts on brainstem Y4 receptors to modulate sympathetic output [36,90,91]. Unlike PYY overexpression, which results in significant loss of bone mass [Boey et al ., submitted], PP overexpression had no significant effects on bone mass or aspects of bone physiology, such as osteoblast or osteoclast functions [62].

Human studies

Patients with Prader-Willi syndrome, characterized by childhood-onset hyperphagia and morbid obesity, consistently exhibit reduced circulating levels of fasting and postprandial PP [92]. By contrast, conflicting data exist for circulating PP levels in other forms of human obesity, with some studies showing a reduction [93,94] and other studies reporting no differences between obese subjects and lean controls [95,96]. Higher fasting circulating PP levels have been reported in patients with advanced malignant disease, suggesting that elevated PP concentration may contribute to the general lack of appetite in these patients [97]. Consistent with this finding, subjects with anorexia nervosa showed an increased diet-induced PP response [98,99]. Moreover, postprandial plasma PP concentrations are significantly less in obese than in lean subjects [100]. A recent study in Pima Indians demonstrated that an increase in postprandial PP levels was associated with decreased weight gain, but surprisingly, the same study showed that high fasting PP levels were associated with increased weight gain [101].

In 2003, Batterham and coworkers demonstrated the anorectic effects of intravenous PP administration in ten healthy volunteers in a randomized, double-blind, placebo-controlled crossover study [102]. Cumulative 24-h energy intake was reduced by 25%, indicating sustained inhibition of food intake. The precise mechanism for the anorectic effect of PP is still unclear, but is hypothesized to be due to direct activation of Y4 receptors in the area postrema [103]. Whether or not part of PP's anorectic effect is mediated by reduced gastric empting is still unclear, with one group reporting no effect on gastric emptying [102], while another reported an inhibition [104]. Importantly, it was demonstrated that subjects with Prader-Willi syndrome responded to the hypophagic effect of intravenous PP infusion [105], providing promise for PP-based ligands as treatment for obesity.

Summary & conclusions

A complex system has evolved to regulate food intake and to maintain energy homeostasis. However, the NPY system - comprising NPY, PYY, PP and the Y receptors - has proven to be one of the most important regulators in feeding behavior and energy homeostasis. In parallel to the development of the different NPY-related peptides, a sophisticated Y receptor system has evolved to mediate the various effects of these peptides. PYY and the Y2 receptor play a role in regulating appetite, food intake and glucose homeostasis. Recent data also suggest that PYY and Y2 receptors regulate bone mass, possibly via central modulation of the hypothalamo-pituitary-somatotropic axis. Studies performed thus far present a possible role for PYY as a treatment for obesity and diabetes, but it is important to first assess whether or not long-term administration of Y2 agonists, such as PYY or related compounds, has any detrimental effects, notably on bone health. In addition, data from animal models suggest that global Y2 receptor antagonism may provide antiobesity benefits and, moreover, provides potentially additional benefits by increasing bone mass through hypothalamic actions.

In addition to PYY and the Y2 receptor, there is some evidence that PP and the Y4 receptor also play a role in the regulation of energy homeostasis, and some work has been done to investigate PP or related Y4 receptor agonists as novel antiobesity treatments. However, an outstanding question remains as to whether or not global Y4 receptor antagonism - particularly combined with Y2 receptor antagonism - may provide even greater benefits for weight loss and bone health than Y2 or Y4 receptor agonism.

Expert commentary

A strong emphasis is put on identifying targets for reducing food intake, either via inhibiting appetite or increasing satiety. The NPY system is central to this problem. On the one hand, NPY in the hypothalamus acts as a strong stimulator of food intake under conditions of low energy storage and, on the other hand, the peripherally released hormones PYY and PP act as satiety signals to counterbalance NPY's action. From these observations, one would predict that Y receptors are an ideal target for intervention in the regulation of energy homeostasis. However, so far, no Y receptor-acting antiobesity pharmaceutical agent has become available. From all the evidence gathered so far, it is our view that the best target for a Y receptor-based antiobesity drug would be a Y2 receptor antagonist. Although actions of such a drug on Y2 receptors in the hypothalamic arcuate nucleus may actually cause an increase in food intake owing to blockade of

PYY's satiety and antiobesity actions, the net benefit is likely to be a reduction in adiposity owing to antagonism of Y2 receptors in nonhypothalamic sites, such as by acting directly in fat tissue. This outcome, combined with the concomitant increase in bone mass, is likely to outweigh any obesogenic effects of central Y2 receptor antagonism.

Again, contrary to the predicted increase in food intake and body weight owing to the lack of PP-mediated satiety, deletion of Y4 receptors in the germline causes a lean phenotype in mice. However, in this case, the effect on energy homeostasis is not extended to bone metabolism and is mostly restricted to effects on adipose tissue. From these results, a next step would be to develop a Y4 receptor antagonist for investigation as an antiobesity drug. This proposal is supported by the fact that double deletion of Y2 and Y4 receptors synergistically reduces body fat content and further increases bone mass even over the level seen in Y2 receptor knockout mice. Combination treatment of Y2 with Y4 antagonists might therefore be the best solution for maximum effect on reducing adiposity and increasing bone mass at the same time. However, it has to be considered what effects Y receptor-specific drugs may have on the CNS.

Five-year view

Studies on transgenic models of the NPY family of ligands and their Y receptors have provided a wealth of information on molecular and behavioral phenotypes over recent years and have finally defined more clearly some of the roles for the individual Y receptors. With the help of these knockout and transgenic models, important new insights have been revealed, particularly in the coordinated regulation of food intake, energy homeostasis, regulation of lean tissues such as bone, and reproduction. However, some refinement of the in vivo targeting mutagenesis techniques will be needed to circumvent problems such as compensation during development. The use of knockin strategies, which will also allow modification rather than complete inactivation of receptor or ligand functions, will be required. More importantly, this will allow selective deletion of the genes in question only in defined tissues or nuclei in adult animals, thereby avoiding complication due to whole body ablation.

Studies in knockout and transgenic animal models provide invaluable insights into the roles and functionality of the NPY system and, over the next 5 years, we envisage that emerging pathways and hypotheses will move towards testing in humans. In particular, whereas activities have been undertaken to test Y2- and Y4-acting agonists such as PYY- or PP-like compounds on weight loss in humans, it will be of great interest to determine whether or not Y2 and Y4 antagonists, either alone or in combination, have even greater effects to promote weight loss and maintain skeletal health in humans.

It is important to note that food intake is not the only determinant of how much fat an organism stores and, in future years, other aspects of energy homeostasis besides hunger and satiety are worthy of more attention. In particular, energy partitioning; the way energy is used in the body and finding compounds that alter how to direct energy into different tissues, might offer some relief to the obesity epidemic. The Y2 receptor system, with its dual role in the regulation of adiposity and bone formation could be an extremely important target. It will be interesting to investigate the role of this receptor in determining the fate of mesenchymal stem cells, which have the ability to develop into either adipocytes or osteoblasts. Y2 receptors could be key players determining the preference of these class of stem cells to develop down one or the other differentiation pathways (e.g., fat or bone).

Key issues

* Peptide YY (PYY) and pancreatic polypeptide (PP) are important satiety signals mediating their effect centrally via the Y2 and Y4 receptors, respectively.
* Obese subjects have reduced circulating levels of PYY and PP and these levels increase upon reduction in body weight.
* Low PYY levels are a predictor for the potential development of Type 2 diabetes and obesity.
* Whole-body ablation of Y2 or Y4 receptors leads to a lean phenotype and, in the case of Y2 receptor knockout mice, it also leads to a strong increase in bone formation and bone mass via actions on the hypothalamus.
* Deletion of both Y2 and Y4 receptors in Y2Y4 receptor double-knockout mice results in greater reductions in adiposity and greater increases in bone volume than in mice with a deficiency of either the Y2 or Y4 receptor alone.

* A novel strategy for antiobesity/antiosteoporotic treatments could be to coadminister Y2 and Y4 receptor antagonists.

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