The Review of DIABETIC STUDIES

REVIEW

Peptides and Their Potential Role in the Treatment of Diabetes and Obesity

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■ Abstract

It is estimated that 347 million people worldwide have diabetes and that over 1.5 billion adults worldwide are overweight. Predictions suggest these rates are increasing. Diabetes is a common complication in overweight and obese subjects, and in 2004, an estimated 3.4 million people died from consequences of high blood sugar. Thus, there is great interest in revealing the physiological systems that regulate body weight and blood sugar. Several peptidergic systems within the central nervous system and the periphery regulate energy homeostasis. A number of these systems have been investigated as potential treatments for obesity and the metabolic syndrome. However, manipulation of peptidergic systems poses many problems. This review discusses the peptidergic systems currently attracting research interest for their clinical potential to treat obesity. We consider first neuropeptides in the brain, including the orexigenic neuropeptide Y and melanin-concentrating hormone, and anorectic factors such as the melanocortins, ciliary neurotrophic factor, and neuromedin U. We subsequently discuss the utility of targeting peripheral gut peptides, including pancreatic polypeptide, peptide YY, amylin, and the gastric hormone ghrelin. Also, we analyze the evidence that these factors or drugs based on them may be therapeutically useful, while considering the disadvantages of using such peptides in a clinical context.

Keywords: gastrointestinal hormone · hypothalamus · neuropeptide · obesity · type 2 diabetes

Introduction

The metabolic syndrome is a collection of medical disorders that together contribute to an increased risk of developing diabetes and heart disease. It is estimated that 347 million people worldwide have diabetes, and in 2004, an estimated 3.4 million people died from consequences of high blood sugar [1]. The metabolic syndrome has many pseudonyms and definitions, and the underlying cause is not completely understood. Although the metabolic syndrome is comprised of many factors, most, if not all, can be improved with weight loss. Over 1.5 billion adults worldwide are overweight. Predictions suggest these rates are increasing. Excess weight and diabetes are conditions associated with a high morbidity and mortality [2].

Understanding the regulation of body weight is thus vital to identify potential therapeutic targets. The control of body weight involves a coordinated regulation of both food intake and energy expenditure to promote stable levels of energy storage in organs such as adipose tissue and liver. This process, referred to as energy homeostasis, is the result of integrating neural and hormonal signals from the periphery and neural signals within the central nervous system (CNS) to defend a relatively consistent body weight. The efficiency of this system is demonstrated by the fact that, following

a period of fasting, food intake increases until body weight recovers, and it returns to baseline levels [3]. The effects of incretins on the regulation of energy homeostasis are extensively discussed in other articles within this issue. The purpose of this article is to discuss other peptidergic systems involved in the regulation of energy homeostasis and their therapeutic potential.

Central peptides regulating energy homeostasis

Energy homeostasis is regulated by peptides synthesized and released within the CNS, which act as neurotransmitters, and by peptide hormones produced peripherally, for example by the gastrointestinal (GI) tract, which act either via the vagus nerve or directly on the CNS (see Figure 1). Within the CNS, the hypothalamus is a key region in the regulation of energy homeostasis. The brain stem also plays a role; it receives several signals:

1. Neural signals from the gut via the vagus nerve.
2. Hormonal signals.

Abbreviations:
α-MSH - alpha-melanocyte-stimulating hormone
ACTH - adrenocorticotropic hormone
AgRP - agouti-related peptide
AMRI - Albany Molecular Research Inc.
ARC - arcuate nucleus
BMS - Bristol-Myers Squibb
C57BL/6 - inbred strain C57 black 6
CNS - central nervous system
CNTF - ciliary neurotrophic factor
DIO - diet-induced obesity
GHS-R - growth hormone secretagogue receptor
GI - gastrointestinal
GLP-1 - glucagon-like peptide 1
ICV - intracerebroventricular
MC1R - melanocortin 1 receptor
MCH - melanin-concentrating hormone
MCH1R - MCH 1 receptor
mRNA - messenger ribonucleic acid
MSH - melanocyte-stimulating hormone
MTII - melanotan II
NDP - 4Nle-D-7Phe
NMU - neuromedin U
NMU-R1 - neuromedin U receptor 1
NPY - neuropeptide Y
PEG - polyethylene glycol
POMC - pro-opiomelanocortin
PP - pancreatic polypeptide
PYY - peptide YY
SNAP- 7941 - selective, non-peptide antagonist at the MCH receptor
STAT3 - signal transducer and activator of transcription 3

Subsequently, the brain stem signals to the hypothalamus to influence appetite.

The hypothalamus comprises several discrete nuclei. They are responsible for integrating and processing both central and peripheral signals of energy homeostasis, and for inducing the changes in feeding behavior and/or energy expenditure required to maintain overall energy homeostasis [4]. The hypothalamic nuclei communicate with each other and with other parts of the brain via release of specific neuropeptides. Some of these hypothalamic neuropeptide systems are putative targets for anti-obesity therapies. The potential systems and therapies are discussed in this article (see Table 1).

Neuropeptide Y

Neuropeptide Y (NPY) is a 36 amino acid peptide. It is produced in several hypothalamic nuclei and is important in controlling energy homeostasis. The NPY-containing neuronal population in the hypothalamic arcuate nucleus (ARC) is the best characterized. The ARC is located adjacent to the median eminence, which has an incomplete blood brain barrier, facilitating sensing of peripheral nutrients and hormones. NPY is expressed in the medial ARC where it is colocalized with another orexigenic neuropeptide, agouti-related peptide (AgRP) [5, 6]. ARC neurones expressing NPY project to several other nuclei, including the paraventricular nucleus, dorsomedial nucleus, and lateral hypothalamic area [7, 8]. They also express receptors for peripheral signals of energy homeostasis, e.g. ghrelin and leptin [9-11].

NPY is a member of the PP-fold peptide family that includes the gut hormones peptide YY (PYY) and pancreatic polypeptide (PP), which are discussed below. The members of the PP-fold family signal through the Y receptors, Y1-Y5. NPY is thought to physiologically increase food intake. Hypothalamic NPY expression is upregulated by fasting, and central administration of NPY increases food intake by acting at the Y1 and Y5 receptors [4]. Although NPY, Y1, and Y5 knockout models do not have the profound alterations in energy homeostasis which might be expected, this is thought to be due to developmental compensation [12-14]. Ablation of ARC NPY/AgRP neurones in adult mice results in hypophagia and weight loss [15-17].

Y1 and Y5 receptor antagonists have thus been designed to antagonize the effects of endogenous NPY on appetite and food intake. Several Y1 receptor antagonists have been administered to ro-

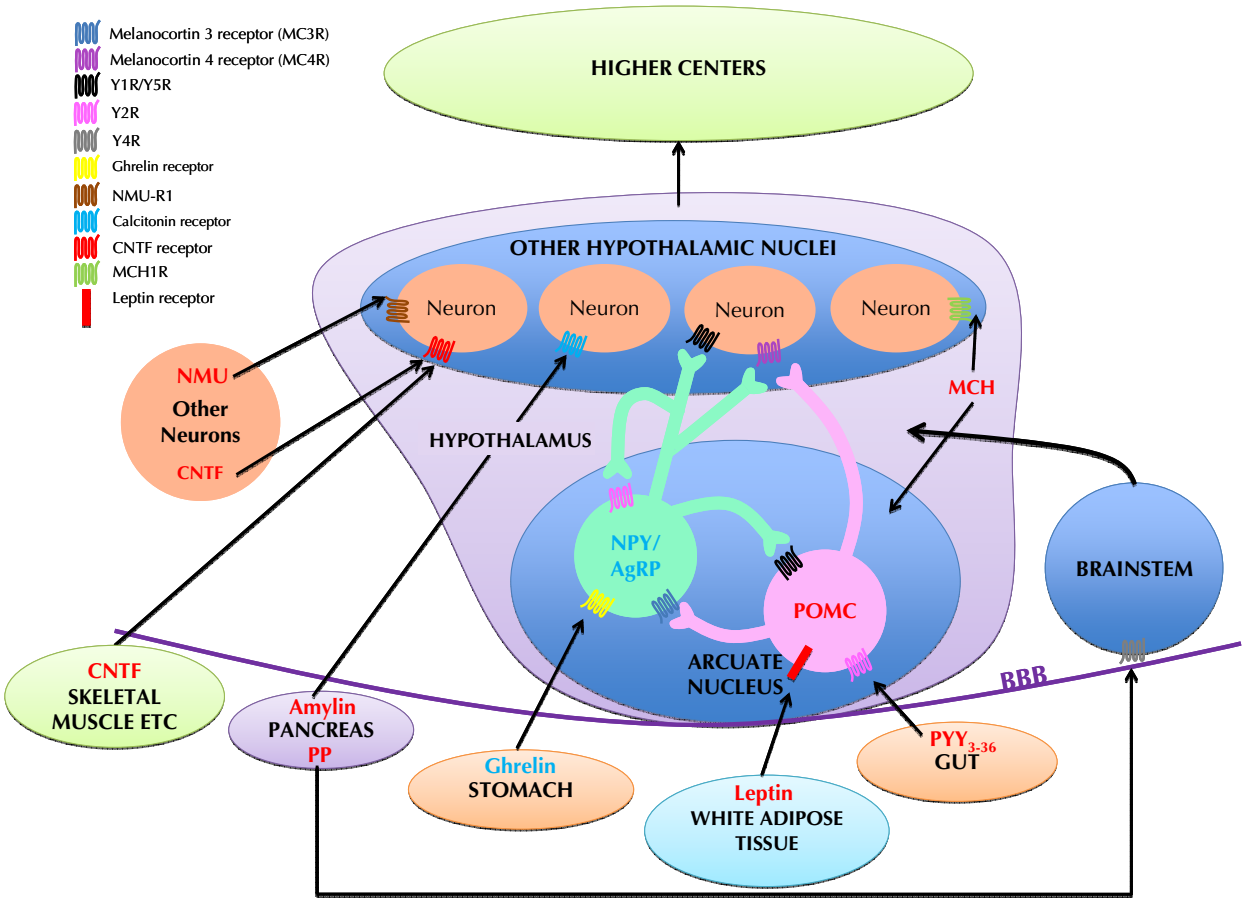

Figure 1. Schematic diagram illustrating the peptidergic signaling pathways regulating energy homeostasis. Peptides colored blue are orexigenic, promoting hunger, whilst peptides colored red are anorexigenic, promoting satiety. POMC: pro-opiomelanocortin. NPY: neuropeptide Y. AgRP: agouti-related peptide. NMU: neuromedin U. CNTF: ciliary neurotrophic factor. MCH: melanin concentrating hormone. PYY3-36: peptide YY3-36. PP: pancreatic polypeptide. BBB: blood-brain barrier.

dents, but most have associated safety concerns, and none have been tested in humans [18, 19]. The Y1 receptor antagonist J-115814 inhibits feeding in lean C57BL/6 mice and in hyperphagic *db*/*db* mice, which lack leptin signaling, and in mice with diet-induced obesity (DIO) [20, 21]. However, J-115814 potently interacts with the I_{Kr} potassium channel [18], suggesting that it could have cardiovascular side effects. Another Y1 receptor antagonist, BMS-193885, attenuates both NPY-induced and spontaneous nocturnal feeding following intraperitoneal administration to rats [19]. Chronic administration of BMS-193885 suppressed feeding and body weight gain. However, although no behavioral side effects were identified, a number of rats fell ill following chronic administration [19]. Also, the drug is not orally available and induced adhesive peritonitis in these rats following intraperitoneal administration due to poor tissue absorption [19].

Several Y5 receptor antagonists have been demonstrated to be effective at reducing DIO, whilst others have shown no effect, as reviewed in [22]. Many of those which are effective have unwanted side effects [22]. Nonetheless, some Y5 receptor antagonists have been tested in humans. The orally active Y5 receptor antagonist MK-0557 induced weight loss statistically greater than placebo control after one year of treatment, but the magnitude of this effect was not clinically meaningful [23]. The orally active Y5 receptor antagonist S-2367 resulted in a small reduction in body

Table 1. Drugs targeting peptidergic systems recently investigated for their potential as anti-obesity therapies

Drug [reference]	Mechanism of action	Stage of development	Efficacy in humans	Safety issues
J-115814 [18, 20, 21]	Y1 antagonist	Pre-clinical	Reduced food intake for up to 2 hours*	Potential cardiovascular toxicity*
BMS-193885 [19]	Y1 antagonist	Pre-clinical	Reduction in food intake and chronic administration suppresses feeding and body weight gain*	Poor tissue absorption inducing peritonitis*
MK-0557 [23]	Y5 antagonist	Phase III completed	Randomized controlled trial showed no meaningful effect	None reported
S-2367 [22]	Y5 antagonist	Phase IIb completed	Small but significant reduction in body weight in obese volunteers	Well tolerated, but small reduction in red cell count, hemoglobin, and hematocrit in some volunteers
Obinepitide [101]	Y2/Y4 agonist	Phase I/II	Reduction in food intake up to 9 hours after subcutaneous dose	Safe and well tolerated, no side effects reported
TM30339 [100]	Y4 agonist	Phase I/II	High efficacy in body weight reduction in diet-induced obese mice*	None reported
ACTH/MSH_{4-10} [42-44]	MC4R agonist	Pre-clinical	Reduction in body weight achieved, but not in obese volunteers nor in children with a POMC deficiency	None reported
MK-0493 [46]	MC4R agonist	Failed in phase I	No reduction in energy intake or body weight	Generally well tolerated, but some nausea and vomiting reported
ALB-127158(a) [63]	MCH1R antagonist	Phase I completed	Reduction in hunger measures and energy intake	Well tolerated
Axokine [69]	CNTF analogue	Abandoned in phase III	Reduction in food intake and body weight, but development of antibodies in 70%	Mild injection site reactions, and development of antibodies precludes use as this may cause neuroprotective issues
NMU [72-74]	Physiologically anorexigenic peptide	Pre-clinical	Reduction in food intake and increased energy expenditure*	Potential role in other systems; so side effects likely*
PEGylated Y2 [97]	PEGylated Y2 agonist	Pre-clinical	Prolonged reduction in food intake	None reported
Symlin + metrepeltin combination therapy [110]	Calcitonin receptor and leptin receptor agonism	Abandoned in phase II	Greater weight loss in obese subjects than with either alone; reduction of 12.7% over 24 weeks	Mild to moderate transient injection site adverse events and nausea

Legend: All agents are believed to act in the central nervous system, in particular on the hypothalamus. * There are no published data regarding administration in humans; so animal data is given.

weight compared to placebo-treated controls when administered to obese subjects in conjunction with a 500-800 kcal/day energy deficit [22]. S-2367 may be more effective at reducing body weight in conditions where NPY signaling might be expected to be higher, for example in *ob*/*ob* mice and in caloric restriction [22]. S-2367 initially appeared to be well-tolerated in humans. However, some studies reported a small, statistically insignificant, reduction in erythrocyte count, hemoglobin and hematocrit, raising concerns of a potential risk of anemia [22].

Therefore, it remains unclear whether antagonizing endogenous NPY receptor signaling will be useful for the treatment of obesity. NPY is the most abundant neuropeptide in the CNS. It is dif-

ficult to specifically target its hyperphagic action because of its wide range of functions. Therefore, it remains problematic to produce an agent that targets NPY signaling to reduce food intake without causing side effects.

Melanocortinergic signaling

The hypothalamic melanocortin system has a key role in the regulation of energy homeostasis [24]. Melanocortins are peptides produced from the gene encoding pro-opiomelanocortin (POMC). They exert their biological effects via five melanocortin receptors (MC1R-MC5R), both in the periphery and the CNS [25]. POMC mRNA levels are decreased in food-deprived rats [26]. Intracerebroventricular (ICV) administration of alpha-melanocyte-stimulating hormone (α-MSH), a 13 amino acid peptide product of POMC which acts as a ligand for the MC3R and MC4R, reduces food intake in rats [27-29]. The physiological importance of melanocortin signaling in body weight maintenance has been demonstrated using a number of animal models; knockout of POMC or MC4R results in obesity [30, 31]. It is thought that α-MSH provides a physiological anorectic tone at the MC4R that restrains food intake. Deletion of MC3R does not result in obesity, but does influence energy homeostasis [32, 33].

Melanotan II (MTII), a melanocortin receptor agonist originally developed as a tanning agent, reduces food intake when injected peripherally to rodents, suggesting it can cross the blood brain barrier [34]. The effects of MTII on food intake appear to be mediated via the MC4R; MTII has approximately a five-fold higher potency at the mouse MC4R versus the MC3R, and MTII does not have anorectic effects in MC4R null mice [35]. Most studies that pharmacologically manipulate central melanocortin signaling to reduce food intake have been conducted in rodents. There is only one published study in non-human primates which shows an anorectic effect of ICV NDP-MSH, a stable α-MSH analogue [36].

The melanocortin system is also important in humans. Individuals with mutations in POMC are hyperphagic and obese, and haploinsufficiency of MC4R has been shown to be the most common known monogenic cause of human obesity [37-39]. Other genetic mutations in melanocortinergic pathways have been linked with obesity [40, 41]. Despite this convincing evidence that MC4R is critical in energy homeostasis in humans, it has not been demonstrated that administration of an MC4R agonist can treat human obesity. Intranasal administration of ACTH/$MSH_{4\text{-}10}$ (representing the core sequence of all melanocortins) reduces food intake in normal weight males, but is ineffective in obese males, and chronic treatment does not reduce body weight in children with POMC deficiency [42-44]. It is therefore possible that obesity induces resistance to the effects of MC4R agonists, worsening obesity and invalidating MC4R agonism as a potential therapy. However, it must be noted that ACTH/$MSH_{4\text{–}10}$ lacks potent activity at MC4R [45]. In a 12 week study of obese human subjects, potent agonism of MC4R with MK-0493 did not reduce energy intake or body weight compared to placebo [46]. This is further evidence that melanocortin resistance may occur, although the compound was not tested in lean individuals. Also, MC4R agonism has been shown to induce off target effects in humans, including detrimental effects in blood pressure and sympathetic function [47]. Further research is required to determine whether such side effects limit the use of MC4R agonists for the treatment of obesity, or whether it is possible to selectively target MC4R appetite pathways.

Melanin-concentrating hormone signaling

Melanin-concentrating hormone (MCH) was originally isolated from the pituitary gland of teleost fish, in which it controls skin pigmentation [48]. MCH was subsequently discovered to be expressed in the hypothalamus and zona incerta [49]. MCH is overexpressed in the hypothalamus of rodents following fasting and in obese *ob*/*ob* mice [50]. Central administration of MCH increases food intake in rats [50]. Also, mice which lack the precursor to MCH are hypophagic and lean [51]. The MCH receptor MCH1R is widely expressed in central and peripheral tissues. The expression pattern of MCH1R suggests that MCH is involved in many physiological processes [52, 53]. Mice with targeted deletion of MCH1R are hyperphagic [54], implicating this receptor in the regulation of energy homeostasis. A further MCH receptor, MCH2R, has been identified in humans, but is not expressed in rodents [55, 56].

In 2002, two papers reported that small molecule antagonists of MCH1R might be useful in the treatment of obesity [57, 58]. Acute intracerebroventricular administration of SNAP-7941 or T-226296 decreased MCH-induced food intake in rats, and chronic administration of SNAP-7941 reduced body weight in rats with DIO [57, 58]. Several MCH1R antagonists have since been created and shown to be effective at reducing body

weight in animal models of DIO, as reviewed in [59].

These effects may not be exclusively mediated through the CNS. Peripheral MCH signaling modulates leptin secretion and insulin release [60, 61]. Systemic MCH1R antagonism may thus modulate energy homeostasis via both central and peripheral mechanisms. MCH1R antagonist-induced weight loss is suggested to be driven by increased energy expenditure in addition to reduced food intake. Administration of an MCH1R antagonist to mice moderately suppressed feeding but resulted in a disproportionately large reduction in body weight [62]. Pair feeding to the MCH1R-treated group resulted in a smaller reduction in body weight, suggesting that MCH1R antagonism also has effects on energy expenditure [62].

One MCH1R antagonist, ALB-127158(a), has recently completed Phase I trials in humans, with promising results. Reports from the pharmaceutical company AMRI have stated that ALB-127158(a) dose-dependently reduced food intake in DIO mice in preclinical testing. The resultant weight loss of up to 18% after 28 days of administration was substantially higher than that achieved with sibutramine. Weight loss correlated with a reduction in food intake, a preferential reduction in fat stores, and improvements in glucose tolerance. At the doses tested in humans, ALB-127158(a) was well tolerated, and subjects reported reduced appetite, hunger, and desire to eat. Subjects who received the compound consumed less at a test meal [63]. Further trials will identify whether ALB-127158(a) can reduce food intake in obese subjects, and whether it promotes weight loss when administered chronically.

Ciliary neurotrophic factor

Ciliary neurotrophic factor (CNTF) was initially described as having trophic actions on motor neurones in the ciliary ganglion, and to influence other motor neurone populations [64, 65]. Clinical trials were conducted to determine whether CNTF is beneficial in patients suffering from motor neurone disease in the mid 1990's. An unexpected outcome was that CNTF induced substantial weight loss. This finding was initially treated with caution, as it was suspected that CNTF might be acting in a manner similar to cachectic cytokines such as interleukin-1. However, subsequent studies suggested that CNTF may play a physiological role in food intake. CNTF and leptin signaling pathways share molecular components, including the STAT3 transcription factor [66, 67]. CNTF receptors are present in hypothalamic nuclei important in feeding, and CNTF causes preferential loss of fat in *ob*/*ob* mice [68]. Importantly, CNTF is also effective in DIO, a state which is similar to human obesity, and which results in resistance to the anorectic effects of leptin [68].

A phase III trial for the use of axokine, a modified version of human CNTF, for the treatment of obesity was initiated. Although there was a small improvement in some patients, approximately 70% of subjects developed antibodies against the drug after three months of treatment [69]. Those who did not develop antibodies lost around 12.5 pounds in a year compared to 4.5 pounds in the placebo group. However, it was feared that development of antibodies to a CNTF-derived molecule might reduce the neuroprotective effects of endogenous CNTF [69], and axokine was abandoned as an anti-obesity agent.

Neuromedin U

Neuromedin U (NMU) is a member of the neuromedin peptide family. It was originally isolated from porcine spinal cord and subtyped 'U' because of its potent ability to cause uterine contraction [70]. NMU is widely expressed throughout the body, with highest expression in the brain and GI tract [71]. NMU does not circulate, and is thought to act as a neuropeptide rather than as a hormone. Hypothalamic NMU mRNA levels are significantly reduced following fasting [72]. ICV administration of NMU reduces food intake in mice [73] and rats [72]. NMU administration also increases both physical activity and non-exercise activity thermogenesis in both DIO and lean rats [74]. NMU null mice display hyperphagia, increased body weight and adiposity, and decreased locomotor activity and energy expenditure, suggesting a role for this peptide in the regulation of energy homeostasis [75]. These mice also develop late-onset hyperglycemia and hyperlipidemia [75]. Transgenic mice that overexpress NMU are hypophagic, and have reduced body weight, low somatic and liver fat, and improved insulin sensitivity [76]. On a high fat diet, these mice remain lighter than wild types and eat less [76]. A variant in the human NMU gene has also been linked to obesity in the human population [77].

NMU signals via two receptors, NMU-R1 and NMU-R2. The distribution of these receptors has not been clearly defined. However, NMU-R1 is thought to be expressed predominantly in the periphery, particularly in the GI tract, while NMU-

R2 is expressed in hypothalamic nuclei known to regulate energy homeostasis [72, 78]. NMU reduces food intake when administered acutely or chronically, and these effects have been determined to require NMU-R1 signaling. The development of NMU-R1 agonists may therefore prove effective in the treatment of obesity [79]. As there is evidence that NMU increases energy expenditure and improves glucose tolerance, such agents might also have positive effects on other aspects of the metabolic syndrome [79]. However, NMU is implicated in the regulation of other physiological systems, including stress, and it is possible that the use of NMU-R1 antagonists may thus have side effects [80].

Peripheral peptides regulating energy homeostasis

A number of peripherally produced peptide hormones signal to the CNS both directly and indirectly, via the vagus nerve, to influence energy homeostasis. The GI tract is a major site of the production of hormones which influence energy homeostasis. Hormones produced in the gut such as glucagon-like peptide 1 (GLP-1) and oxyntomodulin, which reduce food intake and improve glycemic control, are discussed in other articles in this issue. However, the gut synthesizes and releases a number of other hormones that affect food intake. Peptide or protein hormones which regulate appetite are also produced in additional peripheral sites around the body, in particular the endocrine pancreas. These hormone systems and their potential as anti-obesity targets are discussed below.

PP-fold gut peptides

The PP-fold peptide family includes NPY and the gut hormones PYY and PP. The orexigenic effects of NPY are mediated by central Y1 and Y5 receptors, as discussed above. In contrast, the Y2 and Y4 receptors mediate anorectic effects of PYY and PP, respectively [81-83]. PYY is an anorectic gut hormone expressed in enteroendocrine L-cells throughout the GI tract, with highest tissue content in the terminal ileum, colon, and rectum [84, 85]. PYY exists in two forms. The amino terminal of the full-length peptide PYY1-36 is cleaved by the enzyme dipeptidyl peptidase IV (DPP-IV) to form the amino-terminally truncated form PYY3-36, which comprises the majority of PYY circulating immunoreactivity [86, 87]. PYY1-36 binds with relatively high affinity to all five Y receptors (Y1-Y5), whereas PYY3-36 is a selective Y2 receptor agonist [82].

PYY is released into the circulation following a meal at levels proportional to calories consumed [84], and circulating levels are reduced by fasting. Peripheral administration of PYY_{3-36} reduces food intake in wild-type rodents and lean and obese humans, but not in mice lacking the Y2 receptor [81, 88]. PP is synthesized and released postprandially from the endocrine pancreas in proportion to the calorie content of a meal, and is postulated to act as a satiety signal via activation of the Y4 receptor. Peripheral administration of PP to both rodents and humans also reduces food intake [89, 90].

While studies demonstrate that Y2 and Y4 agonists can reduce appetite acutely, the circulating half-lives of PYY and PP are short, suggesting that the native peptides may be difficult to use as agents to chronically reduce body weight. Also, it has been shown that central administration of PYY_{3-36} or PP in rodents stimulates appetite [91-94]. This is thought to be due to high local concentrations of these hormones pharmacologically stimulating orexigenic Y receptors. This suggests that sufficiently high peripheral doses of PYY and PP might result in orexigenic effects. In addition, the therapeutic window for PYY_{3-36} is relatively narrow, with low doses proving ineffective and high doses causing nausea [95].

Preprandial intranasal PYY_{3-36} was tested in obese patients for two weeks, but was not found to reduce body weight. Intranasal administration resulted in transiently high circulating levels of PYY associated with nausea [96]. PYY analogues selective for the Y2 receptor, and which show moderately increased but persistent circulating levels following administration, may be more useful in the treatment of obesity. A PEGylated selective Y2 receptor agonist has been shown to outperform PYY_{3-36} in reducing food intake in mice [97], suggesting that PEGylation of specific Y2 receptor agonists may improve their therapeutic potential.

PP has not been found to cause nausea so it is possible that PP will prove a more useful therapeutic target than PYY [90]. Also, PP-releasing neuroendocrine tumors are not associated with nausea [98]. A fatty acid acylated human PP analogue specific for Y2 and Y4 receptors had improved pharmacokinetic properties compared to a non-lipidated human PP analogue [99]. The lipidation also increased the duration of food intake reduction in mice, suggesting that it might be useful in the treatment of obesity [99]. Thus, structural modification and acylation have proved useful as

tools in increasing the efficacy of PP-fold gut peptides. The Y4 receptor agonist TM30339 has proved efficacious at reducing body weight in diet-induced obese mice, and is currently undergoing phase I/II trials [100]. The same pharmaceutical company is also carrying out phase I/II trials of a dual Y2 and Y4 receptor agonist, obinepitide, which has been shown to inhibit food intake in obese individuals up to nine hours after subcutaneous administration [101].

Amylin

Amylin, or islet amyloid polypeptide, is a peptide hormone secreted by pancreatic β-cells concurrently with insulin to synergistically control blood glucose levels [102]. It activates the calcitonin receptor when dimerized with a receptor activity-modulating protein [103]. In addition to its effects on blood glucose, peripheral administration of amylin reduces food intake in wild type, *ob*/*ob*, and *db*/*db* mice [104]. Rats receiving daily doses of amylin for 6 days prior to the dark phase had a reduced food intake and body weight [105]. Amylin also slows gastric emptying, and there is evidence that it enhances the action of other peptides such as cholecystokinin.

The clinical utility of amylin is restricted by its instability and its tendency to self-aggregate. Many diabetics are deficient in amylin. Pramlintide (symlin), a synthetic analogue of amylin which is soluble and non-aggregating, is currently approved to be used with insulin by type 1 and type 2 diabetics. Treatment of type 1 and type 2 diabetic patients with symlin at doses which mimic the plasma levels of non-diabetics for one year results in sustained weight loss [106, 107]. Symlin is currently being tested in non-diabetics as a treatment for obesity. One disadvantage of using symlin as an anti-obesity drug is that it has to be injected at meal times, rather than using more user-friendly administration protocols.

A recent study has suggested that amylin might be used in combination with leptin as a weight-loss therapy. Leptin is a protein adipostat hormone produced in adipose tissue which circulates at levels proportional to body fat to regulate energy homeostasis [108, 109]. Concurrent infusion of amylin and leptin to DIO rats reduces food intake and body weight, specifically fat mass [110, 111]. Similar results have also been demonstrated in humans; coadministration of symlin and the leptin analogue metreleptin to overweight/obese subjects results in a greater weight loss than either analogue alone [110]. It has been suggested that amylin might restore or enhance sensitivity to leptin in obesity [112, 113]. However, this development program has now been abandoned at phase II. It has been shown in rats that removal of either amylin or leptin results in regain of all body weight lost following amylin and leptin coadministration, indicating that both agents are required [114]. It is possible that using other anorectic gut hormones in combination with leptin might also demonstrate additive or synergistic effects. Combination therapies are an attractive strategy, as weight loss evokes powerful compensatory mechanisms which are responsible for recovering the lost body weight [115]. Targeting multiple signaling systems may attenuate the effects of these compensatory pathways. However, current drug-testing legislation does not facilitate the identification of novel effective drug combinations for the treatment of obesity, or indeed for any disease.

Ghrelin

Ghrelin is synthesized in the stomach and is the only gastrointestinal hormone known to stimulate appetite [116]. It was first discovered as the endogenous ligand for the growth hormone secretagogue receptor (GHS-R) [117]. Ghrelin is also expressed in the hypothalamic ARC and periventricular area [117, 118]. The GHS-R is expressed in many tissues throughout the body, including the gut, hypothalamus, and pituitary [119]. Ghrelin levels rise before meals and fall postprandially [120]. Acutely, central or peripheral administration of ghrelin potently stimulates feeding in rodents [121]. Chronic administration induces weight gain [116]. Intravenous ghrelin infusion increases energy consumption during a buffet meal in humans [122].

Ghrelin acts via the orexigenic ARC NPY neurones in the hypothalamus [123, 124], though it may also act on the vagal brainstem pathway in addition to having a direct effect [123]. It is also thought to target the mesolimbic dopamine system to enhance food reward. Ghrelin and GHS-R knockout mice are resistant to DIO. Ghrelin also inhibits glucose-stimulated insulin release. Therefore, antagonizing ghrelin signaling could improve glucose tolerance [125]. Blocking GHS-R signaling has thus been proposed as a treatment for obesity and diabetes.

Ghrelin spiegelmers (single stranded oligonucleotide 'mirror images' of the peptide to be inhibited) have been created to inhibit the function of endogenous ghrelin. Intravenous administration to rats of NOX-B11, a ghrelin spiegelmer, sup-

pressed ghrelin-induced neuronal activity and ghrelin-induced food intake [126]. Another novel approach to reduce the effect of circulating ghrelin is the use of anti-ghrelin vaccines. Rats injected with rat ghrelin linked to a large immunogenic carrier molecule mounted an immune response to ghrelin. After one week, rats with higher ghrelin-specific antibody titres gained less weight and had lower levels of body fat [127]. However, such vaccination is theoretically permanent and so there is a danger that too much body weight could be lost, with detrimental effects on health. A number of GHS-R antagonists have also been described, but have not been demonstrated to reduce food intake in animals.

Ghrelin is implicated in other physiological systems besides energy homeostasis, including growth, learning, and blood glucose regulation [117, 123, 125], which may result in side effects for agents modulating the ghrelin system. Although the animal evidence supporting the use of agents blocking GHS-R signaling is compelling, the efficacy of ghrelin antagonists has yet to be proven in clinical trials. Ghrelin levels are reduced in obese subjects [128], which may explain why it is difficult to reduce obesity using this strategy. It is possible that such agents may be useful in conditions of hyperghrelinemia such as Prader-Willi syndrome.

Conclusions

A number of peptidergic systems have been shown to influence food intake and energy homeostasis. Manipulation of several of these systems reduces food intake and body weight in preclinical models. However, in clinical trials, many of these agents failed to meaningfully reduce body weight [46, 69, 96]. This is due to a number of reasons, including lack of efficacy and side effects. These two issues are related; it may be impossible to administer such an agent at the dose required to reduce body weight because of the associated side effects. The relatively wide expression of many peptidergic system components, and their associated diversity of function, makes their manipulation for the treatment of obesity fraught with difficulties.

There are also difficulties with the routes of administration. For example, agents targeting CNS receptors must have a means of crossing the blood brain barrier. If a drug needs to be taken often, the route of administration becomes a patient issue. It is inconvenient and painful to have to inject a drug; orally active small molecule treatments are preferable. However, by their very nature, peptide hormones are difficult to mimic with small molecules. In particular, many gut hormone receptors require large ligands for receptor interaction, and residues essential for receptor interaction can be widely distributed throughout their secondary structure.

Furthermore, antibodies may develop against drugs based on peptides. This carries the risk that the immune response extends to the endogenous peptide, as shown for axokine. As most of these peptidergic systems have multiple functions, this could have extremely deleterious effects. Nevertheless, despite these difficulties, recent studies have produced promising results. Therefore, peptidergic systems remain an attractive research target in the fight against obesity.

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