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REVIEW

Can neuropeptides treat obesity? A review of neuropeptides and their potential role in the treatment of obesity

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Obesity is a major worldwide public health issue. The physiological systems that regulate body weight are thus of great interest as targets for anti-obesity agents. Peptidergic systems are critical to the regulation of energy homeostasis by key regions in the hypothalamus and brainstem. A number of neuropeptide systems have therefore been investigated as potential treatments for obesity. Blocking orexigenic peptide signals such as neuropeptide Y, melanin-concentrating hormone, orexins, relaxin-3 and galanin-like peptide or stimulating anorectic signalling pathways used by peptides such as the melanocortins, ciliary neurotrophic factor and brain-derived neurotrophic factor, are approaches that have shown some promise, but which have also highlighted possible concerns. Manipulation of central peptidergic systems poses a number of therapeutic problems, including brain access and side effects. Given that the homeostatic defence of body weight may limit the effectiveness of any single-target therapy developed, a combination therapy approach may offer the best hope for the effective prevention and treatment of obesity.

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Abbreviations
α-MSH, α-melanocyte-stimulating hormone; ACTH, adrenocorticotropic hormone; AgRP, agouti-related peptide; ARC, arcuate nucleus; BDNF, brain-derived neurotrophic factor; CART, cocaine and amphetamine regulated transcript; CNTF, ciliary neurotrophic factor; DMN, dorsomedial nucleus; EMEA, European Medicines Agency; FDA, Food and Drugs Administration; GALP, galanin-like peptide; LHA, lateral hypothalamic area; MCH, melanin-concentrating hormone; MSH, melanocyte-stimulating hormone; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; PP, pancreatic polypeptide; PVN, paraventricular nucleus; VMN, ventromedial nucleus

Introduction

Obesity is a major public health issue worldwide. It is estimated that more than 1.5 billion individuals are overweight and more than 400 million adults are obese (World Health Organization, 2011). These figures are anticipated to rise, with predictions that by 2050, 60% of men, 50% of women and 25% of children under 16 in the UK will be obese (Foresight Report, 2007). Increased weight predisposes to and can aggravate many clinical conditions, including cardiovascular disease, type II diabetes mellitus, restrictive lung disease, certain cancers and infertility (Kopelman, 2000). Obesity is a multifactorial condition in which genetic, environmental, behavioural and socio-economic conditions all contribute to determine body weight, adipose tissue mass and distribution (Bray, 1992).

Current therapeutic options

For a new drug to be considered efficacious in the treatment of obesity by the US Food and Drug Administration (FDA), it

must meet certain criteria in randomized controlled trials carried out over a minimum period of 1 year. The mean placebo-subtracted weight loss must be at least 5% from baseline, or alternatively, at least 35% of patients in the drug-treated group must lose more than 5% of baseline body weight (and the loss must be statistically significant and double that of the placebo group; FDA, 2007). The European Medicine Agency (EMEA) suggests that efficacy is demonstrated with a loss of at least 10% baseline body weight, statistically significant compared to placebo, following a minimum 1 year treatment period (EMEA, 2006).

Currently, the only approved drug for the long-term treatment of obesity in the UK is orlistat (Xenical), which reduces the absorption of dietary fat by inhibiting intestinal lipases (Hauptman *et al.*, 1992; Sjöström *et al.*, 1998). There are a number of anti-obesity drugs, which are internationally approved but which are not licensed for use in the UK. These include appetite-suppressing amphetamine analogues such as phentermine, which are only approved for short periods (less than 12 weeks) due to concerns regarding tolerance and dependency (Ioannides-Demos *et al.*, 2011). Qsymia, a combination of phentermine and topiramate, and lorcaserin, a selective 5-HT_{2C} receptor agonist (receptor nomenclature follows Alexander *et al.*, 2011), have recently been approved for the treatment of obesity by the FDA (FDA, 2012). Two drugs previously approved for the treatment of obesity, which target the CNS, have been withdrawn in recent years due to their unacceptable side effects. Sibutramine (Reductil), a 5-HT and noradrenaline reuptake inhibitor was withdrawn in 2010 due to its cardiovascular side effects including increased blood pressure and heart rate associated with an increased risk of stroke and myocardial infarction (James *et al.*, 2000; Kim *et al.*, 2003). Rimonabant, an inverse agonist for the cannabinoid CB_1 receptor was withdrawn in 2009 due to reports of severe depression and suicidal ideation (Van Gaal *et al.*, 2005; Topol *et al.*, 2010).

The withdrawal of these drugs illustrates the need for more selective drug targets in order to minimize adverse effects. Obesity is associated with numerous co-morbidities and any treatment for obesity is likely to be required long term; the therapeutic window of any new pharmacotherapy must therefore be closely scrutinized before approval. The classical neurotransmitter and endocannabinoid systems are widespread within the CNS, reflecting multiple physiological roles, and manipulation of these systems carries the risk of unwanted effects. Neuropeptides can act in the CNS as neuromodulators of the classical neurotransmitter systems. Typically expressed by discrete clusters of neurons, they project diffusely throughout the CNS, and modulate postsynaptic neurons in a way that can alter their responses to classical neurotransmitters. All CNS neurons that produce neuromodulators also release classical neurotransmitters, for example, neuropeptide Y (NPY) and galanin regulate central adrenergic transmission within the hypothalamus (Tsuda *et al.*, 1989). Neuropeptides tend to be larger than classic neurotransmitters, and usually have more receptor recognition sites or motifs, leading to greater binding affinity and higher receptor selectivity, theoretically making pharmacological intervention less prone to side effects. Certain neuropeptidergic systems are more discretely localized than others and are thought to play more specific roles than classical neurotransmitters. Neuropeptides thus appear potentially more promising drug targets. However, despite the advances made over the past 40 years in our understanding of the neuropeptide systems controlling appetite, the numerous drug discovery programmes targeting these systems have not yielded an anti-obesity agent for clinical use (Table 1) (Elmquist *et al.*, 1999; Gautron and Elmquist, 2011). The majority of these programmes, discontinued at a later stage, were abandoned due to safety issues or a lack of efficacy. In part, this may reflect the increased emphasis on safety for such agents over the period, and in some cases, the lack of efficacy is likely due to the lower doses being required because of safety concerns associated with higher doses (Greenway and Caruso, 2005). It may also reflect the difficulties in designing peptide-based agents, which can access the brain, and of developing small-molecule agonists for particular families of the GPCRs that mediate the effects of many of these neuropeptides (Miller *et al.*, 2004). The problem of translating acute anorectic effects into long-term reductions in appetite and body weight may be due to tachyphylaxis and/or the tendency of other compensatory systems to act to oppose body weight loss. Thus, while stimulation of anorectic neuropeptide systems or blockade of orexigenic neuropeptide systems may be viable options for the design of new drugs, they have yet to prove their utility. Understanding of the complex pathways that regulate energy balance and the redundancy of these systems is critical to the identification of the most promising novel therapeutic targets and to identify potential safety concerns.

The purpose of this article is to discuss CNS peptides and their therapeutic potential in the treatment of obesity. Brain-derived neurotrophic factor (BDNF), though a growth factor, has been included as there is evidence to suggest that its neuromodulatory properties regulate energy homeostasis (Nicholson *et al.*, 2007). The effects of peripheral signals on the regulation of energy homeostasis, including those which act centrally to modulate appetite and energy expenditure, are not within the scope of this review, but are described in detail elsewhere (Murphy *et al.*, 2006). We will examine the neuropeptide systems identified as potential targets for anti-obesity agents, reflect on the outcomes of clinical trials of agents that targeting these systems and consider the possible safety implications of manipulating these neuropeptidergic systems.

Central appetite regulation

Despite wide variation in daily food intake and energy expenditure, most individuals are able to maintain a remarkably stable body weight (Leibel, 1990). This homeostatic regulation is achieved by hypothalamic centres that receive signals from other CNS regions and from the periphery (summarized in Figure 1). Such peripheral signals can reflect body fat stores and nutritional state, and include nutrients, adipose tissue-, pancreas- and gut-derived peptides, such as insulin, leptin, ghrelin, glucagon-like peptide-1, peptide YY and cholecystokinin. The hypothalamus integrates and processes signals of energy homeostasis and induces appropriate changes in appetite and energy expenditure.

The hypothalamus comprises several discrete nuclei, which communicate with each other, and with other parts of

Table 1

Agents targeting neuropeptidergic systems investigated for their potential as anti-obesity therapies

Agent	Mechanism	Stage of development	Efficacy	Safety issues	References
NGD95-1	Y receptor antagonist	Discontinued – Phase 1b	Not released	Elevated liver enzymes	Clapham *et al.*, 2001
BMS-193885	Y_1 receptor antagonist	Preclinical	Chronic administration reduces food intake and body weight gain in rats	Rats developed adhesive peritonitis	Antal-Zimanyi *et al.*, 2008
J-115814	Y_1 receptor antagonist	Preclinical	Reduction in 24 h food intake and body weight in obese mice	Potential cardiovascular side effects	Kanatani *et al.*, 2001; Mashiko *et al.*, 2009
Y1-718	Y_1 receptor antagonist	Preclinical	Reduction in 30 min food intake in fasted mice	None reported	Kameda *et al.*, 2009
Compound 2f	Y_1 receptor antagonist	Preclinical	Reduction in 12 h food intake in lean rats	None reported	Griffith *et al.*, 2011
MK-0557	Y_5 receptor antagonist	Discontinued – Phase III	1.1–1.6 kg placebo-subtracted weight loss over 52 weeks in obese individuals	None reported	Erondu *et al.*, 2006; Erondu *et al.*, 2007a; Erondu *et al.*, 2007b
S-2367	Y_5 receptor antagonist	Phase III	2.8–3.0 kg placebo-subtracted weight loss over 52 weeks in obese individuals	Reduced haematological levels	Smith *et al.*, 2009; Puopolo *et al.*, 2009
T-226296	MCH_1 receptor antagonist	Preclinical	Reduction in 24 h food intake and body weight in obese rats	None reported	Kowalski *et al.*, 2004
SNAP-7941	MCH_1 receptor antagonist	Preclinical	Chronic administration (4 weeks) reduces body weight gain by 26% in obese rats	None reported	Borowsky *et al.*, 2002
NGD-4715	MCH_1 receptor antagonist	Discontinued – Phase I	Not available	None reported	Neurogen, 2007
ALB-127158(a)	MCH_1 receptor antagonist	Discontinued – Phase I	Reduced feelings of hunger and desire to eat plus a reduction in energy consumption	None reported	Moore *et al.*, 2011
BMS-830216	MCH_1 receptor antagonist	Phase I/II	Not available	None reported	NCATS, 2012
$MSH/ACTH_{4–10}$	MC_4 receptor agonist	Phase II	0.8 kg reduction in body weight after 6 weeks in lean individuals – no effect in obese	None reported	Fehm *et al.*, 2001; Hallschmid *et al.*, 2006
MK-0493	MC_4 receptor agonist	Discontinued – Phase II	1.2 kg placebo-subtracted weight loss over 18 weeks in obese individuals	Nausea and vomiting	Krishna *et al.*, 2009
RM-493	MC_4 receptor agonist	Phase I	Chronic administration (8 weeks) reduces body weight gain by 13% in obese primates	None reported	Kievit *et al.*, 2010
AZD2820	MC_4 receptor agonist	Discontinued – Phase I	Not available	Serious adverse event	Palatin Technologies, 2012
TTP-435	AgRP receptor antagonist	Phase II completed	Not available	None reported	ClinicalTrials, 2011
Axokine	CNTF analogue	Discontinued – Phase III	4.2 kg placebo-subtracted weight loss over 12 weeks in obese individuals	Development of antibodies to CNTF	Ettinger *et al.*, 2003

All agents are believed to act in the CNS. Preclinical studies are shaded in grey.

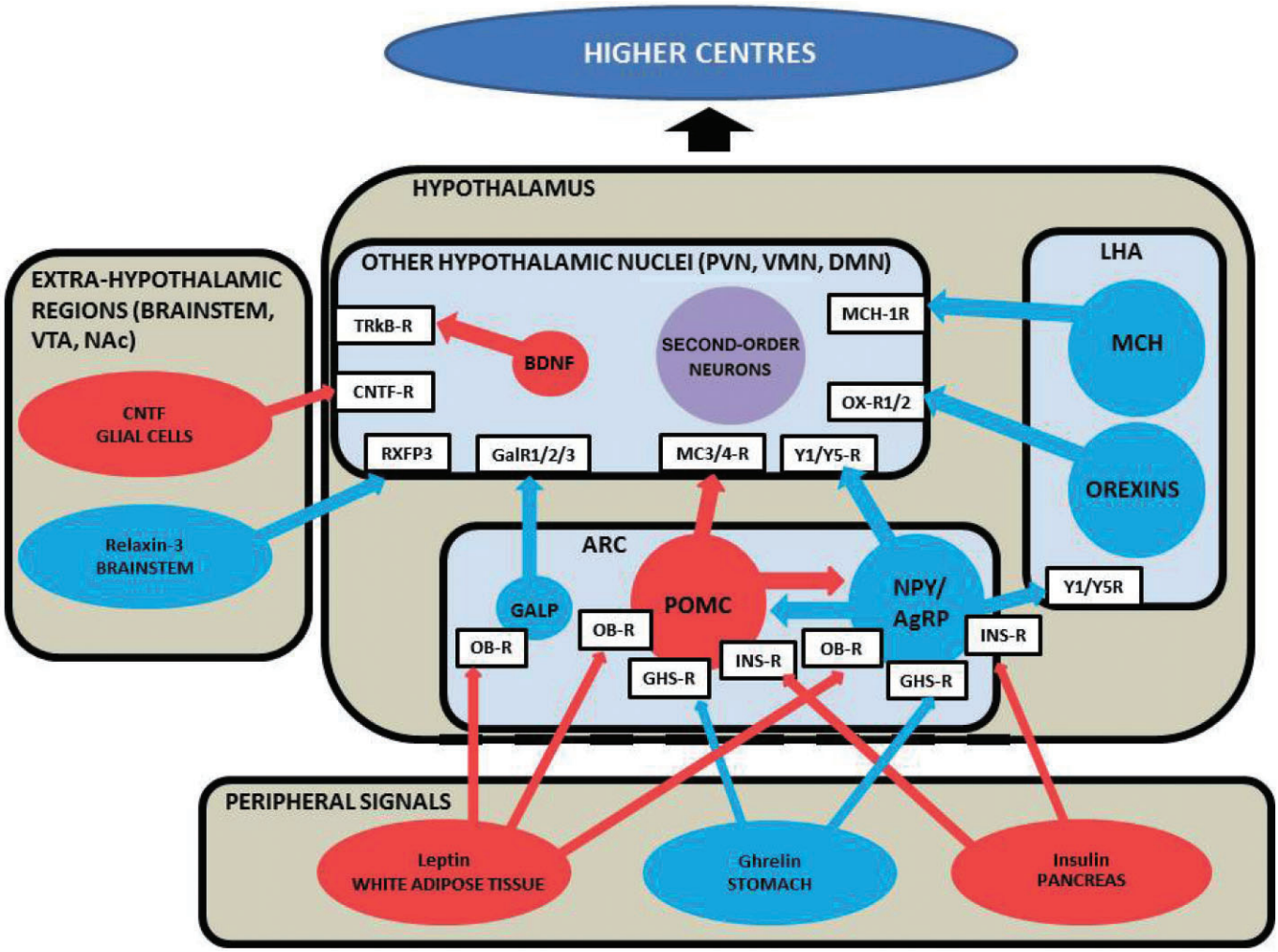

Figure 1

Schematic diagram illustrating the neuropeptidergic signalling pathways regulating energy homeostasis. Hypothalamic nuclei integrate and process signals of energy homeostasis from the periphery and other CNS regions and induce appropriate changes in appetite and energy expenditure. Peptides coloured blue are orexigenic, promoting food intake, while peptides coloured red are anorexigenic, promoting satiety. Receptors are in white boxes. Purple indicates neurons, which can be either orexigenic or anorexigenic. Non-peptidergic neurotransmitters are not included in this diagram for clarity. The peripheral factors in the diagram are reviewed elsewhere (Murphy *et al.*, 2006). CNTF-R, ciliary neurotrophic factor receptor; GalR1/2/3, galanin receptors 1,2 and 3; GHS-R, growth hormone secretagogue receptor; INS-R, insulin receptor; MC3/4-R, melanocortin 3/4 receptors; NAc, nucleus accumbens; OB-R, leptin receptor; RXFP3, relaxin/insulin-like family peptide receptor 3; TRkB-R, tyrosine-related kinase B receptor; VTA, ventral tegmental area; Y1/Y5-R, neuropeptide Y_1/Y_5 receptors.

the brain via release of specific neuropeptides. These neuropeptide systems are potential targets for anti-obesity therapies. The hypothalamic nuclei important in the regulation of energy homeostasis include the arcuate nucleus (ARC), the paraventricular nucleus (PVN), the dorsomedial and ventromedial nuclei (DMN and VMN) and the lateral hypothalamic area (LHA; Figure 1).

The hypothalamus also contains and receives signals from neuronal circuits utilizing non-peptidergic neurotransmitters, including noradrenergic, dopaminergic, serotonergic, histaminergic and endocannabinoid signals. It is these systems that have predominantly been targeted in the treatment of obesity to date. Extra-hypothalamic brain regions important in regulating energy homeostasis, and which communicate with the hypothalamus, include the brainstem (which itself receives neural signals from the gut via the vagus nerve and hormonal signals), the ventral tegmental area within the midbrain and the nucleus accumbens in the striatum (Contreras *et al.*, 1984; Hommel *et al.*, 2006; Figure 1). The latter two brain regions are thought to be important in non-homeostatic regulation of feeding behaviour, modulating motivated behaviour, addiction and reward.

The hypothalamic ARC is thought to be pivotal to the integration and interpretation of signals of energy balance. Located at the base of the hypothalamus next to the median eminence with its incomplete blood–brain barrier, the ARC is able to respond to circulating hormones such as leptin, insulin and ghrelin (Banks, 2006). There are two well charac-

terized neuronal populations within the ARC known to be involved in energy homeostasis (Cone *et al.*, 2001). One population expresses the orexigenic peptides NPY and agouti-related peptide (AgRP; Broberger *et al.*, 1998; Hahn *et al.*, 1998). The second population produces the anorexigenic peptides α-melanocyte-stimulating hormone (α-MSH), cleaved from the pro-opiomelanocortin (POMC) precursor molecule and a peptide encoded by cocaine and amphetamine regulated transcript (CART; Elias *et al.*, 1998a). The expression of NPY and AgRP mRNA in the ARC is increased by fasting (Hahn *et al.*, 1998), and the activity of these neurons is directly inhibited by leptin and insulin (Sipols *et al.*, 1995; Stephens *et al.*, 1995) and stimulated by ghrelin (Kohno *et al.*, 2003; Figure 1). POMC/CART expressing neurons are also regulated by leptin and insulin (Cheung *et al.*, 1997; Kristensen *et al.*, 1998), and their selective ablation causes hyperphagia and obesity (Xu *et al.*, 2005). The expression of POMC and CART mRNA in the ARC is decreased by fasting (Kristensen *et al.*, 1998; Smith and Funder, 1988). Administration of leptin directly into the ARC inhibits feeding (Satoh *et al.*, 1997) and lesions of the ARC attenuate the anorectic effect of peripheral leptin administration (Tang-Christensen *et al.*, 1999), suggesting that the ARC is critical in mediating leptin's effects on energy homeostasis.

Hypothalamic orexigenic neuropeptides

Neuropeptide Y

NPY is a 36 amino acid neuropeptide, which belongs to the pancreatic polypeptide (PP) fold family of proteins, together with the gut hormones peptide YY and pancreatic polypeptide (Glover *et al.*, 1984). The PP fold peptides bind to members of the Y receptor family (Y_1 to Y_5; Blomqvist and Herzog, 1997). NPY is one of the most abundant neuropeptides in the CNS (Allen *et al.*, 1984). In the hypothalamus, it is synthesized by several hypothalamic nuclei but the NPY neurons in the ARC are best characterized and known to project to the PVN, the DMN and the LHA (Bai *et al.*, 1985; Chronwall *et al.*, 1985). NPY is the most potent orexigenic neuropeptide known (Woods *et al.*, 1998). Injection of NPY into the hypothalamus increases food intake acutely in rats (Clark *et al.*, 1984; Stanley and Leibowitz, 1984), while chronic administration results in hyperphagia and obesity (Stanley *et al.*, 1986; Zarjevski *et al.*, 1993). Hypothalamic NPY synthesis and release is increased in response to fasting, and is regulated by peripheral signals that reflect body nutritional status, such as ghrelin, insulin and leptin, suggesting that NPY plays a physiological role in the regulation of feeding (Sahu *et al.*, 1988; 1995; Schwartz *et al.*, 1992; Kamegai *et al.*, 2001). NPY binds preferentially to the Y_1 and Y_5 receptors, and administration of Y_1 or Y_5 receptor antagonists attenuates feeding in fasted rats (Kanatani *et al.*, 1996; Criscione *et al.*, 1998). Surprisingly, NPY knockout mice do not display any profound alterations in their food intake and body weight, and respond normally to leptin, suggesting developmental compensation by other neuronal pathways (Erickson *et al.*, 1996; Qian *et al.*, 2002). In addition, Y_1 and Y_5 receptor knockout mice exhibit only slight alterations in energy homeostasis developing late onset mild obesity (Marsh *et al.*, 1998; Pedrazzini *et al.*, 1998). However, postnatal ablation of ARC NPY/AgRP neurones leads to hypophagia and weight loss in mice, and knockdown of NPY expression in the adult rat ARC reduces food intake and body weight gain, confirming an important physiological role for this neuronal population in energy homeostasis (Bewick *et al.*, 2005; Gardiner *et al.*, 2005; Gropp *et al.*, 2005; Luquet *et al.*, 2005).

Given the role of Y_1 and Y_5 receptors in mediating the orexigenic effects of NPY, many specific antagonists of these receptors have been developed as putative anti-obesity agents (see Yulyaningsih *et al.*, 2011). NGD95-1, an early non-selective NPY receptor antagonist reached clinical trial stage but the trial was suspended following the detection of elevated levels of liver enzymes in the blood of subjects (Clapham *et al.*, 2001).

Selective Y_1 receptor antagonists have been investigated in animal studies but have not yet progressed to clinical trials in humans. BMS-193885 is a potent and selective Y_1 receptor antagonist, which reduces food intake, and which results in an 8.5% reduction in body weight following 44 days of administration in rats. However, it lacks oral bioavailability as it is poorly absorbed by the intestine. BMS-193885 exhibited no behavioural side effects but a significant number of rats developed adhesive peritonitis following chronic administration, thought to result from poor tissue absorption of the compound (Antal-Zimanyi *et al.*, 2008). J-115814, a 2,4-diaminopyridine-based selective Y_1 receptor antagonist, reduced 24 hour food intake by 50–90% and body weight at 24 hours by approximately 5% in both leptin-resistant (*db/db*) and diet-induced obese (DIO) mice following peripheral administration (Kanatani *et al.*, 2001; Mashiko *et al.*, 2009). It has been suggested that this compound may be associated with cardiovascular side effects due to its interaction with hERG channels (Fermini and Fossa, 2003) and no further data has been released, suggesting its development has been terminated. A similar molecule, Y1-718, was subsequently designed to eliminate activity at these channels (Kameda *et al.*, 2009) and has been used to validate a PET ligand in studies examining Y_1 receptor distribution in the monkey brain (Hostetler *et al.*, 2011). However, there are currently no suggestions of its use as a potential therapeutic agent. More recently, a series of pyrazolo[1,5-a]pyrimidine derivatives with high binding affinity and selectivity at Y_1 receptors has been designed. Peripheral administration of compound 2f of this series resulted in a 12% reduction in nocturnal food intake, 12 hours after administration in lean rats (Griffith *et al.*, 2011). The effects of chronic administration have not yet been reported.

Many Y_5 receptor antagonists have been designed and have shown efficacy at reducing food intake and body weight in rodent models of obesity (Criscione *et al.*, 1998; Daniels *et al.*, 2002; Kakui *et al.*, 2006; Mashiko *et al.*, 2008; Sato *et al.*, 2008; Omori *et al.*, 2012). However, of these, only two (MK-0557 and S-2367) have progressed to clinical trial stage, and MK-0557 was recently discontinued due to its modest effect. MK-0557, an orally available Y_5 antagonist, reduced body weight gain by 40% in mouse models of obesity after chronic peripheral administration for 35 days (Erondu *et al.*, 2006). Clinical trials demonstrated that MK-0557 induced weight

loss of 2 kg in obese individuals over a 12 week period and was generally safe and well tolerated. A longer-term study lasting 52 weeks in a cohort of 1661 overweight and obese individuals resulted in only a 1.1 kg placebo-subtracted weight loss (Erondu *et al.*, 2006). Furthermore, MK-0557 treatment in combination with a hypocaloric diet for 52 weeks or with co-administration of sibutramine or orlistat for a period of 24 weeks failed to produce weight loss of greater than 1.6 kg over and above the effects of energy restriction or sibutramine/orlistat alone (Erondu *et al.*, 2007a; 2007b). S-2367 (Velneperit), another orally active Y_5 receptor antagonist, which reduced body weight in DIO mice (Yukioka *et al.*, 2006) induced a 2.8 kg placebo-subtracted reduction in body weight and reduced waist and hip circumference in obese subjects after 52 weeks of treatment. The efficacy of S-2367 was enhanced to a 3 kg placebo-subtracted weight loss after 52 weeks when administered to subjects following a low-calorie diet (Puopolo *et al.*, 2009; Smith *et al.*, 2009). S-2367 was well tolerated with no significant safety issues. However, there are reports of a small reduction in the levels of erythrocyte count, haemoglobin and haematocrit in the majority of the trial subjects, raising a potential risk of anaemia that requires further investigation (Flach *et al.*, 2007).

Considering the potent effect of NPY on appetite and energy balance, the results of clinical trials have been relatively disappointing. Poor oral bioavailability and brain penetrability, toxicity and lack of long-term effects have prevented the progress of many of these compounds into a clinical setting. It is apparent that NPY-induced feeding is mediated via more than one receptor subtype and that redundancy of such a critical system is likely to exist between Y_1 and Y_5 receptor signalling, whereby blockade at individual Y receptors may be overcome by increased activity at other Y receptors. Simultaneous targeting of more than one Y receptor with the aim of synergistic effects may therefore prove more successful in treating obesity, though to date, no dual Y_1/Y_5 receptor antagonists have reached a clinical trial stage. It is also important to consider the other roles of the NPY system, including the modulation of cardiovascular function, anxiety, seizures, memory, circadian rhythm, bone density and sexual functions (Brothers and Wahlestedt, 2010), which may be also be affected by long-term administration of NPY receptor antagonists.

Melanin-concentrating hormone

Melanin-concentrating hormone (MCH) is produced by neurons of the LHA and the zona incerta. MCH neurons project to other hypothalamic nuclei and to limbic areas reflecting its involvement in feeding, arousal and sensorimotor integration (Bittencourt *et al.*, 1992). MCH expression is increased with fasting (Qu *et al.*, 1996) and chronic MCH administration leads to hyperphagia and obesity in rodents (Della-Zuana *et al.*, 2002; Gomori *et al.*, 2003). Transgenic mice overexpressing MCH have an obese hyperphagic phenotype (Ludwig *et al.*, 2001) and conversely, targeted deletion of MCH results in a hypophagic, lean phenotype with increased metabolic rate (Shimada *et al.*, 1998). MCH binds to the MCH_1 receptor, which is widely distributed throughout the rodent brain, and highly expressed in hypothalamic nuclei (Marsh *et al.*, 2002). A second MCH receptor exists in humans (MCH_2), although its function remains relatively obscure, as it is absent in rodents (Tan *et al.*, 2002). Selective MCH_1 receptor antagonists were first developed in 2002 and have demonstrated efficacy at reducing food intake and body weight in animal models of obesity (Borowsky *et al.*, 2002; Takekawa *et al.*, 2002).

Several pharmaceutical companies have subsequently created MCH_1 receptor antagonists, which have been assessed for their efficacy and safety in human clinical trials (reviewed in Luthin, 2007). NGD-4715, a small molecule MCH_1 receptor antagonist developed by Neurogen reportedly displayed anorectic effects in animal studies and completed phase I clinical trials in humans (Neurogen, 2007). While it was reported to be safe and well tolerated, details regarding its efficacy have not been released and no further trials have been pursued (Luthin, 2007). ALB-127158(a), a selective high-affinity MCH_1 receptor antagonist developed by Albany Molecular Research, Inc. has been shown to reduce food intake by up to 30% and body weight by 18% following 28 days of administration in DIO mice (Guzzo *et al.*, 2010). This molecule has recently completed phase I trials in humans and has been reported to be safe and well tolerated (Moore *et al.*, 2011). Subjects reported an approximately 30% reduction in feelings of hunger and desire to eat, and a reduction in test meal consumption was observed after 14 days of treatment (Moore *et al.*, 2011). A later report suggested that as higher doses than those originally predicted would be required, further studies with this molecule would not be carried out. It is possible that similar molecules with better CNS penetrance, and thus requiring lower doses, will be developed (AMRI, 2011). BMS-830216 is an orally bioavailable pro-drug of BMS-819881, a selective and potent antagonist of the MCH_1 receptor developed by Bristol-Myers-Squibb. Preclinical studies demonstrated a reduction in food intake and a 6% reduction in body weight in both lean and DIO rats (NCATS, 2012). This molecule recently underwent phase I/II trials in humans and while details of its efficacy are awaited, reports released by the company suggest that it is generally safe and well tolerated (NCATS, 2012).

While the development of MCH_1 receptor antagonists for the treatment of obesity has received a great deal of interest, the majority of drug programs targeting this system have been discontinued. It is important to consider the potential effects of manipulation of the MCH system. Although MCH mRNA has rather limited expression in the brain, MCH-containing fibres are widely distributed within the CNS, and MCH has been attributed a diverse range of functions (Luthin, 2007). Antagonism at the MCH_1 receptor may therefore potentially cause numerous adverse effects. Most significantly, MCH has been demonstrated to reduce the incidence of seizures (Knigge and Wagner, 1997) and although there have been no reports of any pro-seizure effects of MCH_1 receptor antagonists in human trials, this is a concern for chronic use. Antagonism at the MCH_1 receptor has also been associated with a profound drop in blood pressure in animal studies (Kym *et al.*, 2006; Lynch *et al.*, 2006) but again, this has not been reported in human trials. MCH also has complex effects on several hypothalamo-pituitary axes (Saito and Nagasaki, 2008) and it will be important to assess whether small molecule MCH_1 receptor antagonists influence the hypothalamic-pituitary-gonadal, -thyroid or -adrenal axes in clinical trials. Other concerns involve roles of MCH in

learning and memory, and in the maintenance of bone density (Luthin, 2007).

Orexins

Orexin A and orexin B are hypothalamic neuropeptides synthesized in cell bodies within the LHA (de Lecea *et al.*, 1998; Sakurai *et al.*, 1998)and orexin-containing fibres project widely throughout the brain (Elias *et al.*, 1998b; Date *et al.*, 1999). The orexins bind to the orexin receptors OX_1 and OX_2; the OX_1 receptor binds orexin A with greater affinity than orexin B, while the OX_2 receptor binds both orexins with equal affinity (Sakurai *et al.*, 1998). Central injection of either of the orexins stimulates food intake in rats (Edwards *et al.*, 1999), and prepro-orexin mRNA expression is up-regulated in fasted rats (Sakurai *et al.*, 1998). The orexins are also involved in arousal; orexin and OX_2 receptor deficient mice exhibit a phenotype similar to human narcolepsy (Chemelli *et al.*, 1999; Tokita *et al.*, 2001), which itself is characterized by a deficiency in orexin production (Nishino *et al.*, 2000).

Orexin receptor antagonists have been developed and progressed through clinical trials for the treatment of sleep disorders. Almorexant is a non-selective orexin receptor antagonist (Owen *et al.*, 2009), which completed phase III clinical trials (Hoever *et al.*, 2012). Further development of this drug was discontinued recently following a review of data from additional clinical studies conducted to further establish the clinical profile of almorexant, including the tolerability profile (Actelion, 2011). MK-4305 (Suvorexant), a second orexin receptor antagonist has also completed phase III trials for its effects on sleep disorders (Cox *et al.*, 2010). There is no data currently available regarding the effects of these drugs on body weight in trial subjects, but it will be interesting to see if these compounds have anti-appetite actions.

Orexins have also been suggested to modulate pleasure/reward pathways in the brain (Harris *et al.*, 2005; Aston-Jones *et al.*, 2010). It seems unlikely that targeting the orexin system will result in a viable therapy for obesity due to the powerful centrally mediated behavioural effects of this system.

Relaxin-3

Relaxin-3 (INSL-7) is a member of the insulin superfamily, a group of structurally related peptide hormones that includes insulin, insulin-like growth factors, relaxins and insulin-like peptides (Bathgate *et al.*, 2002). Relaxin-3 mRNA is predominantly expressed in the brainstem in neurons that project to brains areas including the hypothalamic nuclei involved in energy homeostasis (Burazin *et al.*, 2002; Tanaka *et al.*, 2005). Relaxin-3 binds to RXFP3 (GPCR135) and also to two other receptors: LGR7 and GPCR142 (Liu *et al.*, 2003a,b; Sudo *et al.*, 2003). RXFP3 is highly expressed in the PVN and the supraoptic nucleus of the hypothalamus, and relaxin-3 neurons also extend to these regions (Chen *et al.*, 2005; Smith *et al.*, 2010a). Central injection of relaxin-3 for 14 days increases food intake threefold and increases body weight gain by approximately 15% in rats, an effect blocked by pretreatment with the selective RXFP3 antagonist (McGowan *et al.*, 2005; 2006; Hida *et al.*, 2006; Kuei *et al.*, 2007). Relaxin-3 knockout mice exhibit a lean phenotype (Sutton *et al.*, 2009). These results suggest therapeutic potential for RXFP3 antagonists to reduce body weight gain in obesity. However, an RXFP3 antagonist given alone had no effect on food intake in satiated rats (Sutton *et al.*, 2009). The discrete localization of the relaxin-3/RXFP3 system in the brain holds the promise of highly selective antagonists that can reduce body weight, although relaxin-3 signalling also modulates an array of circuits involved in arousal, stress responses, affective state and cognition (see van der Westhuizen *et al.*, 2008). A more complete understanding of the biological roles of the relaxin family peptides and their receptors will be useful in determining their utility as therapeutic targets.

Galanin-like peptide

Galanin-like peptide (GALP) is a member of the galanin peptide family originally discovered as another endogenous ligand for the galanin receptors (Ohtaki *et al.*, 1999). The distribution of GALP mRNA is restricted in the CNS to the ARC and the median eminence (Larm and Gundlach, 2000; Jureus *et al.*, 2001; Takatsu *et al.*, 2001). Central administration of GALP increases 2 hour food intake by up to 300% in both lean and DIO rats (Lawrence *et al.*, 2002; Matsumoto *et al.*, 2002; Tan *et al.*, 2005). Counter-intuitively for an orexigenic peptide, GALP mRNA expression is decreased in response to fasting and is positively regulated by insulin and leptin, suggesting the actions of GALP on energy homeostasis are complex (Jureus *et al.*, 2000; Fraley *et al.*, 2004; Johansson *et al.*, 2008). The increase in GALP expression in response to leptin may reflect a role in neuroendocrine regulation rather than in energy homeostasis. In addition to its effects on energy homeostasis, GALP also has a role in the regulation of the hypothalamo-pituitary gonadal axis (see Lang *et al.*, 2007). There is an interest in the development of GALP as a therapeutic agent for the treatment of obesity due to its restricted distribution in the CNS and relatively specific biological activity (Shiba *et al.*, 2010). However, GALP is known to bind to several receptors, increasing the chance of side effects, and it may not be possible to separate the effects of GALP on food intake from its effects on the reproductive axis. Until the biology of the galanin peptide family is completely understood, pharmacologically manipulating the GALP system remains problematic.

Hypothalamic anorectic neuropeptides

Melanocortins

The hypothalamic melanocortin system plays a critical role in energy homeostasis. The melanocortins are peptide products of the POMC polypeptide precursor, and include adrenocorticotrophic hormone (ACTH) and α, β and γ-MSH (Bertagna, 1994). The melanocortins mediate their effects via five GPCRs (MC_1-MC_5), though only MC_3 and MC_4 receptors are thought to regulate energy homeostasis. POMC mRNA is expressed in the ARC and the brainstem and is decreased in response to fasting (Smith and Funder, 1988). POMC and MC_4 receptor knockout mice are hyperphagic and obese, suggesting a critical physiological role for the melanocortin circuitry in the regulation of energy homeostasis (Huszar *et al.*, 1997; Yaswen *et al.*, 1999).

α-MSH is an agonist at both the MC_3 and MC_4 receptors (Adan *et al.*, 1994), and inhibits feeding after central injection in food-deprived rats (Tsujii and Bray, 1989). Unusually, the MC_3 and MC_4 receptors also have an endogenous antagonist, AgRP (Fong *et al.*, 1997). Central injection of AgRP increases food intake by blocking the anorectic effect of α-MSH at MC_4 receptors (Rossi *et al.*, 1998), and transgenic mice overexpressing AgRP have a similar phenotype to MC_4 receptor knockout mice (Graham *et al.*, 1997; Ollmann *et al.*, 1997). Melanotan-II, a melanocortin receptor agonist with increased potency at MC_4 receptors reduces food intake in mice, an effect which is attenuated in MC_4 receptor knockout mice (Chen *et al.*, 2000). Furthermore, MC_3 receptor knockout mice do not have altered body weight, but do have increased adipose mass (Butler *et al.*, 2000). These studies suggest that it is the MC_4 receptors that mediate an anorectic tone important in the restriction of food intake. The physiology of the melanocortin system is similar in humans to rodents, and individuals with genetic mutations in POMC or the MC_3 and MC_4 receptors are hyperphagic and obese (Vaisse *et al.*, 1998; Yeo *et al.*, 1998; Biebermann *et al.*, 2003). Indeed, mutations in the MC_3 and MC_4 receptors are the most common known monogenetic causes of human obesity, although only with a prevalence of 4% in a population of morbidly obese patients (Vaisse *et al.*, 2000).

Preclinical studies with synthetic melanocortin ligands have demonstrated potent effects on food intake and energy expenditure in animal models. However, few of these compounds have been investigated for their safety and efficacy in humans. Intranasal administration of the melanocortin core fragment $MSH/ACTH_{4–10}$, an agonist at MC_4 receptors, reduced body weight by 0.8 kg and body fat by 1.7 kg in normal weight individuals following 6 weeks of treatment, but caused no significant reduction in body weight or body fat in overweight subjects or in patients with mutations in the POMC gene (Fehm *et al.*, 2001; Krude *et al.*, 2003; Hallschmid *et al.*, 2006). A potent and selective MC_4 receptor agonist MK-0493 reached phase II clinical trials but was associated with only a non-significant, 1.2 kg placebo-subtracted weight reduction in obese subjects after 18 weeks of treatment and further studies are not planned (Krishna *et al.*, 2009). These two studies with negligible effects on weight loss suggest that obesity may be associated with melanocortin resistance.

RM-493 (formerly BIM 22493) is a small peptide agonist with high selectivity for MC_4 receptors. In preclinical studies, obese primates treated with RM-493 for 8 weeks displayed reduced food intake, a 13% reduction in body weight and improved glucose tolerance. This study also showed improvements in both heart rate and blood pressure, probably attributable to the improved body weight (Kievit *et al.*, 2010). RM-493 is currently undergoing a phase I clinical trial as a potential new treatment for obesity (Rhythm Pharmaceuticals, 2011). Recently, AZD2820, an MC_4 receptor partial agonist administered by s.c. injection, entered phase I trials. The trial was halted due to a serious adverse event suspected to be an allergic reaction after a first dose (Palatin Technologies, 2012). A small molecule inhibitor of AgRP, TTP-435 has been developed and has completed phase II clinical trials (ClinicalTrials.gov, 2011) but information regarding its efficacy has not yet been released.

Despite a considerable effort spent on the development of selective MC_4 receptor agonists to treat obesity, clinical trials have yet to demonstrate efficacy. There are a number of issues relating to the development of drugs targeting the melanocortin system, including the need for a full agonist at MC_4 receptors with receptor subtype selectivity. Although POMC is discretely expressed within the CNS, it still has multiple roles. Furthermore, the MC_4 receptor is widely distributed within the CNS and agonism has been demonstrated to increase blood pressure and sympathetic function (Greenfield, 2011). It is possible that the adverse effects associated with MC_4 receptor agonism may be avoided by new agents selectively targeting the appetite regulating pathways, activated by MC_4 receptors .

Ciliary neurotrophic factor

Ciliary neurotrophic factor (CNTF) is a 22kD protein expressed in Schwann cells and astrocytes, originally described as a trophic factor important for motor neuron survival (Lin *et al.*, 1990; Sendtner *et al.*, 1991). Investigations into its neuroprotective properties in the management of motor neurone disease revealed a significant 2.9% placebo-subtracted body weight loss in the non-obese participants (The ALS CNTF Treatment Study Group, 1996). Further studies demonstrated that CNTF and leptin act through a common intracellular signalling molecule:,the STAT3 transcription factor (Peterson *et al.*, 2000). In addition, the CNTF receptor is expressed in the hypothalamic nuclei known to be involved in appetite regulation (Gloaguen *et al.*, 1997). CNTF causes a 10–20% placebo-subtracted body weight loss in both leptin-deficient *ob/ob* mice and also in mouse models of DIO in which leptin administration had little effect (Gloaguen *et al.*, 1997; Lambert *et al.*, 2001). This mouse study reflects the disappointing results of leptin administration in obese humans, in whom circulating leptin levels are already elevated, suggesting a leptin-resistant state (Heymsfield *et al.*, 1999). The potential for CNTF to bypass this resistance was recognized and a modified version of CNTF, axokine, proceeded to clinical trials. Subjects receiving axokine in a phase III trial demonstrated a 4.2 kg placebo-subtracted weight loss over 12 weeks; 25.1% of those receiving axokine lost greater than 5% of their baseline body weight. However, approximately 70% of recipients developed antibodies to the drug (Ettinger *et al.*, 2003). While this was not associated with a loss of efficacy, concerns regarding the potential for antibodies to interfere with the neuroprotective role of endogenous CNTF led to Regeneron abandoning development of axokine as a potential anti-obesity agent.

Brain-derived neurotrophic factor (BDNF)

BDNF is a member of the neurotrophin family of growth factors (Leibrock *et al.*, 1989). There is strong evidence that BDNF can regulate energy homeostasis and it is possible that BDNF possesses neuromodulatory properties in addition to its role as a growth factor (Nicholson *et al.*, 2007). The biologically active form is mature BDNF, which mediates its effects via the tropomyosin-related kinase B (TrkB) receptor (Klein *et al.*, 1991). BDNF is widely expressed throughout the CNS, including in several hypothalamic nuclei known to be involved in energy homeostasis, the ARC, PVN, VMN and

DMN (Conner *et al.*, 1997). Expression of BDNF mRNA in the VMH is positively regulated by leptin (Komori *et al.*, 2006). Central injection of BDNF for 3 weeks reduces cumulative food intake by approximately 30% and causes a 15–20% body weight loss in rats, in addition to increasing locomotor activity and metabolic rate (Pelleymounter *et al.*, 1995; Wang *et al.*, 2007; 2010). Knockout of BDNF is embryonically lethal, but postnatal deletion of brain BDNF in mice is not and leads to an obese and hyperactive phenotype (Rios *et al.*, 2001), and mice expressing significantly reduced levels of TrkB are hyperphagic (Xu *et al.*, 2003). It has been postulated that BDNF lies downstream of MC4R signalling, and in accord with this, central BDNF administration was able to ameliorate obesity in MC_4 receptor knockout mice (Xu *et al.*, 2003).

Human studies also support a critical role of the BDNF/TrkB pathway in energy homeostasis. A human mutation causing loss of function of the TrkB receptor leads to hyperphagia and obesity (Yeo *et al.*, 2004), and this phenotype is also seen in humans with haploinsufficiency for the BDNF gene (Gray *et al.*, 2006; Han *et al.*, 2008). Furthermore, genome-wide association studies have identified single nucleotide polymorphisms in the BDNF gene associated with a higher body mass index (Speliotes *et al.*, 2010). A number of studies have demonstrated a role for BDNF/TrkB in hedonic feeding, regulating motivation and the reward (see Cordeira and Rios, 2011). Disruptions in BDNF signalling in this circuitry may also contribute to obesity.

BDNF also has an important role in neuronal plasticity and neurogenesis and a phase III clinical trial has been carried out investigating the clinical efficacy of BDNF in the treatment of motor neurone disease. Safety and tolerability were demonstrated in these studies; however, there was no significant survival advantage in subjects receiving BDNF (The BDNF Study Group, 1999). No effects of BDNF on body weight of trial participants were reported.

Further preclinical studies are required to elucidate the precise role of BDNF/TrkB signalling in hypothalamic neuronal populations. Additionally, the role of BDNF elsewhere in the brain influencing eating behaviour warrants further investigation. However, given that mutations that interfere with BDNF signalling cause obesity within the human population, BDNF is an important potential target in the treatment of obesity, and clinical trials are highly anticipated.

Pipeline therapies

Currently, the majority of the new drugs seeking approval for the treatment of obesity are based on classical neurotransmitter systems, despite the widespread nature of these systems leading to concerns regarding their potential side effects. Lorcaserin (Belviq) a selective 5-HT_{2C} receptor agonist very recently received FDA approval for use in the treatment of obesity in select groups of obese patients. Placebo-subtracted weight loss of greater than 5% baseline was achieved by 27.2% and 68% of patients maintained that weight loss following 2 years of treatment (Smith *et al.*, 2010b; Fidler *et al.*, 2011). The mechanism behind the effects of lorcaserin on body weight is not completely understood, but may be mediated by hypothalamic POMC activation (Halford *et al.*, 2007). Clinical trials revealed only mild side effects with lorcaserin treatment, most commonly headache (Smith *et al.*, 2010b).

Contrave is a combination of bupropion and naltrexone, which is currently seeking FDA approval. Phase III trials demonstrated that Contrave, when given in conjunction with a diet and exercise programme over 56 weeks, was associated with a 4.8% placebo-subtracted body weight loss and 39–48% of subjects receiving Contrave lost greater than 5% of their baseline body weight (Greenway *et al.*, 2010). Bupropion is a dopamine and noradrenaline reuptake inhibitor and is already used in the treatment of depression and smoking cessation, while naltrexone is an opioid receptor antagonist used to treat opiate and alcohol addiction. Both drugs have individually shown evidence of weight loss, with placebo-subtracted weight losses of 1.4% with naltrexone and 5.9% with bupropion (Greenway *et al.*, 2009), and it is hypothesized that their combination will have a synergistic effect due to the different mechanisms by which they mediate weight loss. Bupropion stimulates hypothalamic POMC neurons, while naltrexone blocks the auto-inhibition of the POMC neurons by endogenous β-endorphins (Plodkowski *et al.*, 2009). A large-scale study of the long-term cardiovascular effects of Contrave is required by the FDA before approval will be considered.

Qsymia (formerly Qnexa) is a combination of phentermine and topiramate, which has been shown to lead to 9.3% placebo-subtracted weight loss in overweight and obese subjects when combined with a diet and lifestyle modification programme (Gadde *et al.*, 2011; Allison *et al.*, 2012). Phentermine is a noradrenaline reuptake inhibitor, which has been shown to cause placebo-subtracted weight loss of 3.6 kg; topiramate an anticonvulsant, has been associated with a placebo-subtracted weight loss of 6.5% (Li *et al.*, 2005). Concerns have been raised about dangerous side effects of Qsymia including suicidal thoughts, heart palpitations, memory lapses and birth defects (VIVUS, 2012); however, it has recently been approved by the FDA for the long-term management of weight loss (FDA, 2012).

Conclusions

There are many neuropeptide systems that influence food intake and energy homeostasis. Manipulation of several of these systems has been shown to reduce food intake and body weight in preclinical studies (Table 1). However, to date, there has been a disappointing lack of anti-obesity agents reaching clinical practice. Possible reasons for this paucity include the difficulties of such agents accessing the CNS, unacceptable side effects or a lack of efficacy. Although the ARC is incompletely isolated from the circulation by the blood brain barrier, targeting central neuropeptide systems in other regions of the CNS requires that drugs must cross the blood–brain barrier. Often a high dose of a drug is required to achieve sufficient penetrance of the CNS to mediate reductions in body weight, and this can lead to an increased risk of side effects.

While neuropeptide systems tend to be more discretely distributed and more restricted in their physiological function than the widespread neurotransmitter circuits, some, such as the NPY system, are equally widely distributed and are associated with a diverse range of functions. Drugs targeting widespread neuropeptide systems often have a limited therapeutic window, and targeting, for example, specific

receptors within the NPY and melanocortin systems, or, the more discrete neuropeptide systems such as the GALP or relaxin-3 systems may represent more promising targets for future therapies. However, despite the discrete expression of POMC, GALP and relaxin-3, these systems have still been reported to be associated with multiple roles, and manipulation, as has been demonstrated with agents targeting the MC4R, may well be associated with side effects (Greenfield, 2011).

The development of therapeutic agents based on neuropeptide systems has been hampered by a lack of efficacy. This may in part be due to redundancy and adaptation to chronic administration protocols within the neuropeptide systems regulating energy balance. The lack of efficacy in humans of many of the drugs undergoing development contrasts with preclinical data from rodent models. Using animal models to examine the role of specific signals in energy homeostasis is necessary, but has limitations. Many of the side effects one might expect from higher doses of anorectic agents include nausea or changes in motivation, which can be difficult to measure in animals.

Peptide hormones are difficult to mimic with small molecules and therefore some of the drugs with clinical efficacy are not orally active. Injection of a drug is possible but the inconvenience and pain associated are more likely to lead to failures of compliance and complications associated with injection sites in patients. The use of long-acting injectable agents with reduced frequency of dosing may ameliorate the disadvantages associated with injectable therapy.

As with any drug based on a peptide, there is a chance that antibodies may develop against the drug. Where the endogenous peptide has important effects other than those relating to energy balance, this may lead to potentially disastrous effects. In addition, neuropeptide systems are often associated with redundancy, which may lead to a lack of efficacy of any single-target therapy. A combination therapy approach may therefore provide the best chance of an effective treatment for obesity.

It is therefore unclear whether neuropeptides can be used to treat obesity. However, despite the significant failure rate of novel anti-obesity agents, there are a number of potential therapies, which target hypothalamic neuropeptide systems that are currently progressing through clinical trials. It remains to be seen whether these agents have greater efficacy and/or more favourable side-effect profiles than those targeting neurotransmitter systems. The early results of these studies have shown promising weight-reducing effects and these agents may prove useful in the future clinical management of obesity.

All nomenclature in this review conforms to BJP's Guide to Receptors and Channels.

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Conflict of interest

The authors report no conflict of interests.

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