Gut hormones and the control of appetite

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Obesity is the main cause of premature death in the UK. Worldwide its prevalence is accelerating. It has been hypothesized that a gut nutriment sensor signals to appetite centres in the brain to reduce food intake after meals. Gut hormones have been identified as an important mechanism for this. Ghrelin stimulates, and glucagon like peptide-1, oxyntomodulin, peptide YY (PYY), cholecystokinin and pancreatic polypeptide inhibit, appetite. At physiological postprandial concentrations they can alter food intake markedly in humans and rodents. In addition, in obese humans fasting levels of PYY are suppressed and postprandial release is reduced. Administration of gut hormones might provide a novel and physiological approach in anti-obesity therapy. Here, we summarize some of the recent advances in this field.

Obesity is a major health problem in the developed world with a dramatic influence on morbidity and mortality, in addition to major economic consequences. Treating the complications of obesity costs the National Health Service in the UK over half a billion pounds per year [1]. Prevention of obesity is a priority for individuals and governments worldwide.

There are several approaches currently used for the treatment of obesity. All have problems and none, except for bariatric surgery, is very effective. The drug Orlistat blocks the absorption of dietary fat and reviews suggest that it results in an additional loss of 3–4% of body weight over diet alone in a two-year period [2]. However, it might lead to socially unacceptable side effects and deficiency in vitamins A and E. Treatments such as Sibutramine act within the central nervous system (CNS) to reduce energy intake and increase energy expenditure [3,4]. Sibutramine has a similar efficacy to Orlistat, but its use is limited by side effects, particularly tachycardia and hypertension [5]. Although these therapies are helpful in the short to medium term, their effectiveness is limited in the long term. The most efficacious and long-lasting treatment for obesity is gastrointestinal bypass surgery. In one long-term study the mean weight loss was 29.5 kg 13–15 years post-bypass [6]. Although surgery initially reduces calorie absorption, its sustained effect results from a reduction in appetite. Bypass surgery alters circulating gut hormones, such as peptide YY (PYY) [7] and ghrelin [8,9], and its long-term action on appetite might be secondary to these alterations in circulating hormones.

Previous work on cholecystokinin (CCK) and ghrelin have suggested that there is a gut nutriment sensor that signals to appetite centres in the brain to reduce appetite after meals. It has become increasingly apparent that the gut endocrine system plays an important physiological role in postprandial satiety. Recent work has identified the gut hormones PYY, oxyntomodulin and pancreatic polypeptide (PP), which inhibit appetite, and ghrelin, which stimulates appetite. These hormones are active within the plasma range seen in humans and represent a novel approach to the treatment of obesity. Here, we examine how these gut hormones influence appetite and whether they could contribute to the treatment of obesity.

The hypothalamic appetite circuits

The hypothalamus and the dorsal vagal complex appear to be important CNS regions directly regulating appetite ([10] and extensively reviewed in Ref. [11]). Recently, some of the main neural circuits involved have been identified. The arcuate nucleus of the hypothalamus plays an integrative role in appetite regulation; for example, by receiving signals from the periphery via the brainstem [10–12]. Receptors for the gut hormones are found on these neuronal populations within the arcuate nucleus. The arcuate nucleus can also be directly influenced by circulating factors, because it is partially outside the blood–brain barrier. There are two well characterized neuronal populations involved – appetite inhibiting proopiomelanocortin and cocaine- and amphetamine-regulated transcript coexpressing neurones, and appetite stimulating neuropeptide Y (NPY) and agouti related peptide coexpressing neurones [10–13]. Both of these neuronal populations project to the paraventricular nucleus (PVN) and other important nuclei involved in the regulation of food intake. The PVN also receives important inputs from other hypothalamic nuclei; for example, melanin-concentrating hormone-producing neurones from the lateral hypothalamic area, and other brain areas, such as the brainstem and amygdala. Mutations disrupting these hypothalamic systems cause obesity in human [14,15].

Ghrelin

Ghrelin is a circulating hormone synthesized in the stomach [16]. It is the endogenous ligand for the growth
hormone secretagogue receptor, which is expressed in the arcuate nucleus and other hypothalamic and brain stem nuclei [17,18]. Ghrelin levels are highest in the fasting state, rising sharply before and falling within one hour of a meal [9]. Ghrelin peaks are of similar magnitude before each meal of the day and ghrelin might be involved in meal initiation [19].

Ghrelin potently stimulates food intake following peripheral administration in humans [20], and similar results have been obtained in rats [21–23]. Peripheral ghrelin potently stimulates feeding in rodents, with the maximum effect being seen within one hour of administration [23]. A dose of ghrelin that stimulates feeding achieves similar circulating levels to those seen after a 24-h fast [24], suggesting that ghrelin regulates day to day food intake. Chronic peripheral ghrelin administration leads to a significant increase in cumulative food intake and body weight gain, with no attenuation in feeding stimulation with multiple injections [22,24]. This weight gain is mainly the result of increased fat deposition, and might also be caused by decreased energy expenditure in addition to increased food intake [22].

In rats, circulating ghrelin acts, at least in part, through the arcuate nucleus [12,25]. However, it is important to note that both the arcuate c-fos neuronal activation and the stimulation of food intake following ghrelin administration require an intact vagal nerve [26]. Ghrelin is suppressed in obesity [27]. It is not known whether this is associated with a reduced or increased sensitivity to ghrelin. However, obesity is associated with an increased arcuate c-fos response to peripheral ghrelin administration in rats [28].

Leptin
In 1994, the adipocyte hormone leptin was discovered [29], which circulates at concentrations proportional to fat mass and inhibits food intake [30]. In addition, circulating leptin falls during starvation and exhibits a diurnal pattern [30,31]. Leptin crosses the blood–brain barrier to act via its receptor to inhibit orexigenic and stimulate anorexigenic neuropeptides in the arcuate nucleus of the hypothalamus, leading to a reduction in food intake [13,32]. Leptin activates other hypothalamic nuclei such as the dorsomedial hypothalamus (DMH), PVN and ventromedial hypothalamus (VMH) [33–35]. However, the relative contribution of these hypothalamic nuclei to mediating the effects of leptin remains controversial. Leptin has been shown to have synergistic actions with CCK [33]. Leptin is believed to play an important role in energy balance, particularly the response of the body to fasting [31]. Clinical trials have shown that administration of leptin has little effect on body weight in the obese [36,37].

Cholecystokinin
CCK is a hormone produced by mucosal endocrine cells in the upper small intestine and released postprandially. It is also a neurotransmitter with a very wide distribution, being the most abundant neuropeptide in the CNS [38]. CCK is known to inhibit food intake in humans and rodents [39,40]. Although CCK was shown in 1973 to alter food intake, it is still not clear whether this effect is physiologically relevant [41–43]. Following peripheral administration of CCK at doses just sufficient to inhibit food intake, synthesis of c-fos, a marker of neuronal activation, is limited to the brainstem, in the nucleus of the solitary tract and the dorsal vagal nucleus [44]. Rats lacking functional CCK_α receptors are diabetic, hyperphagic and obese [45]. However, the CCK_α receptor-deficient mouse has normal body weight [46].

Peptide YY
In 1985, it was reported that a newly extracted gut peptide, a member of the NPY family with a tyrosine at both ends, named peptide YY (PYY), was produced by gut endocrine cells and released into the circulation after meals [47]. Both PYY 1–36 and PYY 3–36 are stored in the gut mucosal endocrine cells and present in the circulation [48].

Peripheral administration of PYY was first reported in 1993 to decrease appetite [49]. In addition, when PYY 3–36 is administered peripherally in the mouse, rat or human there is a marked inhibition of food intake [50]. The pattern of c-fos synthesis in the brain after peripheral administration of PYY 3–36 showed a marked induction of c-fos in the arcuate nucleus. Injection of PYY 3–36 directly into the arcuate nucleus inhibited food intake and chronic administration of PYY 3–36 leads to a decrease in food intake and body weight. Addition of PYY 3–36 to ex vivo hypothalamic explants inhibited release of NPY and stimulated release of α-melanocyte stimulating hormone (α-MSH). Peripheral administration of PYY 3–36 in rats caused a decrease in the synthesis of mRNA encoding arcuate NPY. PYY 3–36 has a high affinity for the Y2R. The appetite inhibitory effect was also seen with a Y2R-specific agonist and was absent in the Y2R-deficient mouse [50]. It appears that circulating PYY 3–36 inhibits appetite by acting directly on the arcuate nucleus via the Y2R, a presynaptic inhibitory autoreceptor [13,50].

In human volunteers, infusion of PYY 3–36 to mimic accurately postprandial concentrations reduced food intake by 30% in a placebo-controlled double-blinded crossover study [50]. These data demonstrate that PYY physiologically inhibits appetite in humans and suggest that it is important in the every-day regulation of food intake. Interestingly, obese subjects have a lower fasting basal PYY and exhibit a diminished postprandial rise in PYY [51]. There is a highly significant negative correlation between PYY and body mass index (BMI) ($r = -0.89$, $n = 24$, $P < 0.001$). Caloric intake during a buffet lunch two hours after the infusion of PYY 3–36 was decreased by 30% in obese subjects ($P < 0.0005$) and 31% in lean subjects ($P < 0.0001$). The PYY infusion also caused a significant decrease in the cumulative 24-h caloric intake in both obese and lean subjects [51]. These recent findings suggest that, unlike leptin, obesity is not associated with PYY resistance. Whether this is the cause or a result of their obesity is unclear.

Pancreatic polypeptide
PP, a hormone produced by the PP cells of the islets of Langerhans, has been reported to reduce appetite in
several animal models [52–55]. The PP receptor (Y4R) is present in both the brainstem and arcuate nucleus [56], regions of the brain known to be involved in appetite regulation. It was unclear to what extent PP might be physiologically involved in appetite regulation in animals and whether it has this effect in humans [57–59]. However, a 90-min PP infusion of 10 pmol kg \(^{-1}\) min \(^{-1}\) in healthy volunteers produces a 25.3%±5.8% reduction in food intake over 24 h [60]. More importantly, the inhibition of food intake was sustained, such that energy intake, as assessed by food diaries, was significantly reduced on both the evening of the study and the following morning [60].

**Glucagon-like peptide-1 (7–36) amide**

Glucagon-like peptide-1 (7–36) amide (GLP-1) is produced by processing of the gene encoding preproglucagon in both the gut and brain [61]. It is released from the gut into the circulation in response to nutrient intake. Physiological actions of peripheral GLP-1 in humans include stimulation of insulin release [62].

GLP-1 receptors are found in the brainstem, arcuate nucleus and PVN. Intracerebroventricular (i.c.v) or direct administration into the paraventricular nucleus of GLP-1 to rats potently inhibits food intake, whereas the specific GLP-1 receptor antagonist, exendin 9–39, causes an increase in food intake [63].

Peripheral administration of GLP-1 in humans and rats inhibits food intake and, in rats, also results in c-fos synthesis in the brainstem [64–66]. These and other findings suggest that the main site for appetite inhibition by peripheral GLP-1 is the dorsal vagal complex, acting, in part, directly through the area postrema. The importance of circulating GLP-1 in appetite control in humans is currently unclear. However, GLP-1 decreases gastric emptying and this might have effects on food intake [67]. Infusions mimicking postprandial concentrations produce only a small, although reproducible, reduction in appetite and food intake [68,69].

**Oxyntomodulin**

Oxyntomodulin (Oxm) is also a product of post-translational processing of preproglucagon in the intestine and the CNS [61]. In addition, it is released postprandially and i.c.v administration of Oxm inhibits food intake in the rat with greater potency than does GLP-1 [70]. Oxm appears to act via a GLP-1-like receptor, because its anorectic actions are blocked by coadministration of the GLP-1 receptor antagonist, exendin 9–39 [70]. Recently, it was shown that Oxm is also a potent inhibitor of food intake when administered intraperitoneally (IP) to rats [71]. IP administration of Oxm resulted in c-fos synthesis in the arcuate nucleus, a region partially outside the blood–brain barrier, but there was little activation of neurons in the nucleus of the solitary tract in the brainstem. These experiments show that Oxm has a very different pattern of neuronal activation from that of GLP-1. When the antagonist exendin 9–39 was injected into the arcuate nucleus, circulating Oxm no longer inhibited food intake, suggesting an arcuate site of action. By contrast, the effect of circulating GLP-1, acting via the brainstem, was unaffected.

Intravenous infusion of Oxm in 13 healthy subjects in a randomized double-blind placebo-controlled crossover study significantly reduced ad libitum calorie intake of a free choice buffet meal (mean decrease 19.3%±5.6%, \( P < 0.01 \)) and caused a significant reduction in hunger scores. In addition, cumulative 12-h caloric intake was significantly reduced by infusion of Oxm (mean decrease 11.3%±6.2%, \( P < 0.05 \)). Fasting levels of ghrelin were significantly suppressed by Oxm (mean reduction 44%±10% of postprandial decrease, \( P < 0.01 \)) [72].

**Gut hormones and obesity**

Obesity can be thought of as a state of chronic adaptation to the hormonal changes of increased fat mass. Results to date suggest that increased BMI is associated with increased plasma leptin and decreased plasma ghrelin and PYY [27,30]. Obese people have a similar sensitivity to the appetite inhibitory effects of exogenous PYY 3–36 infusion as lean people [51]; that is, no ‘PYY resistance’. However, the sensitivity to ghrelin infusion remains to be established. Although the gastric oxyntic glands appear to be capable of producing ever-increasing concentrations of ghrelin in states of under-nutrition, could it be that the low levels of PYY in obesity are the result of L-cell failure? PYY replacement could therefore provide a realistic therapeutic anti-obesity treatment.

The only treatment so far that has achieved lasting weight reduction is gastric and intestinal bypass surgery. In one series, mean weight loss at 15 years post-bypass surgery was 29.5 kg [6]. However, the morbidity and mortality associated with bypass surgery, in addition to practical and financial constraints, usually limit this approach to the severely obese patient (BMI >40 kg m\(^{-2}\)). Interestingly, the success of bypass surgery can be as much hormonal as mechanical, because induced malabsorption is usually only temporary, whereas the reduction of appetite is permanent. Plasma ghrelin levels were recently measured in patients who had undergone gastric bypass. Circulating ghrelin levels were 77% lower in the bypass group compared with BMI-matched controls, and the usual pre-meal peaks were lost [9]. However, more recent reports suggest that the effects are more complex and for full review see [8]. After bypass surgery, there is a significant rise in plasma PYY [73]. A recent study has shown that, in rats, bypass surgery results in a threefold increase in circulating PYY, with a 21% reduction in body weight after 28 days [74].

Therefore, the success of bypass surgery might, in part, be the result of a decrease in circulating ghrelin and an increase in circulating PYY. These changes in gut hormones act on the arcuate nucleus of the hypothalamus, either through the median eminence or via the brainstem. Thus, altered gut signals following bypass surgery could explain why bypass patients frequently describe amazingly reduced hunger after surgery.

**Summary and conclusions**

Several new chemical entities are being developed by pharmaceutical companies to target receptor systems
involved in appetite regulation. However, these same systems also affect many other CNS functions, because they use the same receptors; for example the serotonin system. The peripheral administration of natural gut hormones as therapeutic agents has the advantage of targeting only the relevant brain appetite systems. Gut hormones are released every day after meals without side effects and continue to exert their effect without escape. Thus, the administration of the naturally occurring gut hormone might offer a long-term therapeutic approach to weight control, without deleterious side effects.

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