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REVIEW

Therapy for Obesity Based on Gastrointestinal Hormones

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■ Abstract

It has long been known that peptide hormones from the gastrointestinal tract have significant impact on the regulation of nutrient metabolism. Among these hormones, incretins have been found to increase insulin secretion, and thus incretin-based therapies have emerged as new modalities for the treatment of type 2 diabetes. In contrast to other antidiabetic treatments, these agents have a positive outcome profile on body weight. Worldwide there are 500 million obese people, and 3 million are dying every year from obesity-related diseases. Recently, incretin-based therapy was proposed for the treatment of obesity. Currently two different incretin therapies are widely used in the treatment of type 2 diabetes: 1) the GLP-1 receptor agonists which cause significant and sustained weight loss in overweight patients, and 2) dipeptidyl peptidase 4 (DPP-4) inhibitors being weight neutral. These findings have led to a greater interest in the physiology of intestinal peptides with potential weight-reducing properties. This review discusses the effects of the incretin-based therapies in obesity, and provides an overview of intestinal peptides with promising effects as potential new treatments for obesity.

Keywords: DPP-4 inhibitors · GLP-1 receptor agonist · incretin hormone · obesity · type 2 diabetes

Introduction

Worldwide, obesity has more than doubled since 1980 [1, 2]. In the USA, more than two thirds of the population is overweight (body mass index (BMI) 25.0-29.9 kg/m^2), or obese (BMI ≥ 30 kg/m^2). The proportion of the European population having weight problems is smaller, but the number continues to increase [1]. The most recent data from the World Health Organization (WHO) indicate that 1.5 billion adults worldwide are overweight, and 500 million are obese. It is particularly concerning that nearly 50 million children under the age of five are overweight [2]. The main problem seems to be the western lifestyle, combined with a genetic predisposition, which leads to obesity, type 2 diabetes, fatty liver disease, and eventually cardiovascular disease. Almost 3 million adults die each year as a result of being overweight, or obese. It is estimated that approximately 44% of the diabetes burden, 23% of the ischemic heart disease burden, and between 7% and 41% of certain cancer burdens are attributable to overweight and obesity [2]. By 2015, WHO projects that worldwide 2.3 billion adults will be overweight, with more than 700 million being obese.

Unfortunately, weight loss is not easily accomplished, or maintained. Meta-analyses of clinical trials on non-pharmacologic strategies for weight reduction report only modest results (i.e. weight reductions of 1 to 6 kg) that are short-lived [3-5]. Pharmacologic weight loss interventions have shown similar limited success [6-8]. Meta-analyses of sibutamine and orlistat trials, report average reductions of 3 to 5 kg; but attrition rates tend to be very high in the included trials, with almost 50% of the patients leaving the trials prematurely

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[6-8]. The high attrition rates, and the fact that many trials had an inadequate control bias, suggest that real-world use of these agents may be less successful [6-9]. Other meta-analyses show that bariatric surgery is associated with robust body weight-reducing effects, and suggest that certain bariatric procedures may reduce long-term mortality in obese patients [10, 11]. Thus, the combined evidence suggests that bariatric surgery may be a beneficial option for some obese patients. On the other hand, the safety and the cost of bariatric surgery limit the use of this intervention for large populations of patients.

The risk of developing diabetes escalates with the degree of excess weight, increasing 3-fold with a BMI of 25.0 to 29.9 kg/m^{2,} and 20-fold with a BMI of ≥35 kg/m^2 (compared with a BMI of 18.5 to 24.9 kg/m^2) [12]. Thus, for each unit of increase in BMI, the risk for developing diabetes increases by approximately 12% [13]. On top of this, most of the currently available drugs for type 2 diabetes are associated with body weight increase (thiazolidinediones, sulphonylureas, and insulin) [14]. However, with the new incretin-based therapies for diabetes, positive outcomes on obesity-related parameters have been reported [15]. The incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are gut hormones secreted from endocrine cells in the intestinal mucosa acting as key regulators of the glucose-dependent alpha-cell and beta-cell responses in the pancreatic islets of Langerhans. Moreover, GLP-1 has body weight-reducing capabilities. In addition to GLP-1, the endocrine cells in the mucosal layer of the intestinal tract produce a wide range of substances known to influence appetite and food intake. This review summarizes the effects of incretin-based therapies in obesity, and also gives an overview of intestinal peptides with promising effects as potential new treatments for obesity.

Abbreviations:
BMI - body mass index
CCK - cholecystokinin
CI - confidence interval
CNS - central nervous system
DPP-4 - dipeptidyl peptidase 4
EMA - European Medicines Agency
GIP - glucose-dependent insulinotropic polypeptide
GLP-1 - glucagon-like peptide-1
GLP-1R - GLP-1 receptor
GOAT - ghrelin O-acyl tranferase
NPY - neuropeptide Y
PP - pancreatic polypeptide
PYY - peptide YY
WHO - World Health Organization
Y - tyrosine

Glucagon-like peptide-1 (GLP-1) physiology

GLP-1 is an incretin hormone released from the endocrine L-cells, situated primarily in the distal part of the ileum and in the colon, in response to ingestion of nutrients [16]. GLP-1 is processed from proglucagon and is rapidly degraded by the enzyme dipeptidyl peptidase 4 (DPP-4) [17]. DPP-4 cleaves off the two N-terminal amino acids, and leaves the molecule inactive, resulting in a half-life of less than 2 minutes [18]. Because of this rapid elimination, native GLP-1 is unsuitable for clinical use. GLP-1 potentiates glucose-induced insulin secretion and inhibits glucagon secretion, thereby improving glucose homeostasis. GLP-1 receptors (GLP-1R) are expressed in many regions of the brain and in particular in the arcuate nucleus and other hypothalamic regions involved in the regulation of food intake [19]. Correspondingly, animal studies using the GLP-1R antagonist, exendin(9-39), have demonstrated that GLP-1R activation is important in the regulation of appetite and food intake [20, 21].

The mechanisms behind the anorectic actions of gut-derived GLP-1 seems to be mediated through both central and peripheral mechanisms [22-25]. Interestingly, in a study where a destruction of the arcuate nucleus was induced, the inhibitory effect of intra-cerebroventricular GLP-1 administration on food intake and appetite disappeared. Whereas, the appetite suppressive effects of peripherally administered GLP-1 was maintained [26]. The study indicates that the arcuate nucleus is essential for the central action of GLP-1, but not for the peripheral action.

GLP-1 is recognized as a neurotransmitter involved in the regulation of appetite and food intake [21, 24]. Furthermore, in rats GLP-1-producing neurons are activated by distension of the stomach and enteroceptive stress [27, 28]. This could indicate a neuronal transmission of satiety signals from the gastrointestinal tract into the brain through the “brain GLP-1 system”. Theoretically, gut-derived GLP-1 could access the brain through leaks in the blood-brain barrier, such as the subfornical organ and the area postrema, as demonstrated in rats [29]. Thus, systemic infusions of GLP-1 have been shown to inhibit food intake in rats, independent of the vagal sensory afferents [30]. On the other hand, studies with large

molecular size GLP-1R agonists (albumin-conjugated GLP-1R agonists) in both humans and mice have demonstrated that these large proteins inhibit feeding, even though they probably cannot cross the blood-brain barrier [31, 32]. These studies support that at least some effects of GLP-1 may not be exerted directly through the central nervous system (CNS), but possibly through the afferent neurons of the vagus nerve [33]. The nerve endings of these nerves are placed in the lamina propria of the intestine just beneath the mucosal surface in the space between the secreting cells and the capillary bed, with cell bodies in the nodose ganglion [33, 34]. Because of the massive and rapid degradation of the GLP-1 by DPP-4, the concentration of active GLP-1 'available' for these nerve endings, which are very close to the secretory origin of GLP-1, must be many times higher than for any other target cell.

Furthermore, inhibition of food intake by GLP-1 could be related to the well-described inhibition of gastric motility and emptying occurring after GLP-1 administration [35, 36]. This consideration is in line with the fact that gastric emptying has a central role in the regulation of food intake [37]. However, this mechanism does not stand alone, since GLP-1 also elicits appetite reduction in fasting humans [38]. On top of this, the effects of gastric emptying do not explain the weight-reducing effects of GLP-1R agonists, as the effect of GLP-1 on the gastrointestinal motility wanes with time in contrast to the continuing effect on appetite and food intake [39, 40].

GLP-1 receptor agonists

The GLP-1R agonists are resistant to degradation, and thereby inactivation by DPP-4, although a high affinity to the GLP-1R is maintained. Two different types of GLP-1R agonists have been developed for drug treatment:

1. Peptides based on the lizard venom-extracted peptide exendin-4 having ~50% amino acid sequence homology with native GLP-1 (e.g. exenatide (Byetta ®); first market authorization in 2005 [41, 42]).
2. Peptides based on a back bone of the amino acid sequence of native GLP-1 having 97% sequence homology with the native peptide (e.g. liraglutide (Victoza®); first market authorization in 2009) [43]).

Exenatide has a half-life of 4-6 hours and must be administered twice daily, whereas liraglutide can be administered once-daily, because of its half-life of 12-14 hours. In October 2011, exenatide once-weekly was launched in Europe (Bydureon®). Other compounds with long half-lives (dulaglutide and albiglutide), are in late clinical development.

A recent meta-analysis investigated the therapeutic effects of GLP-1R agonists in the treatment of obese subjects with, and without, diabetes [15]. The analysis included twenty-five randomized controlled trials, including a total of more than 6,400 overweight participants treated with exenatide, exenatide once-weekly, or liraglutide for at least 20 weeks. It showed that patients randomized to GLP-1R agonists versus comparator (placebo, sulphonylurea, thiazolidinedione, DPP-4 inhibitor, or insulin) obtained a greater weight loss (weighted mean difference -2.9 kg; 95% confidence interval (CI) -3.6 kg to -2.2 kg). This treatment effect was seen in patients without diabetes (-3.2 kg, 95% CI: -4.3 kg to -2.1 kg) and in patients with type 2 diabetes (-2.8 kg, 95% CI: -3.2 kg to -2.1 kg). These results suggest that GLP-1R agonists might be beneficial as a new treatment of obesity, but long-term studies are needed to verify the effects and to answer safety issues.

In accordance with the meta-analysis mentioned above, a newly published study shows the superior effect of liraglutide compared to orlistat, the only anti-obesity-drug available in Europe [44]. After two years of treatment, a significant mean difference of 3 kg (95% CI: 1.3 kg to 4.7 kg) between the two drugs was seen in favor of liraglutide. In addition to the superior weight loss, liraglutide demonstrated beneficial effects on systolic and diastolic blood pressure, plasma levels of cholesterol and liver enzymes, and glycemic control. On the other hand, liraglutide was associated with nausea, diarrhea, and vomiting [44]. After the approval of exenatide and liraglutide, post-marketing reports of several incidents of acute pancreatitis in patients treated with GLP-1R agonists have been observed [45]. Current knowledge is insufficient to determine whether the incidence of acute pancreatitis is higher in those patients receiving exenatide or liraglutide compared with the background population of patients with type 2 diabetes. The latter group have an almost 3-fold increased risk of pancreatitis compared to a non-diabetic population [46]. It is recommended that GLP-1R agonists should not be used in subjects with a history of or increased risk of pancreatitis. In carcinogenicity studies with liraglutide, thyroid C-cell tumors were observed in mice and rats [47]. However, recent data has identified key differences between rodent models and humans regard-

ing this. Up to the present date, no changes in thyroid function have been reported in clinical trials with GLP-1R agonists [48]. However, the long-term safety of sustained GLP-1R activation in human thyroid tissue requires continuing pharmaco-vigilance [49]. Lately, a debate has arisen on the plausible risk of pancreatic cancer in patients treated with exenatide, as compared to other anti-diabetic medications [50]. The European Medicines Agency (EMA) recently concluded that a causal relationship between GLP-1R agonists and pancreatic malignancies could not be confirmed nor excluded; but again this area requires careful pharmaco-vigilance [51]. Furthermore, large clinical studies are on the way, aiming to assess and confirm the cardiovascular safety of both exenatide and liraglutide.

DPP-4 inhibitors

The effects of GLP-1 can also be exploited by protecting endogenous GLP-1 from degradation by the enzyme DPP-4 [52]. Oral administration of inhibitors of this enzyme increase the circulating levels of active GLP-1 which is associated with anti-diabetic effects [53]. DPP-4 inhibitors are small molecules that are active upon oral administration. They have shown a clinically significant and sustained effect on glycemic control [54]. However, DPP-4 inhibitors have little effect on body weight, presumably because the plasma concentrations of active GLP-1 are not elevated sufficiently to exert this effect [55]. Several DPP-4 inhibitors are undergoing clinical development. Currently four of them (sitagliptin (Januvia®, Merck Sharp & Dohme), vildagliptin (Galvus®, Novartis), saxagliptin (Onglyza®, AstraZeneca/Bristol-Myers Squibb), and linagliptin (Trajenta®, Boehringer Ingelheim)) have been approved as medications for type 2 diabetes. Presumably, none of these agents will be approved for treatment of obesity, because of their overall weight-neutrality.

Perspectives on other intestinal hormones

GLP-1 is not the only intestinal hormone possessing weight-regulating properties. A handful of peptides secreted by the intestinal linings has proven impact on body weight in preclinical settings. Currently, several potential receptor agonists (and also receptor antagonists) of these peptides are being developed. Some of these are even dual receptor agonists with an affinity for two distinctive receptors.

Oxyntomodulin

Oxyntomodulin is a native peptide with affinity for both the glucagon receptor and the GLP-1R [56-58]. It is thus a glucagon-GLP-1 dual agonist. As for GLP-1, it originates from the cleavage of proglucagon, and is released from intestinal L-cells immediately after meal ingestion, with plasma concentrations being closely related to calorie intake [57]. The amino acid sequence of oxyntomodulin corresponds to the entire 29-amino acid sequence of the glucagon molecule, with a C-terminal extension of eight amino acids [59]. Therefore, it was formerly known as enteroglucagon.

The peptide is rapidly degraded by DPP-4 and neprylsin with a half-life of approximately 12 minutes [60, 61]. The effects of acute administration of oxyntomodulin in humans include inhibition of gastric emptying, gastric and pancreatic exocrine secretion, and food intake [60, 62]. Also, repeated subcutaneous administration causes marked weight loss in obese subjects [63]. Weight loss occurs as a result of reduced food intake, and possibly because of increased energy expenditure [64]. As mentioned above, oxyntomodulin acts as an agonist on the glucagon receptor, but with a 10 to 100-fold decreased affinity than glucagon [56, 57].

Nevertheless, an oxyntomodulin analog with an increased affinity for the glucagon receptor in mice, demonstrated increased potency regarding inhibition of food intake and body weight reduction compared to the native oxyntomodulin [65]. This suggests that this potentiated effect was mediated via the glucagon receptor. On the other hand, the central effect of native oxyntomodulin seems to be at least mediated primarily through the GLP-1R, since the effect of oxyntomodulin infused in the rat brain is blocked by the GLP-1R antagonist exendin(9-39) [62]. Also, the effect of oxyntomodulin is abolished in GLP-1R knock-out mice [66].

Peptide YY

Peptide YY (PYY) is a 36-amino acid peptide released by mucosal enteroendocrine L-cells (along with GLP-1 and oxyntomodulin) in response to intraluminal nutrients (postprandial plasma responses are strictly correlated to energy intake) [67]. Carbohydrates, proteins, and lipids all stimulate the secretion of PYY [68]. PYY was originally isolated from porcine intestine, and is characterized by tyrosine (Y) residues at both ends of the

molecule, therefore the name [69]. PYY shares considerable homology with both pancreatic polypeptide (PP) and the neurotransmitter neuropeptide Y (NPY) in sequence and tertiary structure. When PYY(1-36) is secreted from the L-cell (or exogenous infused) it is readily cleaved by DPP-4 to form PYY(3-36), which is the major circulating form of PYY [70, 71]. PYY activates three of the mammalian NPY receptors (Y1, Y2, and Y5). PYY(1-36) has a relatively high affinity for all three receptors, whereas PYY(3-36) is a selective Y2 receptor agonist [72]. In humans, PYY(1-36) infusion delays gastric emptying [73], and inhibits gastric acid secretion and gallbladder contraction [74]. However, the anorectic effect of PYY seems to be mediated by by PYY(3-36), but not by PYY(1-36). This appears to be due to the specific affinity of the dominating peptide PYY(3-36) for the Y2 receptor, and due to the fact that the Y1 and Y5 receptors are associated with increased feeding behavior [75].

The fact that PYY(3-36) seems to be the active 'body weight-reducing' form might partially explain the failure of DPP-4 inhibitors to reduce weight to the same extent as the GLP-1R agonists, since an active DPP-4 enzyme is essential for the metamorphosis of PYY(1-36) to PYY(3-36) [76]. In humans, intravenous infusion of PYY(3-36) reduces food intake in both lean and obese subjects [77]. Unfortunately, adverse effects like nausea and vomiting are very common. In a recent study by Sloth *et al*. only 4 out of the first 9 participants succeeded in completing the high dose PYY(3-36) infusion (0.8 pmol/kg/min) due to the effects of nausea, vomiting, stomach pain, and flushing [77]. Thus, the dose of PYY(3-36) had to be decreased markedly. Similarly, a pharmacologic formulation of PYY(3-36) failed because of side-effects (nausea and vomiting) [78]. Recently, PYY(3-36) was suggested for use as anti-obesity treatment in combination with oxyntomodulin [79]. This would allow augmented action on appetite suppression through additive effects of PYY(3-36) and oxyntomodulin. Since both substances are L-cell derivatives and probably co-secreted this might correlate more with the native anorectic L-cell signal.

Cholecystokinin

Cholecystokinin (CCK) is a gut hormone secreted from the enteroendocrine I cells in the small intestine. It is also a neuropeptide localized at nerve terminals in the CNS and in the peripheral nervous system [80]. CCK is processed in different lengths (e.g. CCK-58, -33, -22, and -8), which predicts its activity. CCK shares its active site and receptors with another gut hormone, namely gastrin [81]. CCK has relative high affinity for the CCK receptor (CCK-A receptor) and a relative low affinity for the gastrin receptor (CCK-B receptor).

In the periphery, CCK primarily exerts its effects through the CCK receptor. It stimulates gallbladder contraction and pancreatic enzyme secretion, and retards gastric emptying [82, 83]. Furthermore, the release of PYY and GLP-1, after lipid ingestion, is dependent on signaling through the CCK receptor [84, 85]. In the CNS, the gastrin receptor seems to be the main receptor; and the effects of CCK-8 and CCK-5 are probably mediated through this receptor as only trace amounts of gastrin are found in the CNS [80, 86]. Intracranial infusion of CCK-8 seems to inhibit food intake in rats, but when it is administrated peripherally these effects are not persistent [87]. When CCK is administered to rats by intra-peritoneal infusion at the start of every meal, reduced meal size is rapidly compensated for by increased meal frequency [88]. Similar results were seen in humans where acute administration of CCK-8 decreased food intake [89].

Nevertheless, the results from phase II trials using CCK receptor agonists have been disappointing to date. Thus, GI181771X (a CCK receptor agonist) did not elicit body weight loss when administered to more than 700 obese subjects (tested in a 24-week, cross-over, double-blinded setting) [90]. On the other hand, animal studies with CCK receptor agonists have demonstrated promising results [91, 92]. CCK has been found to enhance cerebral uptake of leptin in rats, wich results in a synergistic effect on weight loss [93]. Also, the synergistic effect in combination with cannabinoid receptor blockage [94] keeps CCK receptor signaling alive as a potential target for obesity treatment.

Ghrelin

Ghrelin is secreted from endocrine X/A-like cells in the gastric fundus and is activated upon acetylation by ghrelin O-acyl tranferase (GOAT) [95, 96]. It is a potent ligand for the growth hormone secretagogue receptor [97]. Also it seems to be an important regulator of appetite [98]. In humans, ghrelin peaks just before a meal intake, and decreases immediately after meal ingestion in lean subjects [99, 100]. On top of this, intravenous infusion of ghrelin in healthy lean subjects leads to heavily increased appetite and food intake [98].

Furthermore, patients with Prader-Willi syndrome (a rare genetic disease characterized by overeating) are characterized by elevated plasma levels of ghrelin [101].

Interestingly, in obese subjects, plasma ghrelin concentrations do not change after a test meal [102]. This suggests that blocking the ghrelin signal could be a tempting target for the treatment of obesity. Although ghrelin receptor knock-out mice are only modestly smaller than wild type mice, appetite and body composition are comparable to that of wild type littermates [103]. However, some data indicate an impaired growth hormone axis in the ghrelin receptor knock-out mice, a finding that raises some concern. Although, preclinical studies with agonists, and antagonists, to the growth hormone secretagogue receptor indicate that it is possible to distinguish between the actions of ghrelin on growth hormone secretion and food intake [104, 105]. These findings indicate that the effects on food intake might be facilitated through an alternative receptor to the well-known growth hormone secretagogue receptor, thus opening the opportunity to develop specific drugs.

Conclusions

The development of GLP-1R agonists for the treatment of type 2 diabetes with improved glycemic control combined with a sustained weight loss, is a major breakthrough, in the medical treatment of type 2 diabetes; and potentially, for the treatment of obesity. The accumulated data on GLP-1R agonists has led to a remarkable interest in other very important and potent gut-derived peptides. Most have been known for quite a while.

At present, the most promising data concern the L-cell derivatives, of which GLP-1 is by far the best described. However, both oxyntomodulin and PYY have gained some attention in recent years, and the idea of using the entire L cell response as template for the treatment of obesity is very attractive. As such, the results from well-conducted studies combining the effects of the three peptides in various ways are much anticipated.

In addition to the L-cell products, both CCK and especially ghrelin are potent peptides in the regulation of appetite, food intake, and body weight. Nonetheless, the road to the development of compounds specifically designed for the treatment of obesity seems to be a longer than for the L-cell derivatives. The development of all these gastrointestinal hormone derivatives into pharmacological agents will certainly provide novel insight and understanding of the role of the gastrointestinal tract in the regulation of appetite, hunger, food intake, and body weight.

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