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Gut Hormones Ghrelin, PYY, and GLP-1 in the Regulation of Energy, Balance, and Metabolism

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The first hormone discovered in the gastrointestinal tract was secretin, isolated from duodenal mucosa. Some years later, two additional gastrointestinal hormones, gastrin and cholecystokinin (CCK), were discovered, but it was not until the 1970s that gastrointestinal endocrinology studies became more prevalent, resulting in the discovery of many more hormones. Here, we examine the role of gut hormones in energy balance regulation and their possible use as pharmaceutical targets for obesity.

Key Words: Ghrelin; PYY; GLP-1; gastrointestinal tract.

Introduction

In addition to its functions in digestion and assimilation of nutrients, the gut also includes endocrine tissues involved in the regulation of energy balance. Various hormones secreted from endocrine cells in the gastrointestinal tract and several neural pathways that communicate information from the signals responsible for the regulation of food intake and energy expenditure are also responsible for these energy balance functions. After receiving the information from the peripheral tissues, the central nervous system (CNS) processes these signals and sends orders to controllers of energy homeostasis.

The first hormone discovered in the gastrointestinal tract was secretin, isolated from duodenal mucosa. Some years later, two additional gastrointestinal hormones, gastrin and cholecystokinin (CCK), were discovered, but it was not until the 1970s that gastrointestinal endocrinology studies became more prevalent, resulting in the discovery of many more hormones. Here, we examine the role of gut hormones in energy balance regulation and their possible use as pharmaceutical targets for obesity.

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Ghrelin

Growth hormone secretagogues (GHSs) are artificial compounds that release growth hormone (GH) in all species, including humans. These molecules mimic an unknown endogenous factor that activates the GHS receptor in the pituitary and the hypothalamus. The cloning of GHS-R *(1)* suggested that an endogenous ligand for this receptor might exist, and Kangawa's group identified the 28-amino-acid peptide ghrelin as the endogenous ligand for the "orphan" GHS receptor *(2)*. Ghrelin takes its name from the proto-Indo-European word "ghre," which means grow, and "relin," relating to its role in the release of GH. Ghrelin results from the cleavage of a precursor called preproghrelin, which is composed of 117 amino acids. Preproghrelin contains a 23-amino-acid signal peptide and the 94-amino-acid proghrelin, which includes the 28-amino-acid mature ghrelin and a 66-amino-acid tail. The greatest GHS receptor activation has been found in stomach extracts *(2)*. Moreover, Tomassetto's group identified this protein separate from the stomach as motilin-related peptide *(3)*. Motilin-related peptide shows an amino acid sequence identical to that of ghrelin, suggesting that the same gene was discovered by two different groups and therefore given two different names. Based on structure and activity similarities, motilin and ghrelin are considered to represent a novel gastrointestinal hormone family in addition to the gastrin, secretin, pancreatic polypeptide (PP), insulin, epidermal growth factor (EGF), and tachykinin families *(4)*.

Two molecular forms of ghrelin are found in the stomach: the 28-amino-acid ghrelin possesses a fatty acid chain modification, an n-octanoylated serine in position 3, and the 27-amino-acid des-Gln14-ghrelin produced by alternative splicing of the ghrelin gene *(5)*. The acylation appears to be essential for the release of GH in both natural forms of ghrelin, although desacyl ghrelin may also be biologically active in the cardiovascular system *(6)*, in the promotion of adipogenesis *(7)*, or in the regulation of insulin sensitivity *(8)*. Other minor forms of ghrelin are present in human plasma and stomach, measured as the total immunoreactivity by conventional radioimmunoassay (RIA) based on the carboxyl-terminal fragment of ghrelin *(9)*. Studies of struc-

ture activity show that the amino-terminal fragment conserving the first five amino acids of the molecule displays full functional activity regarding calcium mobilization in vitro. However, structural heterogeneity of ghrelin appears minor, and human ghrelin is identical to rat ghrelin except for two residues *(2)*.

Distribution of Ghrelin

Roughly 65% of plasma ghrelin is produced in the stomach by the X/A-like cells within the oxyntic glands of the gastric fundus mucosa, and at least one third is produced in the small intestine. The brain, hypothalamus, pituitary, pancreas, kidney, lymphocytes, lung, placenta, testis, and ovary also express significant levels of ghrelin and will be discussed below. The ubiquitous expression of ghrelin in all of these tissues suggests that it has local paracrine and/or autocrine actions (revised in refs. *6* and *10*).

Small Intestine

Although Northern blot analysis of rat tissues showed that preproghrelin mRNA occurs only in the stomach, RT-PCR analysis and RIAs have detected it in the intestine as well *(11)*. Using *in situ* hybridization, immunohistochemistry, immunohistochemical double staining, and electron microscopy immunostaining, the authors investigated the ghrelinproducing endocrine cells in the digestive systems of rats and humans. Ghrelin expression was most abundant in the oxyntic mucosa of both species. Four types of endocrine cells—ECL, D, enterochromaffin (EC), and X/A-like cells—have been identified in the oxyntic mucosa *(11)*. The relative percentages of these four cells in rat oxyntic gland are 60–70% for ECL cells, 20% for X/A-like cells, 2–5% for D cells, and 0–2% for EC cells; those in the human oxyntic gland are 30% for ECL cells, 20% for X/A-like cells, 22% for D cells, and 7% for EC cells *(12)*. The localization of ghrelin-immunoreactive cells in the gastrointestinal tract suggests that X/A-like cells produce ghrelin. Ghrelin cells in the oxyntic mucosa are closed-type cells that have no continuity with the lumen, suggesting that they respond to physical stimuli from the lumen, chemical stimuli from the basolateral site, or both. Ghrelin cells are closely associated with the capillary network running through the lamina propria. However, the mechanisms that govern the biosynthesis and secretion of this peptide in the gastrointestinal tract remain unknown.

Hypothalamus

Although initially discovered in the ventral–lateral border of the arcuate nucleus (ARC) *(2)*, further analysis of its neuroanatomical distribution in the brain *(13)* showed bodies of ghrelin-immunopositive cells in the interstitial areas among the dorsomedial hypothalamus (DMH), the ventromedial hypothalamus, and the walls of the third ventricle. Ghrelin-immunopositive fibers were closest to cells that were immunoreactive for neuropeptide Y (NPY), pro-opiomelanocortin (POMC), thyroid-releasing factor (TRH), and corticotrophin-releasing factor (CRH) in nuclei related to food intake control such as the DMH, the lateral hypothalamus, and the periventricular hypothalamus. Ghrelin may stimulate the release of orexigenic peptides and neurotransmitters, thus representing a novel regulatory circuit controlling energy homeostasis.

Pituitary

In one study, ghrelin expression was localized in the pituitary using real-time RT-PCR *(14)*. Another study used double immunofluorescence to demonstrate the specific adenopituitary cell types that express ghrelin; these authors observed immunostaining in lactotrophs, somatotrophs, and thyrotrophs, whereas the peptide was absent in the remaining cell types *(15)*. The ghrelin gene was expressed in the male rat pituitary throughout postnatal development, with the highest levels detected in infantile–prepubertal pituitary samples, and the lowest levels detected in adult tissues. Moreover, the authors observed that pituitary ghrelin mRNA levels were decreased in hypothyroid rats, and after the administration of glucocorticoids in normal rats. Ghrelin was also modified by GH levels but not by fasting, which indicates that the regulation of ghrelin gene expression is tissue-specific *(15)*. Finally, immunohistochemistry revealed positive staining of both acyl and desacyl ghrelin in both normal and abnormal human pituitary, but corticotroph tumors showed significantly less expression of ghrelin *(14)*.

Pancreas

Which pancreatic-type cells express ghrelin is still not clear. Whereas Date et al. *(16)* used immunohistochemistry to observe that ghrelin was colocalized with glucagons in human and rat pancreas, another group used immunohistochemistry and *in situ* hybridization to observe that ghrelin colocalized with insulin in human islets *(17)*. In contrast, a third group used immunohistochemistry and *in situ* hybridization to show that human pancreatic ghrelin cells did not express any pancreatic hormones, such as insulin, glucagons, somatostatin, or pancreatic polypeptide *(18)*. The differences in these results may be due to the different antibodies used by each group.

What seems clear is that ghrelin can produce hyperglycemic effects as well as inhibit the secretion of insulin *(19,20)*. These findings concur with previous studies showing that chronic treatment with GHS induces hyperglycemia and insulin resistance *(21)*. Moreover, ghrelin secretion may be suppressed by increased plasma glucose or insulin levels. However, ghrelin could exert some effect on glycogenolysis, and this action would be mediated by other GHS-Rs because other GHS-R type 1a agonists do not affect this process *(20)*.

Kidney

It has been demonstrated through RP-HPLC coupled with RIA that the kidney contains both ghrelin and the desacyl

peptide *(22)*. Yoshimoto et al. described the gene expression of preproghrelin and ghrelin receptor in the kidney and suggest that ghrelin has possible roles in the kidney as part of an endocrine and/or paracrine system. Moreover, a recent study has described a significant increase in plasma ghrelin in patients with chronic kidney disease compared to patients with normal renal function, as well as a correlation between plasma ghrelin and both S-creatinine and GH *(23)*. Moreover, nephrectomy in mice induced a marked increase in plasma ghrelin without significant changes in ghrelin mRNA levels in the stomach. This finding suggests that the kidney is important in ghrelin clearance, but further studies are needed to clarify the role of ghrelin and its possible effects in chronic kidney disease.

Lymphocytes

There are many studies which have demonstrated that immune system cells, especially inflammatory cytokines, may play an important role in the regulation of food intake and body weight. Previous studies have described mRNA expression of GHS-R in lymphoid organs *(24)*. Recently it was shown the specific location of GHS-R in human T cells *(25)*. Using cultured human T cells, the authors demonstrated a significant and specific rise in intracellular Ca^{2+} in response to ghrelin, while desacyl ghrelin did not induce calcium release. Furthermore, ligation of G protein–couple receptors (GPCRs) is often accompanied by a marked remodeling of the actin cytoskeleton and cell surface molecules and leads to polarization and, in many cases, the directional migration of immune cells *(26)*. In this work, ghrelin produced an important increase in broad membrane structures characteristic of lamellipodia, with typical polarization of F-actin. Taken together, these data demonstrate that on the surface of human T cells, GHS-R is a functional receptor. In these cells, ghrelin was able to decrease IL-1β, IL-6, and TNF-α expression. These actions were mediated by GHS-R, suggesting that the ghrelin/GHS-R system could mediate anti-inflammatory actions *(25)*.

Lung

Ghrelin expression was investigated in human fetal, infant, and adult lungs using immunohistochemistry, *in situ* hybridization, and RT-PCR *(27)*. Ghrelin protein was found in the endocrine cells of the fetal lung in decreasing amounts from embryonic to late-fetal periods. Its expression was maintained in newborns and children under 2 yr, but was virtually absent in older individuals (although the authors were able to observe ghrelin mRNA using RT-PCR). Thus, it seems that the fetal lung is an additional source of circulating ghrelin, although its functions at the respiratory-tract level remain to be elucidated.

Itoh et al. investigated whether plasma ghrelin was associated with clinical parameters in patients with chronic obstructive pulmonary disease (COPD) *(28)*. Plasma ghrelin was significantly higher in underweight patients with COPD than in normal-weight patients and healthy control subjects. In addition, plasma ghrelin correlated positively with a cachectic state and abnormality of pulmonary function *(28)*. In another study it was observed that baseline plasma ghrelin levels were elevated in cachectic patients with lung cancer, and follow-up plasma ghrelin levels increased after chemotherapy in patients with anorexia. Considering the positive energy effects it induces, increased ghrelin production may represent a compensatory mechanism under catabolic–anabolic imbalance in cachectic patients with lung cancer *(29)*.

Placenta

Ghrelin was identified in human and rat placenta using immunohistochemistry and molecular approaches such as RT-PCR and Northern and Southern blot analyses. In placenta, its expression was mainly located in cytotrophoblast cells in humans and labyrinth trophoblast cells in rats, while other cell types such as syncytiotrophoblast cells showed lower expression *(30)*. In human placenta, ghrelin appears to be mainly expressed in the first half of pregnancy, whereas it could not be detected at term. In the rat placenta, expression increases through pregnancy and appears to be present at the later stages of gestation. Although the role of ghrelin in this tissue is not clear, the discovery of pregnancy-related expression in placenta, a condition not paralleled in gastric tissue, suggests that ghrelin may have physiological functions in gestation *(30)*.

Testis

Initial analyses identified a testis-specific ghrelin-gene-derived transcript (GGDT) in mice *(31)*. A later study demonstrated the expression of ghrelin in rat and human testes *(32)*. In the rat testis, ghrelin expression was selectively detected in Leydig cells at advanced stages of maturation. Similarly, immunohistochemical analyses showed that ghrelin is strongly expressed in interstitial mature Leydig cells of the human testis *(33)*.

Regarding its biological activity in this tissue, ghrelin can inhibit human choriogonadotropin- (hCG-) and cAMP-stimulated testosterone secretion in a dose-dependent manner. The inhibitory effect of ghrelin on testosterone secretion was associated with a significant decrease in hCG-stimulated levels of the mRNAs encoding several key factors in the steroidogenic pathway, such as steroid acute regulatory protein and the enzymes involved in P450 side-chain cleavage, 3β-hydroxyl steroid dehydrogenase (HSD), and testis-specific 17β-HSD type III *(32)*.

Furthermore, ghrelin inhibits the expression of the gene encoding stem cell factor, which is a Sertoli cell product regarded as the major paracrine stimulator of germ cell development and a survival factor for spermatogonia, spermatocytes, and spermatids in the adult rat seminiferous epithelium *(34)*. Indeed, using in vivo models, this group has demonstrated that ghrelin can inhibit the proliferative activity of immature Leydig cells during pubertal development *(34)*.

Ovary

In the rat, ovarian expression of the ghrelin gene was demonstrated throughout the estrus cycle, with the lowest levels in proestrus and peak expression at diestrus (i.e., during the luteal phase of the cycle). In rats, ghrelin immunoreactivity was predominantly located in the luteal compartment of the ovary *(35)*. Likewise, strong ghrelin immunostaining was observed in young and mature corpora lutea of the human ovary *(36)*, whereas ghrelin signal was absent in ovarian follicles at all developmental stages. Ghrelin was also detected in interstitial hilus cells, an androgen-secreting cell found in the ovary *(36)*.

In addition, there are several studies regarding ghrelin levels in polycystic ovarian syndrome (PCOS). In women with PCOS, serum ghrelin levels were significantly lower than in healthy controls, both lean and obese *(37)*. Another study supported these results, indicating that obese women with PCOS had lower ghrelin levels than would be expected based on the presence of obesity. Moreover, in these obese women with PCOS, ghrelin negatively correlated with insulin sensitivity *(38)*, whereas an anti-androgen treatment increased their circulating-ghrelin levels *(39)*. On the other hand, other groups were unable to see any differences in plasma ghrelin levels between PCOS and control subjects *(40)*.

Ghrelin Regulation

The regulation of ghrelin can occur at different levels, including at the gene transcription, translation, and the post-translational modification levels. Regulaton also occurs during ghrelin secretion from the point ghrelin is secreted from several tissues and as a result of enzymatic processes that affect circulating ghrelin; at the point of its clearance or degradation by the kidney or liver; and at the receptor (GHS-R) level regarding the binding, transport, expression in target tissue, and influence of different agonists and/or antagonists of the GHS-R.

Nutritional Status

Ghrelin induces food intake and adiposity *(41)*. Gastric ghrelin production is regulated by nutritional and various hormonal factors. Fasting leads to increased expression in the stomach and higher plasma concentrations *(42)*, and its levels are reduced immediately following food intake. Postprandial ghrelin reduction is proportional to the ingested calorie load *(43)*. Ghrelin levels change throughout the day, reaching high levels before food intake and during the night, suggesting that ghrelin is an important factor in meal initiation *(44,45)*. After ingestion, plasma ghrelin levels are rapidly suppressed, and Williams et al. *(46)* observed that gastric dis-tension did not affect ghrelin suppression after food intake; however, this meal-related suppression of plasma ghrelin requires postgastric stimulation.

Circulating ghrelin levels are lower in obese than non-obese human and rodent subjects *(47,48)* and are higher in patients with anorexia nervosa and in animals after fasting or in states of cachexia *(49,50)*. Moreover, subjects with a low body mass index (BMI) have higher ghrelin levels than obese subjects *(44,51)*.

Contrary to expectations, some studies have revealed that circulating ghrelin levels are decreased after gastric bypass surgery *(52)*. Another surprising result was found in patients with Prader–Willi syndrome, which is a genetic disorder characterized by mild-to-moderate mental retardation, hyperphagia and obesity, GH deficiency, hypogonadism, and sleep and thermoregulation disturbance. Although obese subjects have low ghrelin levels appropriate for their BMI, patients with Prader–Willi syndrome have high ghrelin levels *(53)*, which might result from the hypothalamic damage observed in these subjects *(54)*.

During pregnancy, a hypermetabolic state characterized by increased food intake and body weight, gastric ghrelin mRNA expression showed a similar expression pattern. Moreover, when rats were food restricted, both ghrelin plasmatic and gastric levels were increased, indicating that ghrelin may have a role in mediating physiological responses to undernutrition and could represent an adaptive response to prevent long-lasting alterations in energy balance and body weight homeostasis *(55)*.

Glucose Homeostasis

Several studies performed in humans and rodents demonstrated that there is a negative correlation between circulating ghrelin levels and insulin secretion *(56–59)*. Plasma ghrelin levels decrease after oral and IV glucose administration *(51)*. An inverse pattern of ghrelin and insulin levels has been noted during 24-h observation in normal subjects *(45)*. Also, a reciprocal relationship between insulin and ghrelin has been observed during hyperinsulinemic–euglycemic clamp studies *(59,60)*. Moreover, studies that evaluated ghrelin concentrations in normal vs type 1 diabetic subjects revealed that insulin is required for prandial ghrelin suppression in humans *(61,62)*.

More recently, an inverse relationship between fasting ghrelin and insulin levels and insulin resistance indices has been reported by several investigators *(37,63,64)*. Postprandial suppression of ghrelin correlated with a rise in insulin *(64)*. Obese patients with type 2 diabetes mellitus have lower fasting plasma ghrelin concentrations than normal-weight patients without diabetes *(51,65)*, suggesting that decreased plasma levels of active ghrelin are significantly associated with abdominal adiposity, hyperinsulinemia, and insulin resistance in type 2 diabetic patients. On the other hand, it has been shown that only prolonged and supraphysiological hyperinsulinemia resulted in suppressed plasma ghrelin levels in lean subjects *(66)*. In that study, the authors observed a reduction in plasma ghrelin values only during pharmacological hyperinsulinemia, suggesting that sustained insulin concentrations would be required to suppress ghrelin in lean subjects. They concluded that insulin at physiological concentrations is not responsible for the reduction in

ghrelin in healthy, lean subjects *(66)*. Moreover, although ghrelin secretion in humans is inhibited by oral glucose–induced hyperglycemia and insulin administration. Glucagon and arginine, two substances known to increase insulin and glucose levels, did not affect ghrelin levels. These findings call into question the assumption that glucose and insulin directly regulate ghrelin secretion *(67)*. Plasma levels of ghrelin and insulin are similar in acromegalic patients with GH-induced insulin resistance and in obese patients *(68)*.

Adipose and Gut Hormones

Leptin is a 16-kDa protein that is produced predominantly by white adipose cells *(69)*. Elevated leptin levels are present in obesity, and this hormone can be considered a signal to the body concerning its energy reserve levels. The reported effects of leptin on ghrelin production have been variable (i.e., increase, no change, or decrease), and may be due to differences in dosage or duration of leptin administration or feeding conditions of the animal subjects *(70–72)*. However, in human patients with leptin deficiency, ghrelin levels are low *(73)*.

Pancreatic polypeptide (PP) is a 36-amino-acid peptide that belongs to a family of peptides including NPY and peptide YY (PYY). Peripherally, but not centrally, administered PP inhibits food intake. PP significantly inhibited mRNA expression of gastric ghrelin in rats deprived of food for 24 h *(74)*, but a PP infusion had no effect on ghrelin levels in humans *(75)*. $PYY_{3\text{-}36}$, a gut peptide that has controverted actions on food intake *(76,77)*, decreased ghrelin levels in both the fasted and postprandial state and directly inhibited ghrelin-activated neurons of the ARC *(78)*. However, a reduction in circulating ghrelin is not requisite for the effects of $PYY_{3\text{-}36}$ *(79)*. Oxyntomodulin IV injection in humans reduced food intake and ghrelin levels *(80)*. Administration of gastrin or CCK resulted in an increase of both acyl and desacyl ghrelin levels in rats *(81)*. Glucagon increased the activity of the ghrelin promoter in vitro *(82)*.

Biological Actions in Energy Balance

Food Intake and Body Weight

As described above, ghrelin is expressed in neurons adjacent to the third ventricle between the dorsal, ventral, paraventricular, and ARC hypothalamic nuclei. These neurons send efferents on to key hypothalamic circuits that regulate energy balance, such as NPY, agouti gene-related protein (AGRP), POMC, and CRH neurons. Both peripheral and central ghrelin administration potently stimulate food intake, leading to body weight gain in rodents *(41,83)*. The increase in body weight is caused by an augmentation in fat mass without changes in longitudinal skeletal growth and with a decrease in lean mass *(41)*. Several studies have suggested that orexigenic effects of ghrelin are mediated via leptin-responsive neurons in the hypothalamus *(83–86)*. Several hypothalamic cells express leptin and/or ghrelin receptors, and there is evidence which suggests that some of these nuclei are critical sites of integration for leptin-responsive and ghrelin-activated pathways. Within the ARC, two distinct leptin- and ghrelin-responsive cell groups exist. One regulatory pathway consists of neurons coexpressing NPY and AGRP, potent stimulators of food intake, while an adjacent set of ARC neurons coexpresses POMC and cocaine- and amphetamine-regulated transcript (CART), which suppress food intake. These cells respond to signals regarding both the long- and short-term energy status of the animal. Leptin, secreted by and in proportion to white adipose tissue, signals the level of energy stores, activates POMC/CART neurons, and inhibits NPY/AGRP neurons, resulting in inhibition of feeding and an increase in energy expenditure. On the other hand, ghrelin activates NPY/AGRP neurons, stimulating feeding and decreasing energy expenditure. Consistent with ghrelin action in the ARC, ablation of the ARC with monosodium glutamate significantly blunts the ingestive behaviors normally stimulated by central delivery of ghrelin *(87)*. Furthermore, when administered at the central level, ghrelin decreases spontaneous locomotor activity *(88)*.

Although the main site of action in the control of food intake by ghrelin seems to be the ARC in the hypothalamus, some studies have found orexigenic activity of ghrelin following administration outside of this area, including the hindbrain. Administration of ghrelin in the fourth ventricle or directly on the dorsal vagal complex resulted in a hyperphagic response with a magnitude similar to that obtained with administration in the third ventricle *(89)*. Moreover, GHS-R expression was found in neuronal cells of the nucleus of the solitary tract, the dorsal motor nucleus of the vagus, the rostral ventrolateral medulla, and the caudal ventrolateral medulla. It has been reported that injection of ghrelin in the hippocampus or dorsal raphe nucleus evoked an increase in food intake. These results suggest that, in addition to areas of ghrelin control in the hypothalamus, ghrelin exhibits orexigenic activity in various zones of the central nervous system, especially in the caudal brain stem.

In addition to the central pathways, ghrelin also can exert its actions on peripheral tissues, but its effects on food intake are less potent and seem to be temporary *(90)*. It has been demonstrated that, in vitro, ghrelin can increase the expression of PPAR-γ2; thus, ghrelin stimulates the differentiation of preadipocytes and antagonizes lipolysis, suggesting that it acts directly on adipocytes to stimulate adipogenesis *(91)*. Furthermore, administration of both ghrelin and des-octanoyl ghrelin in the bone marrow also stimulates adipogenesis *(7)*. Ghrelin also has direct effects on brown adipose tissue, decreasing adiponectin expression *(92)*.

There is some published evidence demonstrating that ghrelin action might be mediated not only by efferent but also by afferent activity of the vagal nerve. In electrophysiological studies, IV-administered ghrelin has been shown to decrease the afferent activity of the gastric vagal nerve at low doses *(70)*. It has been reported in humans that ghrelin

has no effect on food intake in patients who have undergone surgical procedures involving vagotomy *(93)*. The described effects are in contrast to those of gastrointestinal satiety peptides such as CCK and may add additional pathways to the growing number of signaling routes with which ghrelin is connected *(4)*.

Gut

Ghrelin is the first gut peptide proven to have orexigenic properties, and the increase in food intake following central injection of ghrelin in rodents occurs rapidly and is more potent than any other orexigenic factor *(94)*. Although motilin can stimulate food intake after central administration, it produces the least effect when administered peripherally. Almost all other gut peptides such as CCK, glucagon-like peptide-1 (GLP-1), PP, and the controvert PYY_{3-36}, as well as neural signals derived from the gut, act as agents of satiation *(95)*. They influence the termination of individual meals and/or relate to the long-term regulation of body weight.

In one study, it was observed that administration of ghrelin in rats increased gastric acid secretion and gastric motility *(96)*, and these effects were abolished by pretreatment with either atropine or bilateral cervical vagotomy. Ghrelin also stimulates gastric emptying *(74)* and gastrin release *(97)*.

Glucose and Insulin

As described above, there is a negative correlation between circulating ghrelin concentrations and insulin secretion. However, the results regarding the influence of ghrelin on insulin secretion are conflicting. Whereas in one study ghrelin induced hyperglycemia and reduced insulin secretion in humans *(20)*, in another study ghrelin increased the cytosolic free Ca^{2+} concentration in beta cells and stimulated insulin secretion when it was administered to isolated rat pancreatic islets *(16)*. Further studies support the effect of ghrelin as an inhibitory factor for insulin secretion *(19,98)* and showed that this effect was dose-dependent *(99)*. Moreover, ghrelin stimulates somatostatin and pancreatic polypeptide release in humans *(100)*, and there is a negative correlation between somatostatin and insulin.

Ghrelin and Ghrelin Receptor Knockout Models

Ghrelin knockout animals are indistinguishable from their wild-type littermates. In studies performed in mice with disruption of ghrelin gene expression, researchers did not detect any changes in food intake, body weight, body size, growth rate, body composition, reproduction, bone density, or organ weight *(101,102)*. However the absence of ghrelin reduced the respiratory quotient in animals fed with a high-fat diet, which means that there was an increase in fat oxidation, suggesting that ghrelin can play a role in the preference of metabolic fuels besides fat *(102)*.

The GHS-R knockout was also created recently. In contrast to wild-type mice, acute treatment of GHS-R–null mice with ghrelin had no effect on GH release or food intake, showing that the GHS-R is a biologically relevant ghrelin receptor. However, the appearance of GHS-R–null mice could not be distinguished from that of their wild-type littermates. GHS-R–null mice were not dwarfs, and food intake and body composition were similar to that of wild-type littermates. Furthermore, in contrast to suggestions that ghrelin regulates leptin and insulin secretion, fasting-induced changes in serum levels of leptin and insulin were identical in wild-type and null mice. The only phenotypic difference was observed in serum insulin-like growth factor 1 levels and body weights of mature (16–24 wk of age) GHS-R–null mice, which were modestly lower compared to wild-type animals; however, there was no significant difference in food intake at these ages. Although it has also been proposed that ghrelin plays a role in testicular and placental function, as described above, breeding of GHS-R heterozygous mice produced normal size litters.

Peptide YY

Peptide YY (PYY) is a 36-amino-acid hormone that is structurally and functionally related to NPY and PP. PYY was isolated from porcine intestine *(103)*, and its immunoreactivity has been localized in the open-ended L-type endocrine cells of the terminal ileum and colon in the rat, dog, and human *(104,105)*, as well as in cells along the periphery of pancreatic islets. But there are important structural differences among the members of this family. The N terminal of PYY is very different from that of PP, allowing PYY, but not PP, to cross the blood–brain barrier.

PYY levels are low in the fasting state and are released in response to food intake, acting to inhibit gastric motility, gastric acid, and insulin secretion *(106)*. PYY levels reaches the highest levels after 1–2 h post-ingestion, with these peak levels influenced by both the number of calories and the composition of the food consumed. PYY is increased by fat at higher levels than proteins or carbohydrates. PYY is also located in the central nervous system in low concentrations *(107)*; PYY-containing neurons are present in the hypothalamus and hindbrain regions *(108)*; and PYY receptors are located with dense localizations in thalamic, limbic, and hindbrain nuclei *(109–111)*. PYY_{1-36} and PYY_{3-36} are the two molecular forms of PYY abundant in the blood *(112)*. Both PYY and PYY_{3-36} show selective affinity to Y1 and Y2 receptors, respectively. PYY_{3-36} is a truncated 34-amino-acid form created through cleavage of N-terminus Tyr-Pro residues by dipeptidyl peptidase IV *(113)*.

Orexigenic Effects vs Anorexigenic Effects

The 70% structural homology between PYY and NPY suggested that these two peptides might elicit similar biological responses. Morley et al. *(114)* have reported that

centrally administered PYY is a more potent orexigenic factor than NPY. However, multiple injections do not attenuate the hyperphagic effect of PYY *(114)*. PYY produces hyperphagia when injected into either of the cerebroventricles, the periventricular nucleus, or the hippocampus, an extra-hypothalamic site not usually associated with ingestive responses. PYY infused into the fourth ventricle yields food intake comparable to that seen after third and lateral ventricle infusions *(115)*. Food intake generally subsides within 4 h post-injection. Among all known orexigenic peptides and neurotransmitters, PYY is the most potent acute stimulator of food intake. However, elevated systemic levels of PYY_{3-36} resulting from gastric bypass surgery have contrary effects, leading to a reduction of food intake *(116)*.

In contrast to its central effects, several reports have shown that peripheral administration of PYY_{3-36} reduces food intake in rodents, primates, and humans *(76,117,118)*. It has been demonstrated that peripherally administered PYY_{3-36} suppressed food intake in rodents in a transient manner after a single dose. Furthermore, PYY_{3-36} also inhibits dose-dependent food intake in rats when administered by continuous IV infusion to non-food-deprived rats. PYY_{1-36} is less potent than PYY_{3-36} in decreasing food intake. PYY_{3-36} reduces food intake by decreasing meal size and increasing the satiety ratio (postprandial interval per meal size). PYY_{3-36} inhibits food intake in sham-feeding rats, indicating that this hormone can reduce food intake independent of inhibiting gastric emptying *(119)*.

In humans, infusion of PYY_{3-36} caused an equivalent inhibition of appetite and food intake in the obese and lean groups, resulting in reduced cumulative 24-h food intake. These findings indicate that, unlike its association with leptin, obesity is not associated with substantial resistance to PYY. Fasting PYY levels were lower in the obese group than in the lean group, and there was a negative correlation between fasting PYY levels and BMI. Furthermore, postprandial PYY release was lower in obese than in lean subjects *(117)*.

However, other laboratories were not able to reproduce the anorexigenic effects of PYY_{3-36} in rodents *(77)*. These groups only detected a transient decrease in body weight in *ob/ob* mice. In fact, they observed a significant reduction in locomotor activity, rearing and sniffing behavior following PYY_{3-36} administration, so energy expenditure tended to decrease, albeit not significant. Thus, these findings support a positive energy balance after PYY_{3-36} treatment.

A possible explanation for this incongruity could be that without proper habituation of the mice, the action of PYY_{3-36} is not evident. This loss of efficacy may result from the effects of stress caused by handling the animals *(120)*. However, Tschop et al. *(77)* did not find any changes in plasma corticosteroids, in hypothalamic c-Fos expression in the paraventricular nucleus area, or by monitoring feeding and other stress-sensitive behavior. Thus, these conflicting results need to be clarified, and further studies should elucidate the exact role of this protein in energy balance and the conditions necessary for it to exert its actions in humans and rodents.

Mechanism of Action

PYY_{3-36} acts at the level of the hypothalamus. Consistent with the converse effects of ghrelin and PYY on food intake, a high percentage (50%) of ARC neurons were activated by ghrelin and inhibited by PYY. In line with this inhibitory action, peripherally injected PYY partly reversed the fasting-induced c-Fos expression in ARC neurons of mice *(78)*. Immunohistochemical studies demonstrated that peripherally administered PYY_{3-36} induced c-Fos expression in ARC neurons expressing POMC.

The notion that POMC neurons were the downstream targets of PYY_{3-36} was strengthened through electrophysiological studies demonstrating that PYY_{3-36} was inhibitory to another population of neurons within the ARC, those expressing the orexigenic NPY *(76)*. PYY_{3-36} acts as an agonist with the Y2-R on NPY neurons and, in doing so, causes elimination of the tonic inhibition on adjacent POMC neurons. Interestingly, in Y2-R knockout mice, PYY_{3-36} could not reduce food intake, indicating that this receptor plays a key role in the effects of PYY_{3-36}. Furthermore, the effects of PYY_{3-36} were associated with an increase in POMC and a decrease in NPY mRNA levels in the ARC *(121)*. However, other studies have demonstrated that both POMC and MC4-R knockout mice are responsive to the anorexigenic effects of PYY_{3-36} *(120,122,123)*, suggesting that the MC4-R is not essential for the activity of this protein. Thus, therefore, the increase of POMC might be not essential for PYY_{3-36} actions.

A later study showed by double-immunostaining that approx 20% of melanocyte-stimulating MSH neurons of the ARC were activated by peripheral administration of PYY_{3-36} *(124)*. MSH is a downstream product of the POMC gene and is colocalized with CART in ARC neurons. Because it is known that CART suppresses feeding independently of the MC4-R system when administered intracerebroventricularly *(125)*, these findings imply that CART may play a role in the PYY_{3-36} cascade. Finally, it was observed that PYY_{3-36} also acts on the reduction in food intake in part via the vagal afferent pathway, because it was found to have no effect on food intake in vagotomized rats or in rats with bilateral midbrain transactions in which the efferent fibers ascending from the NTS were ablated *(124)*. However, a recent report showed that peripherally administered PYY_{3-36} activates neurons in the area postrema and NTS, brainstem areas known to mediate effects of certain aversive stimuli. This study also demonstrated that peripheral administration of PYY_{3-36} causes conditioned taste aversion in mice. Thus, inhibition of food intake by PYY_{3-36} may result in part from induction of an aversive response *(125)*.

Glucagon-like Peptides

Glucagon-like peptide-1 (GLP-1) is a hormone that regulates blood glucose levels through its combined actions on the stimulation of glucose-dependent insulin secretion and the inhibition of glucagon secretion, gastric emptying, and food intake *(126)*. Glucagon-like peptide-2 (GLP-2) is a 33-amino-acid peptide that regulates energy homeostasis via acute and chronic effects on gut motility and nutrient ingestion and absorption *(126)*.

GLP-1 and GLP-2 are derived from posttranslational modification of the larger precursor molecule proglucagon *(127)*. Proglucagon is synthesized within the enteroendocrine L cells in the intestines, primarily the ileum and colon. The major stimulus for GLP-1 and GLP-2 secretion is the ingestion of nutrients, including glucose, fatty acids, and dietary fiber *(128)*. When nutrients are ingested, the release of GLP-1 and GLP-2 into the circulation occurs in a biphasic manner, consisting of a rapid (within 10–15 min) early phase followed by a more prolonged (30–60 min) second phase *(129)*. Two different mechanisms are responsible for this biphasic pattern. In the first, the vagus nerve, the neurotransmitter gastrin-releasing peptide, and the hormone glucose-dependent insulinotropic peptide contribute to the rapid release of GLP-1 and GLP-2 from distal L cells in response to nutritional stimuli *(128)*. In contrast, a direct stimulation of the L cells by digested nutrients is responsible for the second phase of peptide release *(129)*. GLP-1 is secreted as GLP-1 (7-37) and GLP-1 (7-36), but its half-life is very short (less than 2 min) *(130)*, whereas GLP-2 is more stable, with a half-life of approx 5–7 min *(131)*. The enzyme responsible for this degradation of both GLP-1 and GLP-2 is dipeptidyl-peptidase IV (DPP-IV), which produces the inactive peptides GLP-1 (9-37) and GLP-2 (3-33).

Biological Effects on Food Intake and Mechanisms of Action

Central or peripheral administration of GLP-1R agonists leads to the inhibition of food intake and reductions in body weight in rodents *(132–134)*. GLP-1 also inhibits food intake and promotes satiety in normal, obese, and diabetic humans *(135–137)*.

It has been proposed that the inhibitory effects of GLP-1 on food intake can be mediated indirectly by its ability to slow gastric emptying, thereby promoting gastric distension and a sensation of satiety. In addition, neurons that express GLP-1 are located in the hypothalamus and NTS, CNS regions that are thought to be important for regulating appetite and satiety *(138,139)*. In rats, central administration of GLP-1 dependently reduces food intake *(140–143)*, an effect that is reversed by coadministration of the GLP-1 receptor antagonist exendin-(9-39) *(142)*. GLP-1 receptors in the hypothalamus mediate the reduction of food intake by acting through the normal pathways that control energy balance *(139,144–146)*, whereas GLP-1 receptors in the amygdala could mediate the reduction of food intake by the activation of aversive signalling pathways that produce visceral illness *(146,147)*.

Peripherally administered GLP-1 also has an anorexigenic effect on healthy *(148)*, obese *(135)*, and diabetic humans *(136,149)*. Because the half-life of active GLP-1 is very short, the reduction of food intake is probably a result of GLP-1's inhibitory effects on gastrointestinal transit and reduced gastric emptying *(150)*. However, peripherally administered GLP-1 can cross the blood–brain barrier *(151)*; thus, its role and relationship within the CNS to food intake needs to be elucidated. Finally, GLP-1R(–/–) mice exhibit normal feeding behavior and body weight *(152)*.

GLP-2 has beneficial effects on intestinal growth *(153, 154)*, and its actions are mediated by a specific GLP-2R *(155)*. In rats, the infusion of GLP-2 prevents the development of small bowel mucosal villous hypoplasia *(156)* and also produces beneficial effects on the gastrointestinal epithelium of rodents *(157)*. However, there is limited evidence that GLP-2 has trophic effects on the stomach, pancreas, or any peripheral organs. Even though GLP-2 is structurally similar to GLP-1, its role on food intake is not clear; some studies have found that central administration of GLP-2 in rats could decrease food intake *(158)*, whereas a study in mice treated with GLP-2 for 9 d found no effect on food intake or weight gain *(159)*. Also, no effect has been observed on food intake or weight gain in humans *(160,161)*.

Oxyntomodulin

Oxyntomodulin (OXM) is a 37-amino-acid peptide also produced by posttranslational processing of preproglucagon in the intestine and the CNS. Similarly to GLP-1, OXM is rapidly released from the L cells of the distal small intestine after food ingestion in proportion to meal calorie intake. ICV administration of OXM inhibits food intake in rats with greater potency than does GLP-1 *(162)*. OXM appears to act via a GLP-1-like receptor, increasing cAMP accumulation and stimulating somatostatin secretion *(163)*. Moreover, OXM anorectic actions are blocked by coadministration of the GLP-1 receptor antagonist, exendin-(9-39) *(162)*; however, the affinity of OXM for the GLP1-R is lower than that of GLP-1, suggesting that the potent action of OXM on food intake could also be mediated by another receptor. Another finding that supports the hypothesis of a second receptor is the stimulation of different areas of the brain by OXM and GLP-1. GLP-1 activates cells in the brainstem and other central autonomic control sites *(164)*, whereas OXM-mediated stimulation is restricted to the ARC *(165)*. Thus, the precise molecular mechanisms by which OXM mediates its biologic actions, including inhibition of food intake, remain inconclusive. Recently, it was shown that OXM is also a potent inhibitor of food intake when administered intraperitoneally to rats *(165)*. Furthermore, in humans, intravenous infusion of OXM significantly reduced

food intake and cumulative 12-h caloric intake, but it did not affect cumulative 24-h energy intake. OXM was able to decrease ghrelin levels by 44% in human subjects, suggesting that ghrelin could mediate OXM actions on energy balance *(80)*. As with GLP-1, in addition to its effects on food intake, OXM also augmented postprandial insulin secretion, inhibited gastric acid secretion, and reduced gastric motility.

Pharmacological Perspectives

Evidence suggests that the gut acts as a nutriment sensor, resulting in the release of several hormones. These hormones initiate hunger or satiety signals, which produce long- and/or short-term responses to control feeding behavior. Therefore, energy intake and, thus, body weight are tightly regulated. This homeostatic control of body weight makes obesity a serious problem. Several approaches are currently used to treat obesity, but all have associated problems and many, in particular pharmacological agents such as orlistat and sibutramine, have limited effectiveness *(166)*.

Because ghrelin stimulates appetite in rodents and humans, it is expected that a ghrelin antagonist could exert beneficial effects in the treatment of obesity; however, such a molecule might be expected to reduce GH levels. Thus, it could be indicated for the treatment of acromegaly, and other effective agents would be available for obesity *(6)*.

Regarding PYY_{3-36}, as the effects of this hormone are not easily reproducible and because it has modest anorexigenic efficacy and a transient effect, it seems that this hormone will not be a successful pharmacological target against obesity. Moreover, its effects may be insignificant relative to the prominent reduction in food intake due to stress, and a novel therapeutic that efficiently reduces food intake and body weight in today's environment is unlikely to be successful if it is dependent on the complete absence of stress. Finally, one of the latest findings showing that PYY_{3-36} induces taste aversion is another argument against its use in clinical trials.

Because it both reduces food intake and stimulates insulin secretion, GLP-1 is a logical candidate as a therapeutic agent in the treatment of obesity and type 2 diabetes. However, as discussed above, the major problems in the potential use of GLP-1 as a treatment are its rapid degradation by DPP-IV and its aversive effects. An alternative strategy is to use compounds that compromise DPP-IV, thus prolonging the half-life of endogenous GLP-1 and eliminating the taste aversion *(167)*. At the moment, it is known that exendin-4 resists degradation by DPP-IV while retaining GLP-1 effects. Moreover, GLP-1 activity is prolonged by conjugation to albumin (liraglutide) or inhibition of DPP-IV.

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