Gastrointestinal Satiety Signals

III. Glucagon-like peptide 1, oxyntomodulin, peptide YY, and pancreatic polypeptide

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Obesity is a growing epidemic that results in an increasing morbidity and mortality. It is caused associatively with a number of serious medical conditions, including diabetes mellitus, coronary heart disease, and a number of cancers. Since leptin was discovered in 1994, advances have been made in our understanding of the peripheral signals that regulate appetite and energy homeostasis. Several peptides synthesized and secreted within the gastrointestinal tract are known to regulate eating behavior. Many of these circulating peptides have direct access to the arcuate nucleus of the hypothalamus and to the area postrema, brain regions involved in the regulation of food intake. These peptides may also work outside the central nervous system (CNS) to influence the activity of neurons such as the vagal nerve, which projects to the nucleus of the solitary tract (NTS) in the brain stem. Food ingestion causes the release of anorexigenic peptides as well as vagal stimulation by mechanical and chemical receptors in the gut. This review considers the anorexigenic peptides PYY, PP, GLP-1, and oxyntomodulin, which decrease appetite and promote satiety in both animal models and humans.

GLP-1 AND OXM

OXM and GLP-1 are products of the preproglucagon gene, which is expressed in the CNS, the L cells of the small intestine, and the pancreas. Preproglucagon is cleaved by prohormone convertases 1 and 2 into different products depending on the tissue. In the pancreas, the glucagon sequence is cleaved out, whereas the part containing the GLP-1 and -2 is secreted as a single, large inactive peptide. The posttranslational processing in the gut and brain are similar. The glucagon sequence remains in a larger peptide, glicentin, thought to be inactive. The two GLPs are cleaved out and secreted separately. Glicentin is later cleaved into glicentin-related pancreatic peptide (GRPP) (inactive NH2-terminal fragment) and OXM. GLP-1 and OXM are released from the L cells of the distal small intestine, 5–30 min after food ingestion and in proportion to meal calorie intake. The secretion of OXM may be in response to fat that has undergone hydrolysis to fatty acids within the gut. L cells may coexpress other anorexigenic peptides such as PYY(3–36) and cholecystokinin.

Increasing plasma levels of GLP-1 and OXM result in postprandial satiety. The raised plasma levels of the two gut hormones also inhibit gastric acid secretion and motility. GLP-1 has been shown to decrease calorie intake in animal models by administration intracerebroventricularly and directly into the hypothalamic paraventricular nucleus (PVN) (8, 19). Chronic administration via this route produces weight loss in rodents (15).

In human subjects, peripheral administration of GLP-1 via an intravenous route results in satiety. An infusion of OXM to normal-weight human subjects produced a reduction in calorie intake of 19.3% (6). A recent meta-analysis of the effect of GLP-1 infusion demonstrated an average reduction in calorie intake of 11.7% (21). The reduction in calorie intake is dose dependant and does not differ between obese and lean individuals.

Both GLP-1 and OXM may exert their effects via the GLP-1 receptor (GLP-1R). Exendin 9–39, an antagonist at the GLP-1R, opposes the effect of GLP-1 and OXM. However, the affinity of OXM for GLP-1R is approximately two orders of magnitude weaker than that of GLP-1, even though OXM exerts a comparable effect on food intake. It is therefore possible that there may be a separate OXM receptor that has not yet been cloned.

The feeling of satiety produced by OXM and GLP-1 is likely due to their effects on the CNS as well as their effect on gastric emptying. The GLP-1 receptor is present in the NTS and arcuate region. The NTS receivesafferent input from the vagal and glossopharyngeal nerves and integrates both neuronal and humoral factors. This area is also able to synthesize GLP-1; thus the GLP-1-containing neurones may influence their own activity.

GLP-1 containing neurones of the NTS project to the arcuate nucleus and hypothalamic nuclei, such as the dorsal medial nucleus (DMN) and PVN, which are involved in appetite.
control. There are two populations of neuronal circuits within the arcuate. One circuit inhibits food intake and consists of neurones that coexpress proopiomelanocortin (POMC), and cocaine and amphetamine-regulated transcript. The other circuit, coexpressing neuropeptide Y (NPY) and agouti-related peptide, stimulates food intake (7). The GLP-1 projections may act on these neurones to inhibit appetite. There is also evidence that the arcuate is influenced by GLP-1 from the periphery via the area postrema and subfornical organ (17). Thus there are several routes by which the gut can communicate with appetite circuits.

OXM may also exert its effects on appetite via suppression of ghrelin, an orexigenic peptide produced by endocrine cells in the oxyntic glands of the stomach. OXM administration, producing plasma concentrations comparable with postprandial levels, reduces ghrelin by ~44% in human subjects (6).

Evidence suggests GLP-1 secretion is reduced in obese subjects and weight loss normalizes the levels (22). The anorectic effects of GLP-1 are, however, preserved in obesity. A reduced secretion of GLP-1 could therefore contribute to the pathogenesis of obesity, and OXM and GLP-1 are potential targets for treatment.

In addition to their effects on satiety, OXM and GLP-1 also promote meal-induced insulin secretion. GLP-1 has been found to upregulate insulin gene expression and potentiate all steps of insulin biosynthesis. An intravenous infusion of GLP-1 is capable of completely normalizing blood glucose levels in patients with long-standing type 2 diabetes who cannot be controlled by sulphfonylurea therapy. Furthermore, a 6-wk subcutaneous infusion of GLP-1 to type 2 diabetics normalizes glycosylated fructosamine and reduces HbA1c by 1.3%. This infusion of GLP-1 was also found to reduce body weight by 2 kg (23). This potential therapeutic application is particularly useful in type 2 diabetes where obesity is commonly a significant issue.

The therapeutic potential of these gut hormones is limited by their rapid breakdown. GLP-1 is deactivated by dipeptidyl peptidase IV (DPP-IV), which cleaves off the two NH2-terminal amino acid residues, transforming the peptide into an antagonist of the GLP-1 receptor. However, recent trials have shown that inhibition of DPP-IV may be an effective treatment for type II diabetes mellitus. Various resistant analogs in development, such as exendin 4 (exenatide, Amylin), and albumin-based forms, such as Liraglutide (NovoNordisk), may improve glycemic control and reduce body weight.

### PP and PYY

PP and PYY were first isolated over 20 years ago, PP from porcine pancreatic extracts in the early 1970s (12) and PYY from porcine intestine in 1980 (18). Both PP and PYY belong to a peptide family that also includes NPY. The peptides have several common features: all are 36-amino acid peptides containing several tyrosine residues, all undergo COOH-terminal amidation, which is necessary for biological activity, and all have a common tertiary structure, the PP fold. This configuration consists of a polyproline helix and α-helix connected by a β-turn resulting in a characteristic U-shaped peptide. There is also marked evolutionary conservation of amino acid sequence between the peptides with 42% homology between rat PP and PYY.

However, there are important differences between PP and PYY. Despite their overall high homology, the NH2 terminus of PP has little similarity to that of PYY. As a consequence, PP interacts poorly with membrane phospholipids and, unlike PYY, does not readily cross the blood-brain barrier (BBB). In animal models, increasing plasma PP concentrations in response to food intake or by infusion does not alter cerebrospinal fluid (CSF) PP levels. In contrast, PYY crosses the BBB freely by nonsaturable mechanisms. A further difference is the presence of an additional 34-amino acid form of PYY, PYY(3–36), created by cleavage of the NH2-terminus Tyr-Pro residues by DPP-IV.

Five cloned receptors for the PP-fold peptide family have been described, Y1–Y5 (nomenclature as recommended by International Union of Pharmacology) (13). They are all seven transmembrane domain receptors coupled to Gi resulting in inhibition of adenylate cyclase. However, Y1 also increases intracellular calcium, and Y2 regulates both calcium and potassium channels.

The receptors are classified according to their affinity for PYY, PP, and NPY fragments and analogs and have diverse distributions and functions (see Table 1 for summary).

Whereas PYY binds with high affinity to all Y receptors, PYY(3–36) shows selectivity for Y2 and Y4 receptors. In contrast, PP binds with greatest affinity to Y4 receptors, where its affinity is greater than that of PYY, but also to Y5 receptors.

### DISTRIBUTION OF PP AND PYY

PP is primarily expressed in the endocrine cells of the pancreas, particularly those in the duodenal portion. Here, PP-immunoreactive cells form ~10% of endocrine cell population. The cells are found at the periphery of the islets, adjacent to somatostatin and glucagon immunoreactive cells. PP cells are also found elsewhere in small numbers, in the exocrine pancreas and in the gastrointestinal tract (mainly in the colon and rectum in humans), and PP immunoreactivity has also been reported in the rat adrenal medulla. The presence of PP in the CNS is unclear. PP immunoreactivity has been reported in porcine hypothalamic extracts, and PP mRNA has been detected by RT-PCR in the rat brain, but it has not been possible to detect PP mRNA by Northern blot analysis in the rodent CNS (9).
In contrast to PP, PYY is widely expressed throughout the gastrointestinal tract in endocrine cells where it is colocalized with GLP-1. PYY immunoreactive cells are almost absent in the stomach, relatively few in the duodenum and jejunum, and dramatically increased in frequency in the ileum and colon, and are at very high levels in the rectum. PYY has been described in the myenteric plexus and endocrine pancreas of many species, but not in humans. PYY immunoreactivity has also been reported in human adrenal medulla. PYY is present in the CNS, with PYY immunoreactive nerve terminals in the hypothalamus, medulla, pons, and spinal cord (9).

**REGULATION OF PP AND PYY RELEASE.**

Plasma PP concentrations are regulated both by food intake and by an intrinsic circadian rhythm. In fasting individuals, circulating PP is lowest at 0200 and peaks at 2100. However, the primary stimulus to plasma PP is food intake, which results in biphasic release. The postprandial PP concentration is proportional to the caloric load, but the contribution of the first and second phases varies. In response to the first meal of the day, there is a relatively small first-phase release of PP, but the contribution of this first phase increases with subsequent meals. However, the overall release with an isocaloric meal remains unchanged. In addition, gastric distension, for example by water ingestion, also significantly increases PP release.

Both diurnal and postprandial PP release appears to be regulated by vagal tone. Propantheline blocks the diurnal changes in plasma PP seen in fasting and reduces postprandial release by 60%, whereas vagotomy abolishes postprandial PP release.

Although ingestion is the main stimulus to PP release, other factors have also been shown to alter circulating PP concentrations. Adrenergic stimulation, for example, due to hypoglycemia or exercise, increases plasma PP concentrations. Other pancreatic and gastrointestinal hormones also regulate circulating PP levels. Ghrelin, motilin, and secretin rapidly stimulate PP release, whereas somatostatin and its analogs significantly reduce plasma PP concentrations.

PPY is also released into the circulation in response to food intake, rising to a plateau after 1–2 h. Release is partly proportional to caloric intake but also influenced by meal composition. Higher plasma concentrations are seen following isocaloric meals of fat compared with intake of protein or carbohydrate. In addition to nutrients, PYY release is also stimulated by gastric acid, cholecystokinin, and infusion of bile acids into the ileum or colon in animal studies. Unlike PP, PYY is not released by gastric distension. An intraduodenal meal increases plasma PYY even before nutrients have reached the PYY-containing cells of the ileum. This suggests release through a neural reflex, probably via the vagus.

Other factors also alter circulating PYY. Plasma PYY concentrations are increased by insulin-like growth factor-1, bombesin, and calcitonin gene-related peptide and decreased by GLP-1.

**ACTIONS OF PP**

The role of PP in appetite regulation has been investigated since the 1970s. In 1977, Malaisse-Lagae et al. (14) noted that ob/ob mice lacked pancreatic PP-producing cells and demonstrated that twice-daily intraperitoneal administration of bovine PP suppressed appetite and body weight in these mice. Subsequently, it was demonstrated that obese rodents were less sensitive to the anorectic actions of PP. More recently, a comprehensive study by Asakawa et al. (1) examined the actions of peripherally administered physiological doses of PP in mice. PP produced a rapid and prolonged reduction in food intake, acting within 20 min of administration and persisting for 24 h. In addition, PP stimulated sympathetic activity and oxygen consumption, suggesting an increase in energy expenditure. These doses were associated with increased vagal activity and delayed gastric emptying. The central responses to peripherally administered PP were also examined. Expression

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<td>Y1</td>
<td>NPY, PYY, Leu[31], Pro[34] NPY/PYY</td>
<td>NPY(2–36), NPY(3–36), NPY(13–36)</td>
<td>PP</td>
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<td>NPY/PYY fragments, PYY(3–36)</td>
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<td>COOH-terminal NPY fragments</td>
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<td>y6 (human)</td>
<td>Deletion in sixth TM domain resulting in truncated non-functioning receptor in man</td>
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<td>Heart, muscle, intestine, spleen</td>
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NPY, neuropeptide Y; PYY, peptide YY; PP, pancreatic polypeptide.
of hypothalamic NPY and orexin mRNA, which encode orexigenic peptides, were reduced, whereas that of the anorectic urocortin was increased following intraperitoneal PP administration. PP also reduced gastric ghrelin expression. Repeated peripheral PP injection reduced body weight and improved metabolic parameters including cholesterol in mice. The role of PP in appetite regulation is also supported by the phenotype of mice overexpressing pancreatic PP. These transgenic animals were lean, with reduced food intake and reduced gastric emptying (20).

The effects of PP on appetite in humans were first examined in subjects with Prader-Willi syndrome, characterized by obesity and marked hyperphagia. Food intake was reduced during intravenous infusion of PP (5). Recently, PP has also been shown to alter appetite in normal-weight individuals. As in rodents, PP infusion resulted in a rapid reduction in food intake, after just 2 h, but also a prolonged action decreasing food intake over the following 24 h (4). However, PP does not appear to alter gastric emptying in humans.

In contrast to the anorectic effects of peripheral PP, central PP administration increased food intake in animal models. Third-ventricle injection of human PP stimulated daytime food intake in satiated rats. Similarly, central injection of PP has the opposite effect to peripheral administration on gastric motility, stimulating rather than inhibiting gastric emptying.

The divergent actions of central and peripheral PP on appetite probably reflect the differential receptor activation. PP is unable to cross the BBB and therefore enters the CNS via regions that have a deficient BBB, such as the area postrema. After intravenous administration, autoradiographic studies demonstrate PP accumulation in the area postrema (AP) and expression of the early gene c-fos is seen in the AP following peripheral administration. Y4 receptors are highly expressed in this region, suggesting that the anorectic actions of PP are mediated by this receptor. The central Y receptors mediating the feeding effects of PP are unclear. Whereas PP binds with high affinity to Y3 receptors, human and bovine PP (but not rat PP) also bind to Y5 receptors. The orexigenic effects of PP are blunted in Y5−/− transgenic mice but not by Y3 receptor antisense oligonucleotides, and the role of Y4 receptors in PP’s central stimulation of food intake has not been confirmed (see Ref. 11 for review).

**ACTIONS OF PYY**

Early studies on the actions of peripherally administered PYY demonstrated numerous effects on the gastrointestinal tract. PYY administration significantly delayed gastric emptying, gastric and pancreatic secretion, and the cephalic phase of gallbladder emptying but increased ileal postprandial fluid and electrolyte absorption. However, the effects of the truncated form of PYY, PYY(3–36), on appetite have only recently been reported. Intraperitoneal PYY(3–36) reduced dark phase and fasting-induced feeding in rats without altering gastric emptying. Repeated PYY(3–36) administration reduced food intake and body weight gain. The anorectic effects of PYY(3–36) are also seen in humans; 2 h after PYY(3–36) infusion, food intake was reduced by >30% in both normal weight and obese volunteers, without any alteration in gastric emptying (3, 4).

The actions of peripheral PYY(3–36) on satiety appear to be mediated by the Y2 receptor expressed in the hypothalamic arcuate nucleus. PYY(3–36) increased arcuate expression of the early gene c-fos, its peripheral actions were mimicked by intra-arcuate injection of PYY(3–36) and a selective Y2 agonist also reduced food intake. In addition, the effects of PYY(3–36) were abolished in Y2−/− mice. The arcuate Y2 receptor acts as a presynaptic inhibitor receptor. Activation of this receptor by PYY(3–36) reduced NPY expression and release. Arcuate NPY inhibits the activity of anorectic POMC neurons. This brake on POMC neurons is therefore reduced by PYY(3–36) binding to arcuate presynaptic Y2 receptors allowing increased POMC neuronal activity and hence a reduction in appetite.

PYY also has direct effects on adipocytes, reducing lipolysis in vitro. Other actions of peripherally administered PYY included decreasing glomerular filtration rate, reduction in plasma renin activity, and, in aldosterone, vasoconstriction and a fall in cardiac output.

In common with PP, PYY administration to the CNS has opposite actions to patients seen with peripheral PYY. PYY injections into the third, lateral, or fourth cerebral ventricles, into the paraventricular nucleus, or into the hippocampus potently stimulated food intake in rodents. Intracerebroventricular PYY(3–36) injection also stimulated food intake; PYY binds with high affinity to Y1, Y2, and Y5 receptors, and PYY(3–36) binds with high affinity to Y2 and with less avidity to Y1 and Y3 receptors. The effects of PYY(3–36) on food intake are diminished in both Y1−/− mice and in Y5−/− mice, suggesting these receptors play a role in central PYY(3–36)-mediated food intake (see Ref. 10 for review). Administration of PYY in the dorsal vagal complex stimulated gastric acid secretion, again the opposite effect to that seen following peripheral administration.

**PP AND PYY IN DISEASE**

Plasma concentrations of PP and PYY are altered in disease and with alterations in body weight. This may, in turn, alter satiety.

The effects of gastrointestinal disease on plasma PYY are diverse. Elevated plasma PYY is seen in those with celiac disease, hepatic cirrhosis, previous ileal resection, or symptomatic Crohn’s disease. However, tissue concentration of PYY is reduced in Crohn’s disease. The changes in plasma PYY reported in active ulcerative colitis vary, being described either as unchanged compared with controls or decreased and with reduced tissue concentrations. The number of colonic PYY-immunoreactive cells is increased in diabetic patients with gastroparesis.

The effects of gastrointestinal surgery are also variable and depend partly on the site of resection. Gastrectomy increased plasma PYY. Ileal resection raised both PYY and PP concentrations, but colonic resection has been variously reported to reduce or increase plasma PYY. PP release is blunted in subjects with chronic pancreatitis, but plasma PYY is increased in postpancreatectomy patients. Gastric bypass has no effect on basal or meal-stimulated PP but increases basal and postprandial PYY.

The most interesting changes in circulating PP and PYY are those seen at extremes of body weight. Plasma PP was increased in individuals with anorexia nervosa. The circulating
levels of PYY in eating disorders are not known, but CSF PYY is raised in those with bulimia nervosa. The effects of obesity on circulating concentrations of PP are conflicting; whereas some report suppressed plasma PP, others have found no difference between lean and obese subjects or between obese subjects before and after weight loss. However, in morbidly obese children with Prader-Willi syndrome, both basal and meal-stimulated PP release are diminished. Circulating PYY is suppressed in patients with morbid obesity and rises to levels seen in nonobese subjects after gastric banding.

In conclusion, the physiological roles of GLP-1, OXM, PP, and PYY are diverse. Recent work suggests their actions are not limited to regulation of gut secretions and motility but that they also play an important role in appetite regulation. Plasma concentrations of GLP-1, PP, and PYY are reduced at extremes of body weight and could contribute to increasing adiposity. All have been shown to be effective in reducing food intake in humans, and, importantly, for both GLP-1 and PYY(3–36), these actions are preserved in obese individuals. Whether these peptides are also effective as long-term modulators of appetite needs to be confirmed.

REFERENCES


