The Gut Hormone Peptide YY Regulates Appetite

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ABSTRACT: The gut hormone peptide YY (PYY) belongs to the pancreatic polypeptide (PP) family along with PP and neuropeptide Y (NPY). These peptides mediate their effects through the NPY receptors of which there are several subtypes (Y1, Y2, Y4, and Y5). The L cells of the gastrointestinal tract are the major source of PYY, which exists in two endogenous forms: $PYY_{1–36}$ and $PYY_{3–36}$. The latter is produced by the action of the enzyme dipeptidyl peptidase-IV (DPP-IV). $PYY_{1–36}$ binds to and activates at least three Y receptor subtypes (Y1, Y2, and Y5), whereas $PYY_{3–36}$ is more selective for Y2 receptor (Y2R). The hypothalamic arcuate nucleus, a key brain area regulating appetite, has access to nutrients and hormones within the peripheral circulation. NPY neurons within the arcuate nucleus express the Y2R. In response to food ingestion plasma $PYY_{3–36}$ concentrations rise within 15 min and plateau by approximately 90 min. The peak $PYY_{3–36}$ level achieved is proportional to the calories ingested, suggesting that $PYY_{3–36}$ may signal food ingestion from the gut to appetite-regulating circuits within the brain. We found that peripheral administration of $PYY_{3–36}$ inhibited food intake in rodents and increased C-Fos immunoreactivity in the arcuate nucleus. Moreover, direct intra-arcuate administration of $PYY_{3–36}$ inhibited food intake. We have shown that Y2R null mice are resistant to the anorectic effects of peripherally administered $PYY_{3–36}$, suggesting that $PYY_{3–36}$ inhibits food intake through the Y2R.

In humans, peripheral infusion of $PYY_{3–36}$, at a dose which produced normal postprandial concentrations, significantly decreased appetite and reduced food intake by 33% over 24 h. These findings suggest that $PYY_{3–36}$ released in response to a meal acts via the Y2R in the arcuate nucleus to physiologically regulate food intake.

KEYWORDS: peptide YY (PYY); Y2 receptor; neuropeptide Y (NPY); pro-opiomelanocortin (POMC); arcuate nucleus; appetite

INTRODUCTION

In response to a meal, hunger is reduced for several hours. However, the mechanisms responsible for this prolonged suppression of appetite are unknown. Intravenous infusion of nutrients does not have this long-lasting effect, suggesting that gut-derived factors are important. The hypothalamic region of the brain receives and integrates neural, metabolic, and endocrine signals from the periphery and orches-

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trates appropriate changes in appetite and energy expenditure.[1] Within the hypothalamus, the arcuate nucleus plays a key role in the regulation of ingestive behavior. The arcuate nucleus is a circumventricular organ, with access to the third ventricle and the portal vasculature and is responsive to a wide range of peripheral hormones and nutrients.[2] Two distinct subsets of neurons controlling food intake are found within the arcuate nucleus: the neuropeptide Y (NPY)/agouti-related peptide (Agrp) neurons and the pro-opiomelanocortin (POMC) neurons. Activation of the NPY/Agrp neurons causes increased food intake and decreased energy expenditure, whereas activation of the POMC neurons decreases food intake and increases energy expenditure.[3] These two neuronal subsets act as sensors, responding to circulating hormones that reflect body energy stores, such as leptin.[4] However, the identity of peripheral factors signaling food ingestion to these feeding circuits remains largely unclear.

PEPTIDE YY

Peptide YY (PYY) is a 36-amino-acid gastrointestinal hormone first isolated from porcine small intestine by Tatemoto in 1980[5] and named PYY because of the presence of an amino acid terminal (Y) tyrosine and a carboxyl terminal tyrosine amide (Y). PYY belongs to the pancreatic polypeptide (PP) family of peptides together with NPY and PP. The L cells of the gastrointestinal tract are the major source of PYY of which there are two main endogenous forms: $PYY_{1–36}$ and $PYY_{3–36}$.[6] The latter is produced by the action of the enzyme dipeptidyl peptidase-IV (DPP-IV), which hydrolyzes PYY at the Pro^2-Ile^3 bond.[7] $PYY_{1–36}$ binds to and activates at least three Y receptor subtypes in rats and humans (Y1, Y2, and Y5). Removing the first two amino acids at the N-terminal changes the receptor selectivity such that $PYY_{3–36}$ is more selective for Y2 receptor.[8] The percentage of these two forms in human blood has been reported to differ according to the feeding status. In the fasted state, the concentration of $PYY_{1–36}$ predominates over that of $PYY_{3–36.}$ In contrast, after a meal, $PYY_{3–36}$ is the major circulating form.[9] Following ingestion of food, plasma levels increase within 15 min, reach a peak at approximately 90 min, and then remain elevated for up to 6 h.[10] Interestingly, PYY levels reflect meal size and the nature of the food, fat being the most potent nutrient in releasing PYY.

There have been conflicting reports regarding the distribution of PYY from immunocytochemical studies, in part due to cross-reactivity of antibodies with NPY and PP, whereas *in situ* hybridization studies have identified PYY messenger RNA (mRNA) only in the gastrointestinal tract, the pancreas, and the brainstem.[11] PYY has been shown to have several biological actions, including vasoconstriction, inhibition of gastric acid secretion, reduction of pancreatic and intestinal secretion, and inhibition of gastrointestinal motility. When injected into the cerebral ventricles, the paraventricular nucleus (PVN) of the hypothalamus, or hippocampus, PYY increases food intake. Indeed, among all the orexigenic peptides and neurotransmitters described to date, PYY is the most potent stimulator of food intake.

Y2 RECEPTOR

The NPY Y2 receptor (Y2R) is a 381-amino-acid, 7-transmembrane-spanning, G-protein-coupled receptor, which inhibits the activation of adenylyl cyclase via

G_i.[12] While it has low homology with other known NPY receptors, there is a high degree of conservation between rat and human Y2 receptors with 98% amino acid identity.[13] The Y2R is widely distributed within the central nervous system in both rodents and man. In the hypothalamus, Y2 mRNA is localized in the arcuate nucleus, preoptic nucleus, and dorsomedial nucleus.[14] Other forebrain areas that contain substantial Y2R mRNA include the posterior hypothalamic nuclei, medial nucleus of the amygdala, parabrachial area, substantia nigra, and the paraventricular thalamic nucleus.[15] Brainstem regions containing Y2R mRNA include the nucleus of the solitary tract and the lateral reticular nucleus, providing both ascending innervation to the hypothalamus and descending projections to the spinal cord. In the human brain the Y2R is the predominant Y receptor subtype in the brain. In addition to the areas described above, Y2R mRNA is also found in the dentate gyrus and the cerebral cortex.[16] Within the arcuate nucleus, over 80% of the NPY neurons co-express Y2R mRNA.[17] Application of Y2-selective agonists have been shown to reduce the release of NPY from hypothalamic slices *in vitro*, whereas the Y2 non-peptide antagonist BIIE0246 increases NPY release.[18] These findings support the role of the Y2R as a presynaptic autoreceptor that regulates the NPY release and hence may be involved in the regulation of feeding.

ROLE OF ARCUATE NUCLEUS Y2R IN FEEDING

To examine the role of the NPY Y2 receptor in the regulation of feeding, we utilized a Y2-selective agonist (Y2A), a C-terminal analogue of NPY (*N*-acetyl [Leu^{28}, Leu^{31}] neuropeptide Y-24–36).[19] Receptor binding studies performed upon distinct cells lines expressing NPY Y1, Y2, and Y5 receptors confirmed that this Y2 agonist

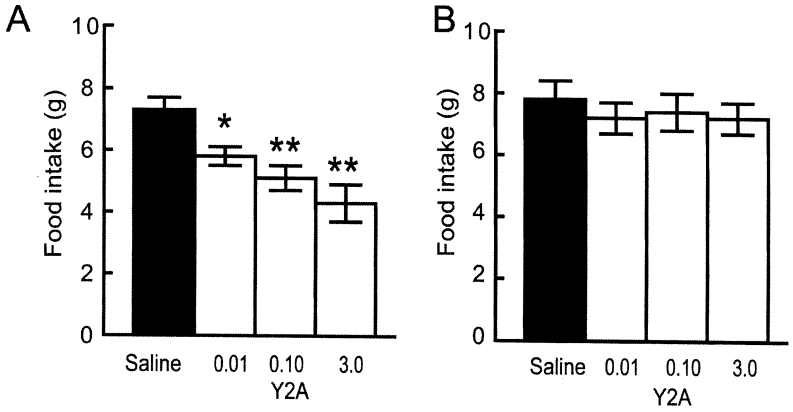

FIGURE 1. Feeding response to Y2A in rats. (**A**) Fasted rats were injected with saline or Y2A into the arcuate nucleus at the doses indicated. Post injection, 2-h food intake was measured. Each result is expressed as a mean ± SEM ($N = 12$ per group), ** = $P < 0.01$ compared to saline. (**B**) Fasted rats were injected with saline or Y2A into the paraventricular nucleus at the doses indicated (nmols). Post injection, 2-h food intake was measured. Each result is expressed as a mean ± SEM.

selectively bound to the Y5 receptor but not the Y1 and Y5 receptors (Y2: EC_{50} of 0.3 nM vs. Y1: EC_{50} > 5000 nM and Y2: EC_{50} > 5000 nM). This clear selectivity for the Y2 receptor provided a useful tool to examine the role of Y2R *in vivo.* Injection of the Y2 agonist into the arcuate nucleus of rats at the onset of the dark-phase caused a reduction in food intake. This effect was seen over a dose range from 100 fmol to 3 nmol and persisted for as long as 8 h post administration. Similarly, intra-arcuate administration the Y2A caused a comparable decrease in re-feeding following a fast (FIG. 1A). In contrast, injection of the Y2A into the PVN had no effect on feeding (FIG. 1B). Using *in vitro* studies on hypothalamic explants, we examined the effects of Y2A on release of the orexigenic peptide NPY and the anorectic POMC gene product α-melanocyte-stimulating hormone (α-MSH), both potent regulators of feeding *in vivo*. Y2A reduced the release of NPY while increasing the release of the α-MSH from POMC neurons, consistent with the proposed role as an inhibitory autoreceptor on NPY neurons.

THE ROLE OF $PYY_{3–36}$ IN THE REGULATION OF FOOD INTAKE

$PYY_{3–36}$ is a Y2R agonist and has been shown to be released by food intake. Therefore, we examined the effects of $PYY_{3–36}$ upon feeding when administered peripherally to rodents. These studies demonstrated that $PYY_{3–36,}$ when given at doses that achieved plasma levels within the normal postprandial range, reduced both dark-phase food intake and re-feeding following a fast. Twice daily administration of $PYY_{3–36}$ for 8 days revealed no attenuation of the inhibitory effect on food intake and resulted in decreased cumulative food intake and reduced body weight gain compared with saline-treated animals (FIG. 2).[20] Taken together these findings suggest that the $PYY_{3–36}$ released postprandially regulates food intake in rodents.

To establish whether the anorectic effect of $PYY_{3–36}$ required the Y2R, we examined the response of mice with targeted gene deletion of the Y2R to peripheral ad-

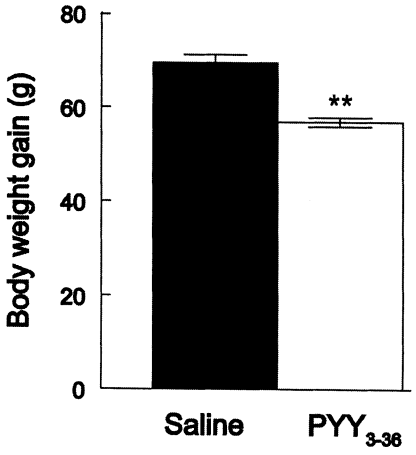

FIGURE 2. The effects of chronic $PYY_{3–36}$ treatment on body weight gain. Rats were injected intraperitoneally with $PYY_{3–36}$ (5 μg/100 g) or saline twice daily for 8 days. Each day, body weight was measured, and the total weight gain for each group was calculated (saline: *filled bar*; $PYY_{3–36}$: *open bar*). Each result is expressed as a mean ± SEM (N = 12 per group), ** = $P < 0.01$ compared to saline.

ministration of $PYY_{3–36}$.[21] Peripheral administration of $PYY_{3–36}$ resulted in a dose-dependent inhibition of food intake in wild-type littermate control mice. In contrast, $PYY_{3–36}$ had no effect on food intake in Y2R knockout mice, suggesting that the anorectic effects of $PYY_{3–36}$ require the presence of the Y2R.

Peripheral administration of $PYY_{3–36}$ resulted in an increase C-Fos expression in the arcuate nucleus and in particular increased the number of POMC neurons that co-localized with C-Fos, suggesting that POMC neurons were being activated. Furthermore, direct intra-arcuate injection of $PYY_{3–36}$ resulted in decreased food intake in fasted rodents, again supporting the hypothesis of the arcuate nucleus as a site of action. Using transgenic mice with targeted expression of green fluorescent protein in POMC neurons,[4] we were able to demonstrate that both the Y2A and $PYY_{3–36}$ depolarized and thus activated POMC neurons.[20]

HUMAN STUDIES

To extend these observations made in rodent models and to study the physiological role of $PYY_{3–36}$ in man, we investigated the effects of a 90-min infusion of $PYY_{3–36}$ (0.8 pmol/kg/min) or saline, on appetite and food intake in 12 healthy subjects (6 males and 6 females; mean age: 26.7 ± 0.7 years: body mass index = 24.6 ± 0.94 kg/m^2) in a randomized crossover study (FIG. 3). During the $PYY_{3–36}$ infusion, plasma levels reached a plateau by 30 min, and peak $PYY_{3–36}$ concentrations were within the normal postprandial range, returning to baseline by 120 min. Food intake assessed from a free choice buffet lunch, 2 h after the termination of the infusion, was decreased 36 ± 7.4% compared with saline (FIG. 4). Volunteers continued to record their food intake for 24 h post infusion. Food intake as assessed by food diaries remained significantly reduced in the post-infusion period in the $PYY_{3–36}$ treated group, resulting in a 33% reduction in total 24-h food intake.

CONCLUSIONS

Our findings suggest that $PYY_{3–36}$, released in proportion to calories ingested, regulates subsequent food intake by modulating the activity of the NPY and POMC neurons in the arcuate nucleus of the hypothalamus. The kinetics of $PYY_{3–36}$ secre-

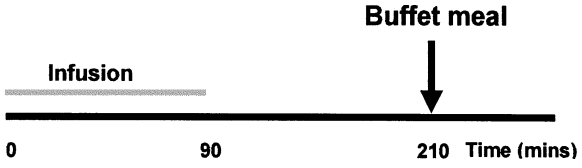

FIGURE 3. Infusion protocol. Fasted subjects received a 90-min infusion of either saline or $PYY_{3–36}$ in a randomized, double-blind, crossover study. Two hours after the termination of the infusion, subjects were offered a buffet meal.

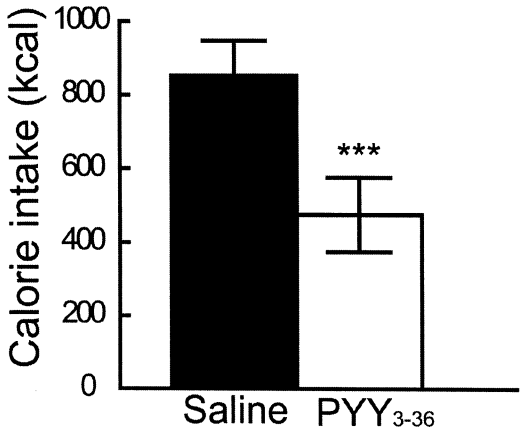

FIGURE 4. Effect of $PYY_{3–36}$ infusion on food intake in humans. Calorie intake from "free-choice" buffet meal 2 h after infusion with saline or $PYY_{3–36}$. Each result is expressed as a mean ± SEM ($N = 12$ per group), *** = $P < 0.001$ compared to saline.

tion and duration of action differentiate it from other classical meal terminating signals such as cholecystokinin (CCK).[22] Previously, factors controlling the regulation of food intake have arbitrarily divided into "short-term regulators" (such as CCK) that act rapidly to influence the termination of individual meals and "long-term regulators" (such as leptin and insulin) that reflect body energy stores. Unlike CCK, the levels of which peak within 30 min of food ingestion, the levels of $PYY_{3–36}$ peak later and remain elevated for several hours following a meal. Together with the long-lasting inhibition of food intake that we have observed, these findings led us to suggest that $PYY_{3–36}$ is involved in the "intermediate" term regulation of food intake.

Our findings suggest that the PYY system may be a therapeutic target for the treatment of obesity. Furthermore, abnormalities of the PYY system may be involved in the pathogenesis of this condition.

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