Imaging the neuroendocrinology of appetite

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Introduction

Effective pharmacotherapy to tackle obesity remains an elusive goal, despite an ever-growing need. In Europe, it is currently estimated that 17% of the population is obese; extrapolation of recent trends predicts that by 2015, this figure could rise to 30%.¹ For the first time in living memory, life expectancy in the West is predicted to decrease as a result of obesity-related health issues, particularly diabetes and cardiovascular disease.² All centrally acting, appetite-inhibiting medications have now been withdrawn from use in the UK because of mood-altering or cardiac adverse events. However, anorectic gut hormones, which are released post-prandially and known to exert effects on appetite control centers in the brain, remain promising therapeutic targets. Two such hormones are peptide YY (PYY) and glucagon-like peptide 1 (GLP-1), both of which are secreted by the gut in response to a meal. The rationale is that they may be used to chronically modulate food intake via physiological pathways, without inducing dangerous side effects. Acute administration of PYY3–36 and GLP-17–36 amide (the active forms of the hormones) to humans is well established to inhibit appetite and reduce food intake.³–⁶ Long-acting GLP-1 analogs, currently licensed for the treatment of Type 2 diabetes, have been shown to be associated with significant weight loss when administered to non-diabetic individuals.⁷ Likewise, long acting PYY analogs are in development. Furthermore, following Roux-en-Y gastric bypass surgery (the most effective current treatment for obesity), patients have elevated post-prandial levels of both PYY and GLP-1, and the combined actions of these peptides are thought to contribute to the greater and sustained weight loss observed following the intervention.⁸,⁹ Thus, clarifying the neuroendocrinology of gut hormones, acting both independently and together, is hugely important for the development of effective obesity treatments.

PYY and GLP-1 Reduce Food Intake and Neuronal Activity in Brain Appetite Centers

In humans, functional MRI (fMRI) imaging offers an opportunity to study the effects of these hormones on the brain. In a recently published study, we examined the independent and combined effects of PYY3–36 and GLP-17–36 amide on brain activity in response to oral glucose challenge, and found that they each induced reductions in brain activity in ventral tegmental area (VTA) and midbrain dopamine system, as well as in prefrontal cortex. These effects were mediated by decreases in food intake, which was significantly correlated with changes in brain activity. Our findings provide new insights into the neuroendocrinology of appetite and offer potential targets for the development of novel anti-obesity treatments.
Comparison of the Effects of PYY and GLP-1 on Neuronal Activity Related to Appetite with Those of Other Appetite-Modulating Hormones

It is of interest to interpret our results in the context of similar human fMRI studies investigating neuronal effects of other appetite-modulating hormones. Consistent trends can be found in reports of modulation of the fMRI BOLD signal when viewing food-related images in the presence or absence of these hormones, with the directionality of neuronal effects seemingly dependent on whether the hormone under scrutiny is anorectic or orexigenic.

Leptin, a hormone secreted by adipocytes, is thought to form part of the feedback loop for maintaining long-term energy homeostasis; when fat stores are replete, increased levels of leptin signal to the brain to reduce subsequent food intake in order to maintain weight neutrality. However, while this is an oversimplification, given the well known phenomenon of PYY₃₋₃₆ and GLP-₁₇₋₃₆ amide on subsequent food intake and neuronal activity in regions of the brain contributing to appetitive processing and behavior.¹⁰ Fifteen healthy, lean subjects were scanned on five separate occasions at least three days apart, during which they received each of the following interventions in random order: a saline infusion following an overnight fast (the fasted, control visit); a saline infusion following a large breakfast (the fed visit); an infusion of PYY₃₋₃₆; an infusion of GLP-₁₇₋₃₆ amide; a combined infusion of PYY₃₋₃₆ + GLP-₁₇₋₃₆ amide (all the gut hormones infusions were performed after an overnight fast). The subjects were then served a free intake buffet lunch at the end of each infusion, in order to assess effects on food intake. As expected, after subjects consumed breakfast, they ate significantly less at lunch than if they had continued fasting through the morning. Administering either of the gut hormones PYY₃₋₃₆ or GLP-₁₇₋₃₆ amide to our fasted subjects also reduced the amount of food they consumed at lunch, although to a smaller extent. Notably, when PYY₃₋₃₆ and GLP-₁₇₋₃₆ amide were co-administered to fasted subjects, food consumption at lunch was comparable to that observed in subjects who had eaten breakfast. While our experimental procedure was not designed to draw conclusions about dose-response relationships for the effects of these hormones, the effects of combined gut hormone infusion appeared to be additive, with no loss of potency when the hormones were administered in combination. We postulate that administration of these anorectic gut hormones in combination induces the satiety similar or identical to that of a meal, but without the associated caloric load. This holds promise for the future development of these hormones as anti-obesity agents.

During the course of each of the infusions described above, using blood-oxygen level-dependent (BOLD) fMRI, we tested for differences in relative activation in several pre-selected brain regions of interest (ROIs) when subjects viewed images of food compared with images of non-food items. This difference in relative brain activation when viewing images of food compared with non-food was attenuated in the insula after a full breakfast, with similar trends in the caudate and putamen (dorsal striatum), nucleus accumbens (ventral striatum) and orbitofrontal cortex. Our findings are largely consistent with recent studies demonstrating that fasting selectively increases BOLD fMRI activation to pictures of high-calorie food compared with pictures of low calorie food in similar ROIs and that this activation is attenuated in the fed state.¹¹,¹² Notably, we did not observe a fed-fasted difference within the amygdala, in contrast to the earlier study by LaBar et al.¹³ In our recent study, PYY₃₋₃₆ or GLP-₁₇₋₃₆ amide infusion resulted in a similar attenuation of activation in all of the above pre-selected ROIs, and infusion of both hormones to fasted individuals resulted in an overall pattern of brain activation comparable with that observed in the fed state. The ROIs were selected based on their known involvement in reward processing and response to food. As with the reduction in food intake, the reduction in BOLD signal after the combined administration of PYY₃₋₃₆ and GLP-₁₇₋₃₆ amide was similar to the sum of the effects of each individual hormone, again suggestive of their action in concert. In summary, our results suggest that PYY and GLP-1 act in the brain to make food intake cues less salient. Combined administration of these anorectic gut hormones to a fasted individual is associated with brain activation and behavioral changes similar to those after eating a full meal.

This fascinating insight into the neuroendocrinology of appetite highlights numerous new questions for future studies. In our study, the lack of any obvious differential activation pattern between PYY₃₋₃₆ and GLP-₁₋₃₆ amide suggests that these hormones may be acting on the brain via a common pathway. One explanation is that circulating gut hormones activate key homeostatic regions in the brainstem and hypothalamus, either directly through action in brain regions without a limiting blood brain barrier, or indirectly via vagal afferents. The functional anatomical localizations of brain activations after hormone administration suggest that ascending connections from vagal nuclei modulate the dopaminergic reward system: it was in the insular cortex (which is situated at an interface of homeostatic and cognitive systems with activity related to establishing salience of external stimuli for appropriate behavioral reactions) that we observed brain activity to be most significantly modulated by both feeding and gut hormone infusion. Another region where we observed changes was in the nucleus accumbens (forming the major part of the ventral striatum): this area is thought to play an important role in the mediation of hedonic drive and action (e.g., food-seeking behaviors).¹⁵

Recent evidence has identified a role for the orbitofrontal cortex in linking food memory with food reward. Connectivities of the orbitofrontal cortex with the hippocampus, amygdala and insula may facilitate a central role in coordinating appetitive behavior. Indeed, a previous fMRI study reported a relationship between orbitofrontal cortex BOLD signal and food intake when PYY₃₋₃₆ was infused, suggesting that the orbitofrontal cortex may mediate a PYY₃₋₃₆ induced switch in the regulation of food intake from homeostatic to hedonic control.¹⁷
of leptin resistance in obesity, in global terms, we may consider leptin to be an anorectic hormone. In an fMRI study that used a protocol similar to ours, when leptin was replaced subcutaneously in individuals with congenital leptin deficiency, BOLD activation was reduced in the caudate, putamen, globus pallidus and nucleus accumbens.\(^{18}\) In another fMRI study testing for brain activation contrasts between viewing high- and low-calorie food images (i.e., slight methodological differences to ours, as we used the signal contrast between all food and non-food images), subcutaneous leptin replacement in congenitally leptin deficient individuals resulted in increased BOLD activation in the insula, visual areas and striatum, along with increased hunger ratings in normal-weight subjects after intravenous ghrelin infusion.\(^{21}\) To investigate the effects of hypoglycemia (another orexigenic stimulus), a recent study reported increased BOLD activation in the nucleus accumbens, fMRI BOLD activation (in response to food vs. non-food images) was reduced in the fusiform gyri, right hippocampus, right temporal superior cortex and right frontal middle cortex.\(^{20}\) The attenuation of BOLD signal in higher cortical centers by insulin is consistent with our data. We speculate that subtle differences in brain responses between insulin and our anorectic gut hormones PYY\(^{3–36}\) and GLP-1\(^{7–36}\) amide may reflect the different roles of these hormones in energy homeostasis: anorectic gut hormones are thought to mediate short-term signaling, whereas insulin may be implicated both in short and longer term signaling of energy homeostasis.

Ghrelin is a potent orexigenic gut hormone. In a BOLD fMRI study similar to ours, relative brain activation with food pictures was increased in the amygdala, orbitofrontal cortex, insula, visual areas and striatum, along with increased hunger ratings in normal-weight subjects after intravenous ghrelin infusion.\(^{21}\) To investigate the effects of hypoglycemia (another orexigenic stimulus), a recent study reported increased BOLD activation in the nucleus accumbens, ventral striatum, putamen, lentiform nucleus, posterior cingulate and dorsomedial prefrontal cortex. Hunger also was reduced after surgery.\(^{22}\) These results are striking, given that we also found similar attenuation of BOLD signal in reward areas when we administered PYY\(^{3–36}\) and GLP-1\(^{7–36}\) amide. Although circulating gut hormone levels were not measured in the gastric bypass study, the authors postulated post-surgical reductions in ghrelin levels as an explanation for their imaging observations. We speculate that elevated levels of PYY and GLP-1 post-bypass would explain their findings. Thus, while functional MRI is at present used only as a research tool in endocrinology, these studies imply translation for the pre-operative work-up of obese patients; it may help highlight those patients who are more susceptible to the anorectic effects of gut hormones and whom therefore benefit most from surgery.

Comparison of the Effects of PYY and GLP-1 on Brain Activity with Those Observed after Gastric Bypass Surgery

In our original article, we reported the plasma levels of the active forms of the hormones, PYY\(^{3–36}\) and GLP-1\(^{7–36}\) amide.\(^{10}\) We also measured total hormone levels (using radioimmunoassays that additionally pick up other forms of each hormone which have lesser activity at the relevant receptors) (unpublished data). Total hormone concentrations are more commonly reported in the literature; review of our total hormone levels thus allows for meaningful comparison with existing relevant data. Our results revealed that during the combined infusion of PYY\(^{3–36}\) and GLP-1\(^{7–36}\) amide the mean total PYY concentration achieved was 70 pM and the mean total GLP-1 concentration was 43 pM. Interestingly, the mean circulating levels of total PYY and total GLP-1 following breakfast were 33 pM and 22 pM, respectively. Therefore, although our infused circulating total PYY and total GLP-1 levels were slightly higher than those observed physiologically after breakfast, they were of a similar order of magnitude. Consistent with this, there was a comparable reduction in ad libitum energy intake at lunch following combined infusion of PYY\(^{3–36}\) and GLP-1\(^{7–36}\) amide and following infusion of saline after a large breakfast (fed visit).

Roux-en-Y gastric bypass is the most effective current treatment for obesity, leading to sustained weight loss of approximately 30%.\(^{23}\) In a study by Le Roux et al.,\(^{9}\) a mean 90 min post-prandial total PYY level of 40 pM was observed following Roux-en-Y gastric bypass. In the same study, a mean 30 min post-prandial total GLP-1 level of 47 pM was observed. These levels are comparable to plasma levels of total PYY and total GLP-1 that were observed in our study. Therefore, although circulating levels achieved in our study may be regarded as somewhat supraphysiological within the normal population, they are of direct functional relevance for understanding the effects of PYY and GLP-1 in patients who have undergone gastric bypass surgery. PYY and GLP-1 appear to be the most important gut hormones implicated in mediating reduced appetite and promoting weight loss following gastric bypass,\(^{24}\) although there are likely to be changes in other gut hormones that are not routinely measured.

It is also of interest to compare our imaging results in healthy, lean individuals to those found in an fMRI study of obese individuals pre- and post-gastric bypass surgery.\(^{25}\) Post-surgery (compared with pre-surgery), there was attenuation of high calorie food cue associated fMRI BOLD signal in the ventral tegmental area, ventral striatum, putamen, lentiform nucleus, posterior cingulate and dorsomedial prefrontal cortex. Hunger also was reduced after surgery.\(^{25}\) These results are striking, given that we also found similar attenuation of BOLD signal in reward areas when we administered PYY\(^{3–36}\) and GLP-1\(^{7–36}\) amide. Although circulating gut hormone levels were not measured in the gastric bypass study, the authors postulated post-surgical reductions in ghrelin levels as an explanation for their imaging observations. We speculate that elevated levels of PYY and GLP-1 post-bypass would explain their findings. Thus, while functional MRI is at present used only as a research tool in endocrinology, these studies imply translation for the pre-operative work-up of obese patients; it may help highlight those patients who are more susceptible to the anorectic effects of gut hormones and whom therefore benefit most from surgery.
Direct interpretation on hypothalamic responses is not yet available. Unfortunately, imaging the hypothalamus is inherently difficult with a BOLD-based fMRI protocol because of its small size and proximity to the intracranial sinuses, which cause local signal loss in the MRI image. Thus, the ascending pathways mediating actions of these circulating appetite suppressants remain incompletely understood. Although our ROIs were chosen based on the results of existing data in the field and also our hypotheses as to their role in food reward, it should be noted that an ROI-based analysis, although statistically providing more sound results, possesses the inherent problem of potentially missing other regions of the brain which are modulated by gut hormones. Despite the above limitations, our data confirm some of the brain areas engaged in appetitive processing and also our hypotheses as to their role in the development of gut hormones as effective anti-obesity therapies. Given the consistency of the imaging data so far, it seems plausible that the administration of combined anorectic gut hormones may provide an effective future medical alternative to a surgical gastric bypass procedure to treat obesity.

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