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Peptide $YY_{3–36}$ and Glucagon-Like Peptide-$1_{7–36}$ Inhibit Food Intake Additively

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Peptide YY (PYY) and glucagon like peptide (GLP)-1 are cosecreted from intestinal L cells, and plasma levels of both hormones rise after a meal. Peripheral administration of $PYY_{3–36}$ and GLP-$1_{7–36}$ inhibit food intake when administered alone. However, their combined effects on appetite are unknown. We studied the effects of peripheral coadministration of $PYY_{3–36}$ with GLP-$1_{7–36}$ in rodents and man. Whereas high-dose $PYY_{3–36}$ (100 nmol/kg) and high-dose GLP-$1_{7–36}$ (100 nmol/kg) inhibited feeding individually, their combination led to significantly greater feeding inhibition. Additive inhibition of feeding was also observed in the genetic obese models, *ob/ob* and *db/db* mice. At low doses of $PYY_{3–36}$ (1 nmol/kg) and GLP-$1_{7–36}$ (10 nmol/kg), which alone had no effect on food intake, coadministration led to significant reduction in food intake. To investigate potential mechanisms, *c-fos* immunoreactivity was quantified in the hypothalamus and brain stem. In the hypothalamic arcuate nucleus, no changes were observed after low-dose $PYY_{3–36}$ or GLP-$1_{7–36}$ individually, but there were significantly more *fos*-positive neurons after coadministration. In contrast, there was no evidence of additive *fos*-stimulation in the brain stem. Finally, we coadministered $PYY_{3–36}$ and GLP-$1_{7–36}$ in man. Ten lean fasted volunteers received 120-min infusions of saline, GLP-$1_{7–36}$ (0.4 pmol/kg·min), $PYY_{3–36}$ (0.4 pmol/kg·min), and $PYY_{3–36}$ (0.4 pmol/kg·min) + GLP-$1_{7–36}$ (0.4 pmol/kg·min) on four separate days. Energy intake from a buffet meal after combined $PYY_{3–36}$ + GLP-$1_{7–36}$ treatment was reduced by 27% and was significantly lower than that after either treatment alone. Thus, $PYY_{3–36}$ and GLP-$1_{7–36}$, cosecreted after a meal, may inhibit food intake additively. (*Endocrinology* 146: 5120–5127, 2005)

THE GUT HORMONES peptide YY (PYY) and glucagon-like peptide (GLP)-1 are cosecreted from endocrine L cells of the small and large bowel. Endogenous concentrations of both hormones are low in the fasting state and rise within 30 min of a meal (1). However, whereas these hormones have been found to inhibit food intake individually (2, 3), their combined effect has yet to be investigated.

It has been reported that peripheral administration of $PYY_{3–36}$ inhibits appetite in rodents (4, 5) and man (2). Peripheral administration of GLP-$1_{7–36}$ inhibits food intake in rodents (6, 7) and man (8). GLP-$1_{7–36}$ and the GLP-1 receptor agonist exendin-4 also stimulate insulin release and inhibit circulating glucagon (6). In contrast, no changes in insulin and glucagon concentrations occur after $PYY_{3–36}$ administration (9).

PYY and GLP-1 may mediate these peripheral effects by altering central nervous system appetite circuits. After ip administration of $PYY_{3–36}$, an increase in c-*fos*, a marker of neuronal activation, has been reported in the hypothalamic arcuate nucleus (ARC) (2). In contrast, GLP-1 is reported to act primarily on brain stem neurons. After iv exendin-4, c-*fos* activation was observed in the area postrema (AP) (10), which, like the ARC, has access to the peripheral circulation. c-*fos* immunoreactivity was also detected in the ARC and paraventricular nucleus (PVN). This hypothalamic activation could be mediated via neuronal projections from the brain stem to the PVN (11) or directly onto ARC neurons.

Both $PYY_{3–36}$ and GLP-$1_{7–36}$ have been administered to humans. Intravenous infusion of $PYY_{3–36}$ was associated with a 36% reduction in energy intake at a free buffet meal (2). $PYY_{3–36}$ has also been reported to inhibit food intake in obese subjects (12). A recent metaanalysis reported that infusion of GLP-$1_{7–36}$ was associated with a small dose-dependent reduction in energy intake in both lean and obese subjects (8). Interestingly, when PYY and GLP-1 were coadministered to man, an additive inhibitory effect on pentagastrin-induced gastric acid secretion was observed (13), although their combined effect on appetite was not examined.

Much work has been performed investigating the effect of exogenous administration of individual gut hormones on appetite to determine their potential physiological role in the control of food intake (4, 14, 15). However, after a meal not one but several gut hormones are elevated, and it may be that the interactions between the changes in levels of different hormones influence food intake more than each hormone individually.

The aim of our study was to investigate whether the combined administration of $PYY_{3–36}$ and GLP-$1_{7–36}$ was more effective in inhibiting food intake than either peptide alone. First, we examined the effects of coadministration of $PYY_{3–36}$ and GLP-$1_{7–36}$ in lean and obese rodents. Second, to investigate potential mechanisms, we quantified *fos*-like immunoreactivity (FLI) in the hypothalamus and brain stem after ip $PYY_{3–36}$ and GLP-$1_{7–36}$ alone and $PYY_{3–36}$ + GLP-$1_{7–36}$ coadministration. Finally, we performed a randomized, dou-

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Abbreviations: AP, Area postrema; ARC, arcuate nucleus; FLI, *fos*-like immunoreactivity; GLP, glucagon like peptide-1; PB, phosphate buffered; PVN, paraventricular nucleus; PYY, peptide YY; WT, wild type.

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ble-blind, placebo-controlled, crossover trial in healthy volunteers to compare the effects of $PYY_{3–36}$, GLP-$1_{7–36}$, and $PYY_{3–36}$ with GLP-$1_{7–36}$ on energy intake in man.

Materials and Methods

Materials

Synthetic human $PYY_{3–36}$ and GLP-$1_{7–36}$ were purchased from Phoenix Pharmaceuticals (Belmont, CA). Unless otherwise stated, all chemicals were purchased from Merck (Poole, Dorset, UK).

Animals

All animal procedures were approved by the British Home Office Animals (Scientific Procedures) Act 1986 (project license no. 70/5516 and 70/5281). Male mice and rats were maintained in individual cages under controlled temperature (21–23 C) and light (12-h light, 12-h dark cycle; lights on at 0700 h) with *ad libitum* access to standard chow (RM1 diet, SDS Ltd., Witham, Essex, UK) and water. All animals were handled daily for 7 d before the first study, particularly in the light of recent evidence that peripheral $PYY_{3–36}$ does not inhibit food intake in stressed animals unaccustomed to their experimental conditions but does so in well-handled, acclimatized animals (5). During the acclimatization period, each animal received two saline injections to minimize stress on the study days.

Intraperitoneal injections

Intraperitoneal injections were administered to mice via a 0.5 ml syringe with a 29-gauge needle (maximum injection volume, 0.1 ml) and rats via a 1 ml syringe with a 25-gauge needle (maximum volume, 0.5 ml). In each study animals received one injection only of equivalent volume for each treatment group. For the combination treatment, GLP-$1_{7–36}$ and $PYY_{3–36}$ were drawn up in a single syringe. In all feeding studies, food intake was measured 1, 2, 4, 8, and 24 h after injection as previously described (7).

Feeding studies in lean mice

C57BL/6 mice (20–25 g) were injected after a 20-h fast. To determine suitable doses for the combined study, dose-response studies were performed for $PYY_{3–36}$ and GLP-$1_{7–36}$ individually. For the $PYY_{3–36}$ study, mice were ip injected with 30, 100, 150, and 300 nmol/kg $PYY_{3–36}$ (n = 11–12). For the GLP-$1_{7–36}$ study, mice were injected with 30, 100, 300, and 900 nmol/kg GLP-$1_{7–36}$ (n = 10). For the combined study, mice were injected with saline, 100 nmol/kg $PYY_{3–36}$, 200 nmol/kg $PYY_{3–36}$, 100 nmol/kg GLP-$1_{7–36}$, 200 nmol/kg GLP-$1_{7–36}$, or 100 nmol/kg $PYY_{3–36}$ + 100 nmol/kg GLP-$1_{7–36}$ (n = 8–9).

Feeding studies in ob/ob and db/db mice

To investigate these effects in obese rodents, $PYY_{3–36}$ + and GLP-$1_{7–36}$ were coadministered to *ob/ob* and *db/db* mice (both on a C57BL/6 background) in a four-way crossover design. After a 20-h fast, *ob/ob* mice (mean weight 54.5 ± 1.4 g) and their wild-type (WT) littermates (32.5 ± 1.0 g) and *db/db* mice (41.8 ± 1.12 g) and their WT littermates (31.0 ± 0.5 g) were ip injected with saline, 200 nmol/kg $PYY_{3–36}$, 200 nmol/kg GLP-$1_{7–36}$, or 100 nmol/kg $PYY_{3–36}$ + 100 nmol/kg GLP-$1_{7–36}$. Four studies were performed with 72 h between each study so that each mouse received all four treatments (n = 10 per treatment group).

Feeding studies in rats

To examine the effect of $PYY_{3–36}$ and GLP-$1_{7–36}$ in another species and during another feeding phase, these peptides were coadministered in rats in the dark phase. Rodents are nocturnal feeders and eat the majority of their food during the night (16). Freely feeding rats (150–200 g) were injected in the hour before lights off. For the $PYY_{3–36}$ dose-response study, animals received ip saline or 1, 3, or 10 nmol/kg $PYY_{3–36}$ (n = 12–13). For GLP-$1_{7–36}$, rats received saline or 3, 10, 30, or 100 nmol/kg GLP-$1_{7–36}$ (n = 12–13). To investigate the combined effect of low doses, 1 nmol/kg $PYY_{3–36}$ and 10 nmol/kg GLP-$1_{7–36}$ were chosen for the joint study (see *Results*). On two evenings, 72 h apart, animals were randomized and injected ip with saline, 10 nmol/kg GLP-$1_{7–36}$, 20 nmol/kg GLP-$1_{7–36}$, 1 nmol/kg $PYY_{3–36}$, 2 nmol/kg $PYY_{3–36}$, or 1 nmol/kg $PYY_{3–36}$ + 10 nmol/kg GLP-$1_{7–36}$ (total n = 19–20 per group) before the dark phase.

Immunocytochemistry

The protocols for c-*fos* staining and quantification were similar to those described previously (17). Briefly, rats (240–265 g) were each handled for 5 min/d for 1 wk to minimize the stress of the procedure. After an overnight fast, rats received ip injections of saline, 10 nmol/kg GLP-$1_{7–36}$, 1 nmol/kg $PYY_{3–36}$, or 1 nmol/kg $PYY_{3–36}$ + 10 nmol/kg GLP-$1_{7–36}$ (n = 5–6 per group). Ninety minutes later, they were terminally anesthetized with ip sodium pentobarbitone (200 mg) (Rhône Mérieux, Harlow, UK) and transcardially perfused with 0.1 M PBS followed by 4% phosphate-buffered (PB)-formalin. The brains were removed and postfixed overnight in PB-formalin and then transferred to PB-sucrose (20% wt/vol) for 48 h. Serial 40-μm coronal brain sections were cut on a freezing microtome and stained for FLI using the avitin-biotin-peroxidase method. *Fos*-positive nuclei were counted using a light microscope (Eclipse E800; Nikon, Tokyo, Japan) by an experienced member of the research team with reference to the Rat Brain Atlas (18), who was unaware of which treatment had been given. The nuclei examined were the PVN, ARC, ventromedial and dorsomedial of the hypothalamus, and the nucleus of the solitary tract and the AP of the brain stem.

Behavioral studies

To determine whether alterations in behavior were associated with treatments of peptides used in the feeding and c-*fos* studies, an observation study was performed (19). After an overnight fast, rats (150–170 g) were injected ip with saline, 10 nmol/kg GLP-$1_{7–36}$, 1 nmol/kg $PYY_{3–36}$, 1 nmol/kg $PYY_{3–36}$ + 10 nmol/kg GLP-$1_{7–36}$, 10 nmol/kg exendin-4, or 1 M lithium chloride (positive control) (n = 10 per group) and their food replaced. Their behavior was assessed for the subsequent 60 min by two observers who were unaware of which treatment had been given. Each observer recorded behaviors of 30 animals (n = 5 per group per observer). Each rat was observed for 15 sec in every 5 min. The 15-sec periods were subdivided into three 5-sec periods. In each or these periods, the observer selected the behavior that most closely resembled what the rat was doing. These options were feeding, drinking, locomotion, grooming, head down, sleeping, and rearing.

In a further experiment, freely feeding rats were ip injected before the dark phase with saline, 10 nmol/kg GLP-$1_{7–36}$, 1 nmol/kg $PYY_{3–36}$, 1 nmol/kg $PYY_{3–36}$ + 10 nmol/kg GLP-$1_{7–36}$, or 10 nmol/kg exendin-4. Infrared beam breaks in two planes x-tot (horizontal movement) and z-tot (vertical movement) were counted for 1 h using an Opto-Max system (Columbus Instruments, Columbus, OH) to assess locomotion.

Human randomized, double-blind, controlled trial

The study was approved by the Hammersmith, Queen Charlotte's, and Chelsea Ethics Committee (reference no. 2002/6261) and performed in accordance with the principles of the Declaration of Helsinki. Healthy lean volunteers were recruited through advertisement. Exclusion criteria were substance abuse, pregnancy, significant medical or psychiatric illness, or regular medication except the oral contraceptive pill. Twelve subjects were recruited, each of whom gave their informed consent to participate in the study. One subject was withdrawn due to violation of the protocol and another was withdrawn due to nausea on a study morning. Thus, 10 subjects (four men and six women) completed the study. Their ages ranged from 22 to 29 with a mean age of 25.6 ± 0.7 yr, and body mass index ranged from 20.5 to 26.4 with a mean of 23.0 ± 0.7 kg/m^2.

Each volunteer received four infusions, saline, GLP-$1_{7–36}$, $PYY_{3–36}$, or $PYY_{3–36}$ with GLP-$1_{7–36}$, each on a separate day at least 5 d apart. Subjects fasted from 2100 h on the evening before infusions. The infusion days were run as previously described (20).

Both $PYY_{3–36}$ and GLP-$1_{7–36}$ were sterile on culture, and limulus amoebocyte lysate assay tests for pyrogen were negative. Two vials were used for each infusion (saline + saline, saline + GLP-$1_{7–36}$, saline + $PYY_{3–36}$, and $PYY_{3–36}$ + GLP-$1_{7–36}$). Both vials were dissolved in 2.5 ml

hemacel (Beacon, Tunbridge Wells, Kent, UK) to minimize peptide adsorption, diluted in 0.9% saline (Bayer, Newbury, Berkshire, UK) and drawn up in a single syringe (total volume 50 ml). The infusion rates chosen were based on the results of previous studies. $PYY_{3–36}$ at 0.8 pmol/kg·min was associated with a 36% reduction in food intake (2). The lowest rate of GLP-$1_{7–36}$ that has been shown to inhibit food intake is 0.75 pmol/kg·min (21). We aimed for a submaximal effect for each individual peptide and so selected rates of 0.4 pmol/kg·min for both GLP-$1_{7–36}$ and $PYY_{3–36}$ infusions and a rate of 0.4 pmol/kg·min $PYY_{3–36}$ + 0.4 pmol/kg·min GLP-$1_{7–36}$ for the combined infusion. Infusions were given for 120 min.

After 90 min infusion, a buffet lunch was served of preweighed food in excess consisting of chicken or vegetable curry, rice, fruit salad, and sweets. Thirty minutes later, the remaining food was removed and weighed and the infusion discontinued. Blood samples (10 ml) were taken 30 min before the start of the infusion, immediately before the infusion, and then every 30 min until 2 h after the meal. Blood was collected into heparin-coated tubes containing 2000 Kallikrein inhibitor units (0.2 ml) of aprotonin (Bayer). After centrifugation, plasma was separated and stored immediately at −70 C until RIA. Subjects were asked to score subjective nausea on a visual analog scales (0–100 mm) (22) at each time point. Pulse and blood pressure were also measured at these time points. Subjects were allowed to leave 2 h after the meal and instructed to eat *ad libitum* and complete a food diary for the following 24 h.

RIAs

All samples were assayed in duplicate and single assays to eliminate the effects of interassay variation. PYY, GLP-1, and insulin concentrations were quantified using established in-house RIAs and antibodies (23–25). Glucose concentrations were measured using a YSI-2300STAT analyzer (Yellow Springs Instruments, Yellow Springs, OH).

Statistical analysis

Results are shown as mean values ± SEM. Data from the animal feeding and immunocytochemistry and behavior studies were analyzed using a one-way ANOVA with a Dunnett's two-sided *post hoc* test. For the human infusions, a random effect, repeated-measures ANOVA was performed and within-subject effect examined under a Greenhouse-Geisser correction. In all cases $P < 0.05$ was considered to be statistically significant.

Results

Coadministration of high-dose $PYY_{3–36}$ and GLP-1 in lean mice

In the $PYY_{3–36}$ dose-response study, food intake in the first hour was significantly inhibited by all doses of $PYY_{3–36}$ administered [food intake 0–1 h (grams): saline, 0.67 ± 0.05; $PYY_{3–36}$, 30 nmol/kg, 0.49 ± 0.04, 100 nmol/kg, 0.44 ± 0.03, 150 nmol/kg, 0.47 ± 0.02, 300 nmol/kg, 0.47 ± 0.04] ($P < 0.05$ for all doses *vs.* saline) (Fig. 1A). PYY appeared to cause maximal feeding inhibition from doses of 100 nmol/kg and above, and this dose was selected for the combined study. Inhibition in cumulative feeding was sustained with all doses for up to 8 h after injection (data not shown).

In the GLP-$1_{7–36}$ dose-response study, food intake in the first hour was significantly inhibited by 100, 300, and 900 nmol/kg GLP-$1_{7–36}$ but not significantly by the 30 nmol/kg dose [food intake 0–1 h (grams): saline, 0.85 ± 0.05; GLP-$1_{7–36}$, 30 nmol/kg, 0.72 ± 0.07, 100 nmol/kg, 0.45 ± 0.03, 300 nmol/kg, 0.31 ± 0.05, 900 nmol/kg, 0.20 ± 0.05] ($P < 0.05$ for 100, 300, and 900 nmol/kg GLP-$1_{7–36}$ *vs.* saline) (Fig. 1B). In contrast with the PYY study, greater inhibition of feeding was observed with increasing doses of GLP-$1_{7–36}$ ($P < 0.05$ for 100 *vs.* 30 nmol/kg GLP-$1_{7–36}$ and for 300 *vs.* 100 nmol/kg GLP-$1_{7–36}$). A dose of 100 nmol/kg GLP-$1_{7–36}$ was selected for the combined study, which achieved an approximately 50% reduction in food intake in the first hour. Inhibition in cumulative feeding was sustained up to 8 h after injection after 100, 300, and 900 nmol/kg GLP-$1_{7–36}$.

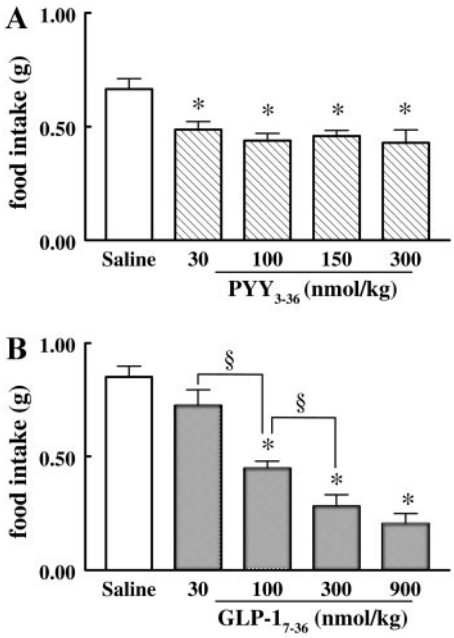

FIG. 1. The effect on food intake in fasted mice in the first hour of ip $PYY_{3–36}$ (30, 100, 150, and 300 nmol/kg) (A) or GLP-$1_{7–36}$ (30, 100, 300, and 900 nmol/kg) (B). *, $P < 0.05$ *vs.* saline. §, $P < 0.05$ for 100 *vs.* 30 nmol/kg GLP-$1_{7–36}$ and 300 *vs.* 100 nmol/kg GLP-$1_{7–36}$.

The doses for the coadministration study were as follows: saline, 100 nmol/kg $PYY_{3–36}$, 200 nmol/kg $PYY_{3–36}$, 100 nmol/kg GLP-$1_{7–36}$, 200 nmol/kg GLP-$1_{7–36}$ and 100 nmol/kg $PYY_{3–36}$ + 100 nmol/kg GLP-$1_{7–36}$. All peptides inhibited feeding in the first hour, compared with saline. Coadministration of $PYY_{3–36}$ with GLP-$1_{7–36}$ led to significantly greater feeding inhibition than all other groups [food intake 0–1 h (grams): saline, 0.74 ± 0.07; 100 nmol/kg GLP-$1_{7–36}$, 0.33 ± 0.05; 200 nmol/kg GLP-$1_{7–36}$, 0.33 ± 0.07; 100 nmol/kg $PYY_{3–36}$, 0.60 ± 0.03; 200 nmol/kg $PYY_{3–36}$, 0.51 ± 0.04; 100 nmol/kg $PYY_{3–36}$ + 100 nmol/kg GLP-$1_{7–36}$, 0.07 ± 0.07: $P < 0.05$ for combined groups *vs.* all other groups] (Fig. 2). Feeding inhibition after the combination treatment was sustained up to 24 h after injection [food intake 0–24 h (grams): saline, 7.0 ± 0.2; 100 nmol/kg GLP-$1_{7–36}$, 6.5 ± 0.2; 200 nmol/kg GLP-$1_{7–36}$, 7.2 ± 0.2; 100 nmol/kg $PYY_{3–36}$, 6.9 ± 0.2; 200 nmol/kg $PYY_{3–36}$, 6.8 ± 0.2; 100 nmol/kg $PYY_{3–36}$ + 100 nmol/kg GLP-$1_{7–36}$, 6.1 ± 0.3: $P < 0.05$ for combined group *vs.* saline].

Coadministration of $PYY_{3–36}$ and GLP-$1_{7–36}$ in ob/ob and db/db mice

Mice were randomized to receive saline, 200 nmol/kg GLP-$1_{7–36}$, 200 nmol/kg $PYY_{3–36}$, or 100 nmol/kg $PYY_{3–36}$ + 100 nmol/kg GLP-$1_{7–36}$. Coadministration of $PYY_{3–36}$ and GLP-$1_{7–36}$ in *ob/ob* mice and their WT littermates led to significant inhibition of feeding, compared with saline and both peptides alone [food intake 0–1 h (grams), *ob/ob* mice: saline,

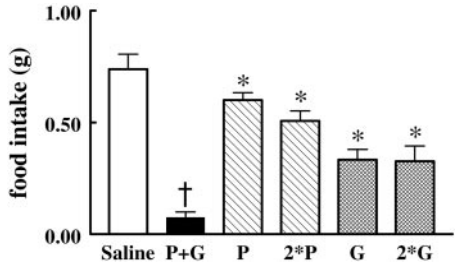

FIG. 2. The effect of ip coadministration of $PYY_{3–36}$ with $GLP\text{-}1_{7–36}$ in fasted lean mice on feeding in the first hour. Mice were injected with saline, P+G (100 nmol/kg $PYY_{3–36}$ + 100 nmol/kg $GLP\text{-}1_{7–36}$), G (100 nmol/kg $GLP\text{-}1_{7–36}$), 2*G (200 nmol/kg $GLP\text{-}1_{7–36}$), P (100 nmol/kg $PYY_{3–36}$), or 2*P (200 nmol/kg $PYY_{3–36}$). *, $P < 0.05$ *vs.* saline. †, $P < 0.05$, compared with all other groups.

0.48 ± 0.09; 200 nmol/kg $GLP\text{-}1_{7–36}$, 0.25 ± 0.03; 200 nmol/kg $PYY_{3–36}$, 0.32 ± 0.05; 100 nmol/kg $PYY_{3–36}$ + 100 nmol/kg $GLP\text{-}1_{7–36}$, 0.14 ± 0.03; $P < 0.05$ for combined group *vs.* all other groups; food intake 0–1 h (grams), WT mice: saline, 1.2 ± 0.08; 200 nmol/kg $GLP\text{-}1_{7–36}$, 0.68 ± 0.09; 200 nmol/kg $PYY_{3–36}$, 0.78 ± 0.069; 100 nmol/kg $PYY_{3–36}$ + 100 nmol/kg $GLP\text{-}1_{7–36}$, 0.33 ± 04; $P < 0.05$ for combined group *vs.* all other groups]. The percentage reduction in food intake with the coadministration treatment, compared with saline, was similar in *ob/ob* (−74 ± 4%) and WT mice (−70 ± 6%) (Fig. 3A). Reduction in cumulative food intake was sustained for up to 24 h in the WT mice and up to 8 h in the *ob/ob* mice (data not shown).

Coadministration of $PYY_{3–36}$ and $GLP\text{-}1_{7–36}$ was also associated with significant reductions in food intake in *db/db* mice and their WT littermates, compared with saline and both peptides alone [food intake 0–1 h (grams), *db/db* mice: saline, 0.59 ± 0.10; 200 nmol/kg GLP-1, 0.45 ± 0.05; 200 nmol/kg $PYY_{3–36}$, 0.36 ± 0.04; 100 nmol/kg $PYY_{3–36}$ + 100 nmol/kg GLP-1, 0.20 ± 0.04; $P < 0.05$ for combined group *vs.* all other groups; food intake 0–1 h (grams), WT mice: saline, 1.17 ± 0.09; 200 nmol/kg $GLP\text{-}1_{7–36}$, 0.99 ± 0.05; 200 nmol/kg $PYY_{3–36}$, 0.76 ± 0.07; 100 nmol/kg $PYY_{3–36}$ + 100 nmol/kg $GLP\text{-}1_{7–36}$, 0.41 ± 0.07; $P < 0.05$ for combined group *vs.* all other groups]. The percentage reduction in food intake with the coadministration treatment, compared with saline, was similar in *db/db* (−65 ± 7%) and WT mice (−63 ± 6%) (Fig. 3B). Reduction in cumulative food intake was sustained for up to 8 h in both WT and *db/db* mice (data not shown).

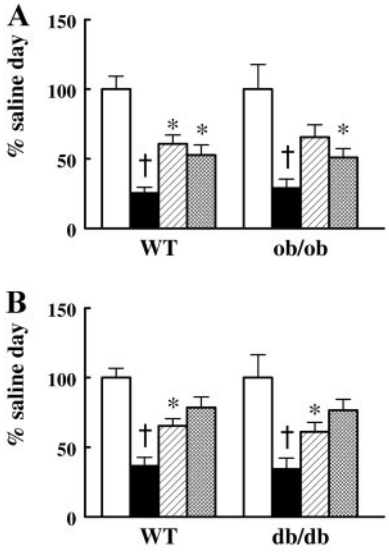

FIG. 3. Food intake in the first hour after ip coadministration of $PYY_{3–36}$ with $GLP\text{-}1_{7–36}$ in fasted WT and *ob/ob* mice (A) and WT and *db/db* (B) mice. Results are presented as a percentage of the mean food intake on the saline day. Mice were injected with saline, 100 nmol/kg $PYY_{3–36}$ + 100 nmol/kg $GLP\text{-}1_{7–36}$, 200 nmol/kg $GLP\text{-}1_{7–36}$, or 200 nmol/kg $PYY_{3–36}$. *, $P < 0.05$ *vs.* saline. †, $P < 0.05$, compared with all other groups. *White column*, Saline; *hatched column*, GLP-1; *striped column*, PYY; *black column*, PYY + GLP-1 (100 nmol/kg $PYY_{3–36}$ + 100 nmol/kg $GLP\text{-}1_{7–36}$).

Coadministration of low-dose $PYY_{3–36}$ and $GLP\text{-}1_{7–36}$ in rats

$PYY_{3–36}$ and $GLP\text{-}1_{7–36}$ dose-response studies were performed in freely feeding rats in the dark phase. In the $PYY_{3–36}$ study, food intake in the first hour of the dark phase was significantly inhibited by 3 and 10 nmol/kg $PYY_{3–36}$ but not significantly by the 1 nmol/kg dose [food intake 0–1 h (grams): saline, 4.9 ± 0.2; $PYY_{3–36}$, 1 nmol/kg, 4.5 ± 0.5; 3 nmol/kg, 3.8 ± 0.3; 10 nmol/kg, 3.5 ± 0.3; $P < 0.05$ for 3 and 10 nmol/kg $PYY_{3–36}$ *vs.* saline] (Fig. 4A). In the $GLP\text{-}1_{7–36}$ study, food intake in the first hour was significantly inhibited by 30 and 100 nmol/kg $GLP\text{-}1_{7–36}$ [food intake 0–1 h (grams): saline, 5.0 ± 0.3; $GLP\text{-}1_{7–36}$, 3 nmol/kg 4.9 ± 0.5; 10 nmol/kg, 4.5 ± 0.3; 30 nmol/kg, 4.1 ± 0.3; 100 nmol/kg, 3.8 ± 0.3; $P < 0.05$ for 30 and 100 nmol/kg $GLP\text{-}1_{7–36}$ *vs.* saline] (Fig. 4B).

The doses of peptides selected for the combined study were 3-fold lower than the minimum doses found to inhibit food intake significantly in the first hour. Rats received ip injections of saline, 1 nmol/kg $PYY_{3–36}$, 2 nmol/kg $PYY_{3–36}$, 10 nmol/kg $GLP\text{-}1_{7–36}$, 20 nmol/kg $GLP\text{-}1_{7–36}$, or 1 nmol/kg $PYY_{3–36}$ + 10 nmol/kg $GLP\text{-}1_{7–36}$. Food intake was signifi-

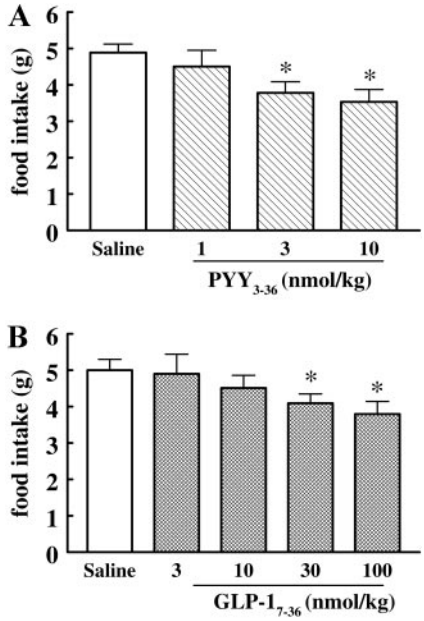

FIG. 4. The effect on food intake in the first hour of the dark phase in freely feeding rats of ip $PYY_{3–36}$ (1, 3, and 10 nmol/kg) (A) or ip $GLP\text{-}1_{7–36}$ (3, 10, 30, and 100 nmol/kg) (B). *, $P < 0.05$ *vs.* saline.

cantly inhibited only in the $PYY_{3–36}$ + $GLP\text{-}1_{7–36}$ group (Fig. 5) [food intake 0–1 h (grams): saline, 4.1 ± 0.2; 10 nmol/kg $GLP\text{-}1_{7–36}$, 3.9 ± 0.3; 20 nmol/kg $GLP\text{-}1_{7–36}$, 4.2 ± 0.3; 1 nmol/kg $PYY_{3–36}$, 4.2 ± 0.3; 2 nmol/kg $PYY_{3–36}$, 4.1 ± 0.2; 1 nmol/kg $PYY_{3–36}$ + 10 nmol/kg $GLP\text{-}1_{7–36}$, 2.8 ± 0.2]. There was significant inhibition of food intake with the combined treatment, compared with all other groups ($P < 0.02$). This effect was most marked in the first hour of the dark phase. Cumulative food intake was inhibited in the coadministration group for up to 4 h, compared with saline ($P < 0.05$) [food intake 0–4 h (grams): saline, 9.8 ± 0.3; 10 nmol/kg $GLP\text{-}1_{7–36}$, 9.7 ± 0.4; 20 nmol/kg $GLP\text{-}1_{7–36}$, 10.0 ± 0.2; 1 nmol/kg $PYY_{3–36}$, 9.9 ± 0.5; 2 nmol/kg $PYY_{3–36}$, 10.1 ± 0.4; 1 nmol/kg $PYY_{3–36}$ + 10 nmol/kg $GLP\text{-}1_{7–36}$, 8.9 ± 0.4]. At these low doses, no differences in food intake were observed between groups at later time points (data not shown).

Immunocytochemistry

To elucidate a possible mechanism for the additive inhibitory feeding effect of PYY and GLP-1, c-*fos* was quantified after low dose individual peptide and combined peptide administration. Doses of PYY and GLP-1 that had been found not to alter food intake individually but had led to significant reduction in food intake after coadministration were chosen. Rats received ip injections of saline, 10 nmol/kg $GLP\text{-}1_{7–36}$, 1 nmol/kg $PYY_{3–36}$, or 1 nmol/kg $PYY_{3–36}$ + 10 nmol/kg $GLP\text{-}1_{7–36}$. In the hypothalamus, there were no significant differences in the number of FLI counts in the PVN, and ventromedial and dorsomedial of the hypothalamus between treatment groups (data not shown). At these low doses, no significant differences were seen in the ARC with either $PYY_{3–36}$ or $GLP\text{-}1_{7–36}$ alone, compared with saline. However, an 85% increase in the number of FLI counts was observed in the ARC after the coadministration treatment, compared with saline ($P < 0.05$) (Fig. 6). Moreover, after the combined $PYY_{3–36}$ + $GLP\text{-}1_{7–36}$ treatment, there were significantly more FLI than after $PYY_{3–36}$ alone ($P < 0.05$) and a trend for more FLI after $GLP\text{-}1_{7–36}$ alone ($P = 0.05$) (FLI-positive neurons in the ARC per section: saline, 35.4 ± 6.18; $GLP\text{-}1_{7–36}$, 42.9 ± 1.2; $PYY_{3–36}$, 36.2 ± 4.4; $PYY_{3–36}$ + $GLP\text{-}1_{7–36}$, 65.6 ± 10.0).

In the brain stem, no significant differences in FLI counts were observed between these low-dose treatments in the nucleus of the solitary tract or AP (data not shown).

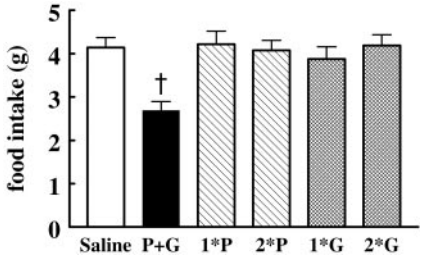

FIG. 5. Food intake in the first hour of the dark phase in freely feeding rats after ip low-dose coadministration of $PYY_{3–36}$ with $GLP\text{-}1_{7–36}$, compared with each peptide alone. Rats were injected with saline, P+G (1 nmol/kg $PYY_{3–36}$ + 10 nmol/kg $GLP\text{-}1_{7–36}$), G (10 nmol/kg $GLP\text{-}1_{7–36}$), 2*G (20 nmol/kg $GLP\text{-}1_{7–36}$), P (1 nmol/kg $PYY_{3–36}$), or 2*P (2 nmol/kg $PYY_{3–36}$). †, $P < 0.05$, compared with all other groups.

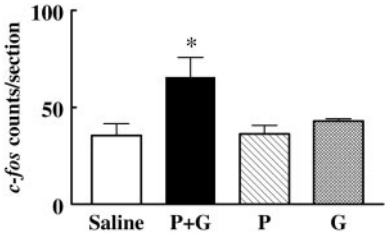

FIG. 6. Expression of FLI in the ARC in response to ip saline, P+G (1 nmol/kg $PYY_{3–36}$ + 10 nmol/kg $GLP\text{-}1_{7–36}$), G (10 nmol/kg $GLP\text{-}1_{7–36}$), and P (1 nmol/kg $PYY_{3–36}$). *, $P \leq 0.05$, compared with all other groups.

Behavioral studies

To determine whether behavioral alteration were associated with doses of $PYY_{3–36}$ and $GLP\text{-}1_{7–36}$ at which an additive feeding effect was observed, an observation study was performed. Fasted rats were ip injected with saline, 10 nmol/kg $GLP\text{-}1_{7–36}$, 1 nmol/kg $PYY_{3–36}$, 1 nmol/kg $PYY_{3–36}$ + 10 nmol/kg $GLP\text{-}1_{7–36}$, 10 nmol/kg exendin-4, or 1 M lithium chloride (positive control) (n = 10 per group). The groups treated with exendin-4 and lithium chloride had significantly fewer feeding episodes and significantly more head-down episodes, compared with saline ($P < 0.05$) (Fig. 7). However, no significant differences in behavior were observed after $PYY_{3–36}$, $GLP\text{-}1_{7–36}$, or $PYY_{3–36}$ + $GLP\text{-}1_{7–36}$.

In further study, locomotion was assessed in freely feeding rats before the dark phase after ip saline, 10 nmol/kg $GLP\text{-}1_{7–36}$, 1 nmol/kg $PYY_{3–36}$, 1 nmol/kg $PYY_{3–36}$ + 10 nmol/kg $GLP\text{-}1_{7–36}$, or 10 nmol/kg exendin-4 (n = 9–10 per group). No significant differences in horizontal movement (x-tot) were observed after the combined $PYY_{3–36}$ + $GLP\text{-}1_{7–36}$ treatment, but there was a slight reduction in vertical movement (z-tot), compared with saline ($P = 0.04$) (Table 1). There were significant reductions in both horizontal and vertical movement after exendin-4, compared with saline ($P < 0.01$ for both comparisons).

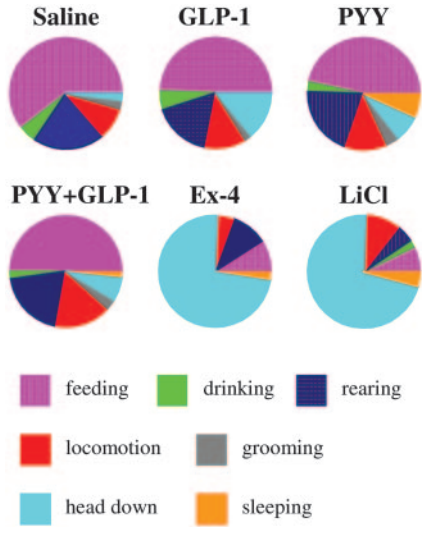

FIG. 7. Number of the following behavior observations: feeding, drinking, locomotion, grooming, head down, sleeping, and rearing after ip saline, 10 nmol/kg $GLP\text{-}1_{7–36}$, 1 nmol/kg $PYY_{3–36}$, 1 nmol/kg $PYY_{3–36}$ + 10 nmol/kg $GLP\text{-}1_{7–36}$, 10 nmol/kg exendin-4 (ex-4), and 0.5 ml 1 M lithium chloride (LiCl). The animals were observed over 60 min after injection, and the total number of observations per animal was 36.

Coadministration of $PYY_{3–36}$ and GLP-$1_{7–36}$ in human volunteers

Infusion of $PYY_{3–36}$ (0.4 pmol/kg·min) was associated with a 15% reduction in energy intake at the buffet meal, compared with saline, although this reduction achieved only borderline statistical significance ($P = 0.07$). There was no significant difference in energy intake with GLP-$1_{7–36}$ infusion (0.4 pmol/kg·min), in keeping with previously published results (8). With the combined $PYY_{3–36}$ and GLP-$1_{7–36}$ infusion, caloric intake at the buffet meal was reduced by 27% (buffet meal energy intake (kilojoules): saline, 4174 ± 285; GLP-$1_{7–36}$, 3978 ± 340; $PYY_{3–36}$, 3551 ± 441; $PYY_{3–36}$ + GLP-$1_{7–36}$, 3033 ± 279; percentage change from saline day: GLP-$1_{7–36}$, −5 ± 5%; $PYY_{3–36}$, −15 ± 9%; $PYY_{3–36}$ + GLP-$1_{7–36}$, −27 ± 3%) (Fig. 8). There was a statistical difference in energy intake among the four infusion groups ($P < 0.01$). The buffet caloric intake on the $PYY_{3–36}$ + GLP-$1_{7–36}$ day was significantly less than on all other infusion days ($P < 0.05$).

Cumulative energy intake over 24 h (buffet + food diary) was significantly reduced with the combined $PYY_{3–36}$ + GLP-$1_{7–36}$, compared with all the other groups (cumulative 24-h energy intake (megajoules): saline, 13.7 ± 0.6; GLP-$1_{7–36}$, 13.6 ± 1.3; $PYY_{3–36}$, 13.5 ± 0.9; $PYY_{3–36}$ + GLP-$1_{7–36}$, 12.0 ± 0.7, $P < 0.05$ for $PYY_{3–36}$ + GLP-$1_{7–36}$ *vs.* all other groups; percentage change from saline day: GLP-$1_{7–36}$, −2 ± 7%; $PYY_{3–36}$, −1 ± 5%; $PYY_{3–36}$ + GLP-$1_{7–36}$, −13 ± 5%). Analysis of the visual analog scales revealed no significant differences in nausea scores between infusion days [nausea score after 90 min infusion (millimeters): saline, 9.4 ± 0.83; GLP-$1_{7–36}$, 1.2 ± 0.05; $PYY_{3–36}$, 3.8 ± 0.18; $PYY_{3–36}$ + GLP-$1_{7–36}$, 6.3 ± 2.2, *P*=NS].

There were significant increases in plasma PYY levels with $PYY_{3–36}$ and $PYY_{3–36}$ + GLP-$1_{7–36}$ infusions and plasma GLP-1 levels with GLP-$1_{7–36}$ and $PYY_{3–36}$ + GLP-$1_{7–36}$ infusions (Tables 2 and 3). Interestingly, our GLP-$1_{7–36}$ infusion inhibited circulating PYY (PYY picomoles per liter at 90 min: saline infusion, 10.3 ± 1.2; GLP-$1_{7–36}$ infusion, 7.7 ± 0.8; $P < 0.05$). However, no significant changes in circulating GLP-1 were observed during $PYY_{3–36}$ infusion.

Fasting plasma insulin was increased by GLP-$1_{7–36}$ infusion at 90 min, compared with saline, and no changes in insulin secretion were observed with $PYY_{3–36}$ alone. Whereas the coadministration $PYY_{3–36}$ + GLP-$1_{7–36}$ infusion enhanced insulin secretion, there was no additional secretion, compared with GLP-$1_{7–36}$ alone (insulin picomoles per liter at 90 min: saline, 53.2 ± 4.9; GLP-$1_{7–36}$, 63.0 ± 3.5; $PYY_{3–36}$, 51.2 ± 5.5; $PYY_{3–36}$ + GLP-$1_{7–36}$, 66.7 ± 4.3; $P < 0.05$ for GLP-$1_{7–36}$ and combined $PYY_{3–36}$ + GLP-$1_{7–36}$ infusions *vs.* saline) (Table 4). Consistent with these changes in insulin levels, fasting plasma glucose was reduced by GLP-$1_{7–36}$ infusion and the joint coadministration infusion but unchanged by $PYY_{3–36}$ alone (glucose millimoles per liter at 90 min: saline, 4.6 ± 0.1; GLP-$1_{7–36}$, 4.2 ± 0.1; $PYY_{3–36}$, 4.5 ± 0.1; $PYY_{3–36}$ + GLP-$1_{7–36}$, 4.1 ± 0.1; $P < 0.01$ for GLP-$1_{7–36}$ and combined $PYY_{3–36}$+GLP-$1_{7–36}$ infusions *vs.* saline) (Table 5). Postprandial insulin and glucose levels were not measured due to the variability of energy intake at the buffet meal.

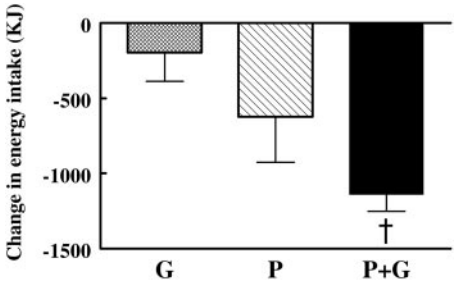

FIG. 8. Energy intake in human healthy volunteers from a free buffet meal with infusion of saline, G (0.4 pmol/kg·min GLP-$1_{7–36}$), P (0.4 pmol/kg·min $PYY_{3–36}$), and P+G (0.4 pmol/kg·min $PYY_{3–36}$ + 0.4 pmol/kg·min GLP-$1_{7–36}$). Results are presented as absolute difference in energy intake from the saline infusion day. †, $P < 0.05$, compared with all other groups.

Discussion

In this paper we investigated the effects of the coadministration of $PYY_{3–36}$ and GLP-$1_{7–36}$ on feeding in rodents and humans.

$PYY_{3–36}$ and GLP-$1_{7–36}$ inhibited food intake individually in mice and rats. $PYY_{3–36}$ appeared to cause maximal feeding inhibition at doses of 100 nmol/kg and above. In contrast, feeding inhibition associated with GLP-$1_{7–36}$ was similar to that seen with CCK-8, with increasing inhibition seen with increasing doses (14). In the dose-response studies with low doses, the minimum doses of $PYY_{3–36}$ and GLP-$1_{7–36}$ found to inhibit food intake were 3 and 30 nmol/kg, respectively. Both these results are consistent with our previously published data (2, 7). In both high- and low-dose coadministration studies, $PYY_{3–36}$ and GLP-$1_{7–36}$ administered together inhibited food intake significantly more than either peptide alone or more than double the dose of either peptide. Thus, $PYY_{3–36}$ and GLP-$1_{7–36}$ appeared to inhibit feeding additively.

$PYY_{3–36}$ and GLP-$1_{7–36}$ were coadministered in genetically obese mice. Examination of the food intake in the saline-injected groups revealed that the *ob/ob* and *db/db* mice ate less in the first hour of refeeding than their WT littermates. This somewhat surprising finding is consistent with previously published work

TABLE 1. Beam breaks in the first hour of the dark phase following IP saline, 10 nmol/kg GLP-$1_{7–36}$, 1 nmol/kg $PYY_{3–36}$, 10 nmol/kg GLP-$1_{7–36}$ + 1 nmol/kg $PYY_{3–36}$, or 10 nmol/kg exendin-4 (n = 8–10)

	Horizontal movement (x-tot)	Vertical movement (z-tot)
Saline	724 ± 60	357 ± 81
GLP-1	664 ± 76	265 ± 56
$PYY_{3–36}$	653 ± 88	291 ± 56
$PYY_{3–36}$ + GLP-1	517 ± 66	167 ± 25[a]
Exendin-4	442 ± 38[a]	128 ± 19[a]

[a] $P < 0.05$ *vs.* saline.

TABLE 2. Plasma levels of PYY in human infusions of saline, 0.4 nmol/kg GLP-$1_{7–36}$, 0.4 nmol/kg $PYY_{3–36}$, and 0.4 nmol/kg $PYY_{3–36}$ + 0.4 nmol/kg GLP-$1_{7–36}$

	PYY levels (pmol/liter) before infusion (fasting)	PYY levels (pmol/liter) after 90 min infusion (premeal)	PYY levels (pmol/liter) 2 h after the meal
Saline	10.5 ± 1.4	10.3 ± 1.2	16.3 ± 1.4
GLP-1	10.9 ± 0.9	7.7 ± 0.8[a]	16.0 ± 1.6
$PYY_{3–36}$	8.9 ± 0.7	59.8 ± 4.2[b]	18.0 ± 1.3
$PYY_{3–36}$ + GLP-1	11.1 ± 1.2	58.4 ± 4.3[b]	23.1 ± 3.3

[a] $P < 0.05$ *vs.* saline.
[b] $P < 0.005$ *vs.* saline.

TABLE 3. Plasma levels of GLP-1 in human infusions of saline, 0.4 nmol/kg GLP-$1_{7–36}$, 0.4 nmol/kg $PYY_{3–36}$, and 0.4 nmol/kg $PYY_{3–36}$ + 0.4 nmol/kg GLP-$1_{7–36}$

	GLP-1 levels (pmol/liter) before infusion (fasting)	GLP-1 levels (pmol/liter) after 90 min infusion (premeal)	GLP-1 levels (pmol/liter) 2 h after the meal
Saline	24.6 ± 2.8	18.8 ± 4.4	62.8 ± 10.4
GLP-1	21.5 ± 1.6	61.5 ± 3.7[a]	78.4 ± 12.3
$PYY_{3–36}$	18.3 ± 1.5	18.2 ± 1.6	47.5 ± 2.9
$PYY_{3–36}$ + GLP-1	23.0 ± 2.2	62.5 ± 5.2[a]	56.8 ± 5.8

[a] $P < 0.005$ *vs.* saline.

TABLE 5. Plasma levels of glucose in human infusions of saline, 0.4 nmol/kg GLP-$1_{7–36}$, 0.4 nmol/kg $PYY_{3–36}$, and 0.4 nmol/kg $PYY_{3–36}$ + 0.4 nmol/kg GLP-$1_{7–36}$

	Glucose levels (mmol/liter) before infusion (fasting)	Glucose levels (mmol/liter) after 90 min infusion (premeal)
Saline	4.44 ± 0.70	4.56 ± 0.07
GLP-1	4.54 ± 0.13	4.22 ± 0.95[a]
$PYY_{3–36}$	4.55 ± 0.58	4.52 ± 0.07
$PYY_{3–36}$ + GLP-1	4.49 ± 0.59	4.11 ± 0.09[a]

[a] $P < 0.05$ *vs.* saline.

(26, 27) and suggests that thermogenic adaptation to fasting reduced the subsequent energy requirement of the obese mouse models (28). Coadministration of $PYY_{3–36}$ and GLP-$1_{7–36}$ treatment led to significantly greater reduction in feeding than twice the dose of either peptide alone in the *ob/ob* and *db/db* and WT mice. Thus, $PYY_{3–36}$ and GLP-$1_{7–36}$ inhibited food intake additively in obese as well as lean rodents.

We quantified c-*fos* after$PYY_{3–36}$, GLP-$1_{7–36}$, and $PYY_{3–36}$ + GLP-$1_{7–36}$ administration to determine a possible mechanism for their additive effect on feeding. There was a significant increase in c-*fos* in the ARC after low-dose combination $PYY_{3–36}$ + GLP-$1_{7–36}$ treatment but no changes in the other treatment groups. Therefore, $PYY_{3–36}$ and GLP-$1_{7–36}$ may inhibit food intake through additive stimulation of ARC neurons. However, c-*fos* mapping cannot identify the pathway(s) through which neuronal activation was mediated. The ARC neuronal activation observed in our study could have been mediated through the blood-brain barrier via the median eminence (29) or the vagus nerve and brain stem (30).

There were no significant differences in observed behavior in rats after $PYY_{3–36}$, GLP-$1_{7–36}$, or $PYY_{3–36}$ + GLP-$1_{7–36}$ at doses at which the combination inhibited feeding. In the locomotion study, no significant differences in horizontal beam breaks were observed after the combined $PYY_{3–36}$ + GLP-$1_{7–36}$ treatment, but there were marginally fewer vertical beam breaks. In their recent publication, Talsania *et al.* (31) suggested that low-dose exendin-4 together with $PYY_{3–36}$ may increase the suppression of food intake in rodents without inducing significant side effects. There were no differences in nausea scores between infusions in our human study. Taken together these results suggest that behavioral change is unlikely to account for the feeding inhibition observed with coadministration of $PYY_{3–36}$ and GLP-$1_{7–36}$. Thus, these peptides may have a specific effect on feeding independent of behavioral change.

In our human study, GLP-$1_{7–36}$ alone (0.4 pmol/kg·min) did not lead to a significant change in energy intake. In human subjects the lowest reported infusion rate of GLP-$1_{7–36}$ associated with a change in energy intake is 0.75 pmol/kg·min (21). However, incretin effects have been observed with a lower rate of GLP-$1_{7–36}$ of 0.5 pmol/kg·min (24). In the current study, we found a small but statistically significant reduction in fasting glucose levels and an increase in the fasting insulin concentration with GLP-$1_{7–36}$ infusion.

$PYY_{3–36}$ infusion (0.4 pmol/kg·min) was associated with a 15% reduction in energy intake, compared with saline infusion, although this did not reach statistical significance. The magnitude of this reduction is in keeping with the larger reduction of 36% reported after higher-rate PYY infusions (0.8 pmol/kg·min) (2).

Coadministration of $PYY_{3–36}$ with GLP-$1_{7–36}$ was associated with a 27% reduction in energy intake from the buffet meal. The combination was more effective in inhibiting appetite then either peptide alone. Cumulative energy intake over 24 h, including the buffet meal, was reduced by 13% after combined infusion, compared with the saline infusion, and was significantly less than that after all other treatment groups. In contrast with the additive inhibitory effect on energy intake, the incretin effect of GLP-$1_{7–36}$ appeared unchanged with the addition of $PYY_{3–36}$.

Our study involving coadministration of $PYY_{3–36}$ and GLP-$1_{7–36}$ suggests an additive inhibitory feeding effect. Interestingly, the GLP-1 receptor agonist, exendin-4, and $PYY_{3–36}$ have been reported to inhibit food intake synergistically in rodents (31). However, it appears that not all combinations of gut hormones behave in this way. Indeed, it has recently been reported that whereas infusion of CCK-33 or GLP-$1_{7–36}$ inhibited food intake when administered individually, no enhanced inhibition was observed when they were infused together (32). Further work is needed to determine whether the other combinations of gut hormones lead to additive alterations in food intake.

We observed a reduction of endogenous PYY levels with GLP-$1_{7–36}$ infusion. This has been previously demonstrated at a higher dose of peripheral GLP-$1_{7–36}$ administration (32). Inhibition of endogenous PYY could result in a reduction in the anorexic action of GLP-1. In contrast, as in previous work (12), no changes in endogenous GLP-1 concentrations were observed after $PYY_{3–36}$ infusion.

In summary, we have demonstrated that $PYY_{3–36}$ and GLP-$1_{7–36}$ may inhibit appetite additively in rodents and man. The mechanism may be through enhanced activation of hypothalamic arcuate neurons. Reduction in energy intake and increase in insulin secretion would make this combination a particularly attractive therapy for patients with type 2 diabetes.

TABLE 4. Plasma levels of insulin in human infusions of saline, 0.4 nmol/kg GLP-$1_{7–36}$, 0.4 nmol/kg $PYY_{3–36}$, and 0.4 nmol/kg $PYY_{3–36}$ + 0.4 nmol/kg GLP-$1_{7–36}$

	Insulin levels (pmol/liter) before infusion (fasting)	Insulin levels (pmol/liter) after 90 min infusion (premeal)
Saline	58.7 ± 4.0	53.2 ± 4.9
GLP-1	56.6 ± 4.4	63.0 ± 3.5[a]
$PYY_{3–36}$	57.3 ± 4.0	51.2 ± 5.5
$PYY_{3–36}$ + GLP-1	58.1 ± 4.0	66.2 ± 4.3[a]

[a] $P < 0.05$ *vs.* saline.

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