Peripheral Exendin-4 and Peptide YY$^{3-36}$ Synergistically Reduce Food Intake through Different Mechanisms in Mice

Tanvi Talsania, Younes Anini, Stephanie Siu, Daniel J. Drucker, and Patricia L. Brubaker

Departments of Physiology (T.T., Y.A., S.S., P.L.B.) and Medicine (D.J.D., P.L.B.) and Banting and Best Diabetes Centre (D.J.D.), University of Toronto, Toronto, Ontario, Canada M5S 1A8; and Ottawa Health Research Institute (Y.A.), Ottawa, Ontario, Canada K1Y 4E9

Glucagon-like peptide-1$^{7-36NH2}$ (GLP-1) and peptide YY$^{3-36NH2}$ (PYY$^{3-36NH2}$) are cosecreted from the intestine in response to nutrient ingestion. Peripheral administration of GLP-1 or PYY$^{3-36NH2}$ decreases food intake (FI) in rodents and humans; however, the exact mechanisms by which these peptides regulate FI remain unclear. Male C57BL/6 mice were injected (ip) with exendin-4$^{1-39}$ (Ex4, a GLP-1 receptor agonist) and/or PYY$^{3-36NH2}$ (0.03–3 μg), and FI was determined for up to 24 h. Ex4 and PYY$^{3-36NH2}$ alone decreased FI by up to 83 and 26%, respectively ($P < 0.05$–0.001), whereas a combination of the two peptides (0.06 μg Ex4 plus 3 μg PYY$^{3-36NH2}$) further reduced FI for up to 8 h in a synergistic manner ($P < 0.05$–0.001). Ex4 and/or PYY$^{3-36NH2}$ delayed gastric emptying by a maximum of 19% ($P < 0.01$–0.001); however, there was no significant effect on locomotor activity nor was there induction of taste aversion. Capsaicin pretreatment prevented the inhibitory effect of Ex4 on FI ($P < 0.05$), but had no effect on the anorexigenic actions of PYY$^{3-36NH2}$. Similarly, exendin-4$^{9-39}$ (a GLP-1 receptor antagonist) partially abolished Ex4-induced anorexia ($P < 0.05$), but did not affect the satiation produced by PYY$^{3-36NH2}$. Conversely, BIIE0246 (a Y2 receptor antagonist) completely blocked the anorexigenic effects of PYY$^{3-36NH2}$ ($P < 0.001$), but had no effect on Ex4-induced satiety. Thus, Ex4 and PYY$^{3-36NH2}$ suppress FI via independent mechanisms involving a GLP-1 receptor-dependent, sensory afferent pathway (Ex4) and a Y2-receptor mediated pathway (PYY$^{3-36NH2}$). These findings suggest that administration of low doses of Ex4 together with PYY$^{3-36NH2}$ may increase the suppression of FI without inducing significant side effects. (*Endocrinology* 146: 3748–3756, 2005)

OBESITY IS A HEALTH problem that is reaching epidemic proportions and is associated with significant morbidity (1). Although relatively few therapeutic approaches have been successful in inducing body weight loss in obese individuals (2), several recent studies have demonstrated that the gut hormone, glucagon-like peptide-1$^{7-36NH2}$ (GLP-1), and long-acting GLP-1-receptor (GLP-1R) agonists, such as exendin-4$^{1-39}$ (Ex4) (3, 4), can acutely reduce food intake (FI) when administered to either rodents or humans (5–9). GLP-1 has recently been established as an effective treatment for patients with type 2 diabetes, stimulating glucose-dependent insulin secretion, as well as inhibiting glucagon release and gastric emptying (10, 11). Consistent with these diverse biological actions, long-term administration of GLP-1 to obese or overweight patients with type 2 diabetes lowers fasting blood sugar and HbA1c levels and significantly reduces body weight despite the improvement in glycemic control (12). Hence, GLP-1 is under investigation as a therapeutic agent for the treatment of type 2 diabetes (10, 11), but has also been proposed as a potential antiobesity

agent (13). Nonetheless, little is known about the mechanism of action of GLP-1 to reduce body weight.

GLP-1 is cosecreted from the intestinal L cell with another hormone, peptide YY$^{1-36}$ (14–17), in response to ingested nutrients (18–21). Once in the circulation, both of these peptides are rapidly degraded by the ubiquitous enzyme, dipeptidyl-peptidase-IV, to form GLP-1$^{9-36NH2}$ and peptide YY$^{3-36NH2}$ (PYY$^{3-36NH2}$), respectively (22, 23). Although the GLP-1 fragment GLP-1$^{9-36NH2}$ is not a potent GLP-1 receptor agonist (24), the truncated form of PYY remains biologically active because it inhibits FI when administered to rodents or humans (25–30). Thus, PYY$^{3-36NH2}$ has also been proposed as a potential therapeutic agent for the treatment of obesity (31).

Intracerebroventricular administration of GLP-1, Ex4 or PYY$^{3-36NH2}$ to rodents has been shown to modulate ingestive behavior through binding of the GLP-1R and the neuropeptide Y (NPY) Y2-receptor (Y2-R), respectively (25, 32). Both of these receptors are found in the hypothalamic arcuate nucleus, which has been implicated in the regulation of appetite, as well as in the dorsal vagal complex, nucleus tractus solitarus (NTS), and area postrema (AP) (33, 34). However, these receptors are also expressed in the nodose ganglion of the vagus nerve (35, 36). Because GLP-1, Ex4, and PYY$^{3-36NH2}$ are all known to cross the blood-brain barrier (BBB) (37–39), it remains unclear as to how these peptides exert their anorexigenic effects when administered into the peripheral circulation. It is also unknown whether GLP-1 and PYY$^{3-36NH2}$ interact to decrease FI, as recently found for several peripheral satiety factors including cholecystokinin (CCK), leptin,

and amylin (40, 41). Therefore the present study was conducted to address the possible synergistic effects and mechanisms of action of peripherally administered Ex4 and PYY$^{3–36NH2}$ to suppress FI.

Materials and Methods

Animals and reagents

Male C57BL/6 mice (for acute studies: 7–10 wk, 20–25 g; for chronic studies: 5 wk, 17–19 g) were obtained from Charles River Laboratories (Montreal, Quebec, Canada). Animals were housed three to four per cage at the University of Toronto Animal Care Facility, where they were allowed to acclimatize, without handling, for a minimum of 1 wk. They were provided with standard Purina Rat chow and tap water *ad libitum*, unless otherwise indicated. The surrounding temperature and humidity were maintained at 21 C and 45–50%, respectively, with a consistent 12-h light, 12-h dark cycle (lights on at 0700 h). All experiments were started between 0900 and 1000 h in a mouse room at the animal care facility, and were performed according to protocols approved by the University of Toronto Animal Care Committee.

Ex4, human PYY$^{3–36NH2}$, and exendin-4$^{9–39}$ [Ex4$^{9–39}$] were purchased from Bachem Inc. (Torrance, CA). (*S*)-N^2-[[1-[2-[4-[(*R*,*S*)-5,11-dihydro-6(6h)-oxodibenz[b,e]azepin-11-yl]-1-piperazinyl]-2-oxoethyl]-cylopentyl] acetyl]-*N*-[2-[1,2-dihydro-3,5(4H)-dioxo-1,2-diphenyl-^{3}H-1,2,4-triazol-4-yl]-ethyl]-argininamid (BIIE0246) was obtained from Tocris (Ellisville, MO), and capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), Tween 20, and dimethyl sulfoxide (DMSO, 99.9%) were from Sigma-Aldrich Canada (Oakville, Ontario, Canada).

Feeding studies

For acute studies, mice were fasted in separate cages for 16–18 h with free access to water. On the experimental day, animals were transferred to cages without bedding and injected ip at t = 0 h with 100 μl of either vehicle (saline), Ex4 (0.03, 0.06, 0.3, 3 μg), or PYY$^{3–36NH2}$ (0.03, 0.06, 0.3, 3 μg) for the individual dose responses. To examine the combined effect of these peptides, mice were treated with 100 μl of either saline, Ex4 (0.06 μg), or PYY$^{3–36NH2}$ (3 μg) alone or in combination. Immediately thereafter, preweighed Purina Rat Chow was placed in a Petri dish within the cage, and FI was determined at 1, 2, 4, 8, and 24 h by measuring the difference between the preweighed chow and the weight of chow plus spill remaining at the end of each time interval. Animals were tested on a maximum of two occasions, separated by a minimum 3-d wash-out period.

Mice were anesthetized with isoflurane and sc administered 100 μl of either vehicle (10% ethanol, 10% Tween 20, 80% saline) or capsaicin (50 mg/kg) (40), followed by an ip injection of sodium pentobarbitol (65 mg/kg) to maintain anesthesia for another hour. A second capsaicin injection was administered 2 d later following the same protocol. One week after the second injection, mice were treated ip with 100 μl of either saline, Ex4 (0.06 μg), or PYY$^{3–36NH2}$ (3 μg) alone or in combination, and FI was measured as described above. Efficiency of the capsaicin pretreatment was verified immediately before euthanasia by the corneal chemosensory reflex test, which consists of monitoring the wiping reflex after ocular administration of a drop of 0.1% NH$_4$OH solution (40).

FI was also measured, as above, in mice injected ip with 100 μl of either saline or the GLP-1R antagonist, Ex4$^{9–39}$ (3, 4) (5 μg), alone or in combination with Ex4 (0.06 μg) or PYY$^{3–36NH2}$ (3 μg). A second set of mice was administered ip 100 μl of either vehicle (50% DMSO) or the NPY Y2-R antagonist, BIIE0246 (42) (50 μg) alone or in combination with Ex4 (0.06 μg) or PYY$^{3–36NH2}$ (3 μg), and FI was measured as previously described.

Gastric emptying

Mice were housed in separate cages and were food deprived for 16–18 h with free access to water. At t = 0 h animals were given free access to preweighed standard chow for 1 h, and then were injected ip with 100 μl of either saline, Ex4 (0.06 μg), or PYY$^{3–36NH2}$ (3 μg) alone or in combination. They were deprived of food for 3 h after ip administration and then euthanized. To determine the amount of food remaining in the stomach after the 3-h postinjection period, the stomach was excised at

the pylorus and cardia, and the contents were collected, frozen, lyophilized overnight, and weighed. Total FI during the 1 h feeding period was determined by measuring the difference between the preweighed standard chow and the weight of chow and spill. Gastric emptying (%) was calculated as: [1 − (dry weight of food recovered from the stomach/total FI)] × 100 (40).

Behavioral studies

Locomotor activity. Mice were fasted for 16–18 h before the start of the study and then injected ip with 100 μl of either saline, Ex4 (0.06 or 3 μg), or PYY$^{3–36NH2}$ (3 μg) alone or in combination (Ex4, 0.06 μg and PYY$^{3–36NH2}$, 3 μg). Locomotor activity was monitored for 1 h using an Opto-Max system (Columbus Instruments, Columbus, OH).

Conditioned taste aversion (CTA). To accustom mice to a water deprivation schedule, animals were allowed access to two water bottles (50 ml each) for 1 h/d (1000–1100 h) for 2 d. This was followed by Conditioning Day 1 in which the water was replaced with two bottles (50 ml each) of either cherry or grape Kool-Aid ["Flavor 1" (0.3% Kool-Aid with 1% saccharin)] for 1 h, and mice were then injected ip with 100 μl of saline. Animals were subsequently given 1 d of normal water access (*e.g.* 1 h/d) as a Rest Day. On Conditioning Day 2, mice received the alternative flavor (two bottles of "Flavor 2"; grape or cherry, respectively) in a counter-balanced manner for 1 h and were then injected with 100 μl of either saline, Ex4 (0.06 or 3 μg), or PYY$^{3–36NH2}$ (3 μg) alone or in combination (Ex4, 0.06 μg and PYY$^{3–36NH2}$, 3 μg). This was followed by a Rest Day, and this procedure was then repeated and followed by two Rest Days. On the Test Day, mice were given access to one preweighed bottle of each flavor, and fluid intake was determined as the change in weight after 24 h. CTA was determined as a "Preference Ratio" [(intake of treatment-paired flavor/total intake) × 100%] (43).

Chronic studies

One day before the start of the study, mice were individually housed and body weights were measured and normalized for each treatment group. Mice were weighed between 0900 and 1000 h and injected ip (10 μl/g of mouse) at 1000 and 1800 h for 14 d with either saline, Ex4 (0.06 μg), or PYY$^{3–36NH2}$ (3 μg) alone or in combination, and were then placed on a diet containing 45% kcal as fat (no. D12451; Research Diet, North Brunswick, NJ) to mimic a typical high-fat diet consumed by humans. Body weight was determined daily at 0900–1000 h, and 8 h FI (1000–1800 h) was measured in fed mice on d 1, 3, 5, 7, 9, 11, and 13, as described above. Magnetic resonance imaging was performed on d 15 to analyze whole body composition (fat and lean mass; EchoMRI, Echo Medical Systems, Houston, TX).

Data analysis

All data are expressed as mean ± SEM. Statistical comparisons between experimental groups were assessed by one- or two-way ANOVA, followed by Student's *t* tests, as appropriate.

Results

The effects of graded doses of peripherally administered Ex4 and PYY$^{3–36NH2}$ on FI were studied in fasted mice. Administration of Ex4 at 0.03 μg produced a significant decrease in FI for a relatively short period of time (1–4 h), during which intake ranged between 56 ± 18 and 71 ± 16% of controls ($P < 0.05$) (Fig. 1A). Mice given 0.06 μg of Ex4 also ate significantly less than controls over 8 h (55 ± 7 to 77 ± 5% of the controls; $P < 0.05–0.01$), whereas higher doses of Ex4 (0.3 and 3 μg) caused a significant suppression of FI over 24 h (Ex4 at 0.3 μg, 28 ± 21 to 63 ± 6%; Ex4 at 3 μg, 17 ± 4 to 65 ± 5% of controls; $P < 0.001$). Although Ex4 at the higher doses (0.3 and 3 μg) induced a profound inhibition of FI, observation of these animals postinjection indicated that these doses of Ex4 caused abnormal behavioral characteris-
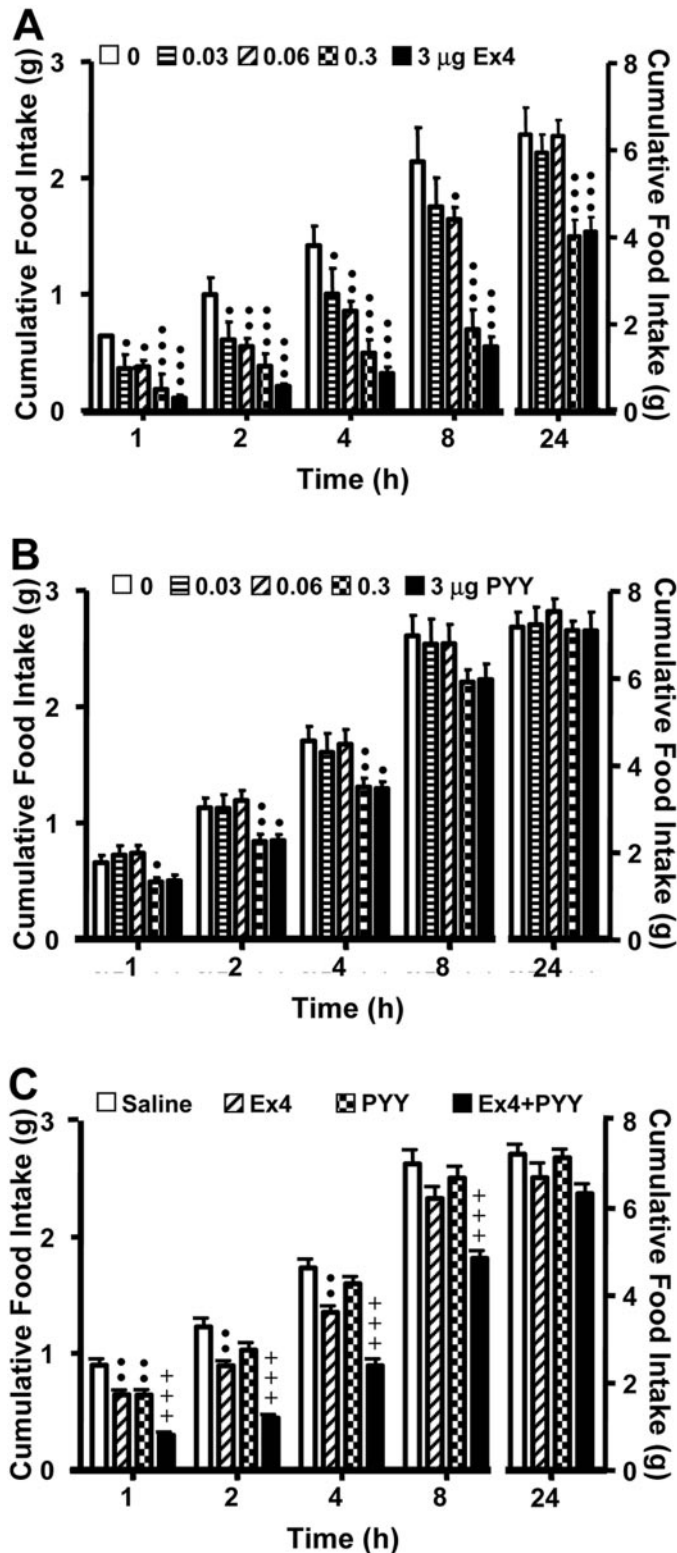
tics (*i.e.* decreased activity). Unlike Ex4, peripherally administered PYY$^{3-36NH2}$ was only effective at producing anorexigenic effects at the two highest doses tested (0.3 and 3 μg; Fig. 1B). Mice injected with 0.3 μg ate significantly less than controls from 1–4 h (74 $\pm$ 6 to 77 $\pm$ 5% of controls; $P <$ 0.05–0.01), whereas 3 μg of PYY$^{3-36NH2}$ induced a significant decrease in FI from 2–4 h (75 $\pm$ 5 to 77 $\pm$ 3% of controls; $P <$ 0.05).

Based on the results of the dose response experiments, doses of Ex4 and PYY$^{3-36NH2}$ that submaximally suppressed FI were selected for further testing in combination. Thus, in fasted mice, administration of Ex4 alone (0.06 μg) significantly decreased FI by 22 $\pm$ 3 to 28 $\pm$ 5% ($P <$ 0.01) at 1–4 h, whereas PYY$^{3-36NH2}$ alone (3 μg) significantly suppressed FI by 29 $\pm$ 5% *vs.* control ($P <$ 0.01) at 1 h (Fig. 1C). However, when given in combination, these peptides significantly decreased FI by 31 $\pm$ 3 to 67 $\pm$ 3% ($P <$ 0.001) over 8 h. Furthermore, the combination of Ex4 and PYY$^{3-36NH2}$ had a significantly greater effect than either Ex4 or PYY$^{3-36NH2}$ alone at 1–8 h ($P <$ 0.001). The decrease in FI produced by the combination was greater than the sum of the individual effects produced by Ex4 and PYY$^{3-36NH2}$ alone ($P <$ 0.001 over the first 8 h), and two-way ANOVA demonstrated a significant interaction between Ex4 and PYY$^{3-36NH2}$ at 2–4 h ($P <$ 0.05), indicating synergistic effects of these peptides. Because the effects produced by Ex4 (at 0.06 μg) or PYY$^{3-36NH2}$ (at 3 μg) either alone or in combination lasted for a maximum of 8 h, all subsequent studies were examined at these doses over an 8-h period only.

To determine whether Ex4 and/or PYY$^{3-36NH2}$ produce their anorexigenic effects through a neural mechanism, mice were pretreated with vehicle or capsaicin to ablate C-type neural fibers (44). At least 1 wk after the last capsaicin treatment, FI was measured in fasted mice after systemic administration of saline, Ex4, or PYY$^{3-36NH2}$ alone or in combination. Capsaicin completely abrogated the anorexigenic effect of Ex4 at 1–8 h ($P <$ 0.05) but had no effect on the suppression of FI produced by PYY$^{3-36NH2}$ alone or in combination with Ex4 (Fig. 2). These results suggest that Ex4 and PYY$^{3-36NH2}$ exert their inhibitory effects through different mechanisms, with only Ex4 requiring sensory afferent pathways.

The GLP-1-related gut peptide, oxyntomodulin is known to require the GLP-1/GLP-1R signaling pathway to exert its anorexigenic effect (45, 46). Therefore, to establish whether PYY$^{3-36NH2}$ produces its effects on FI through interaction with the Ex4 signaling pathway, and specifically through the GLP-1R, fasted mice were peripherally administered vehicle or the GLP-1R antagonist, Ex4$^{9-39}$, alone or in combination with Ex4 or PYY$^{3-36NH2}$, and FI was measured. Pilot studies were conducted to determine a dose of Ex4$^{9-39}$ that blocked the effects of Ex4 but that had no independent effect on FI [$P >$ 0.05 for 5 μg Ex4$^{9-39}$ alone *vs.* saline controls; Fig. 3A]. Ex4$^{9-39}$ partially blocked the inhibitory effect of Ex4 ($P <$ 0.05) but had no effect on the suppression of FI produced by PYY$^{3-36NH2}$. Alternatively, to determine whether Ex4 re-

FIG. 1. Effects of peripheral Ex4 or PYY$^{3-36NH2}$ alone or in combination on FI. Male C57BL/6 mice (7–10 wk; 20–25 g) fasted for 16–18 h were injected ip with increasing doses of saline (□, n = 6) or Ex4 (0.03 μg ▤, n = 6; 0.06 μg ▨, n = 9; 0.3 μg ▧, n = 5; 3 μg ■, n = 6) (A), with saline (□, n = 13) or PYY$^{3-36NH2}$ (0.03 μg ▤, n = 9; 0.06 μg ▨, n = 11; 0.3 μg ▧, n = 18; 3 μg ■, n = 9) (B), or with saline (□, n = 9) or Ex4 (0.06 μg ▨, n = 8) or PYY$^{3-36NH2}$ (3 μg ▧, n = 9) alone or in combination (■, n = 9) (C). Cumulative FI was measured at 1, 2, 4, 8, and 24 h postinjection. •, $P <$ 0.05; ••, $P <$ 0.01; •••, $P <$ 0.001 *vs.* saline-treated mice; +++, $P <$ 0.001 *vs.* mice treated with Ex4 or PYY$^{3-36NH2}$ alone.

FIG. 2. Effects of capsaicin pretreatment on the inhibitory effects of Ex4 and PYY$^{3-36\text{NH2}}$ on FI. Male C57BL/6 mice (7–10 wk; 20–25 g) were pretreated with vehicle (Veh) or capsaicin (Cap) (sc). After 1 wk of recovery, vehicle- and capsaicin-treated mice fasted for 16–18 h were injected ip with saline (S; Veh, n = 24; Cap, n = 31), Ex4 (0.06 μg; Veh, n = 16; Cap, n = 18), or PYY$^{3-36\text{NH2}}$ (3 μg; Veh, n = 15; Cap, n = 14) alone or in combination (Veh, n = 10; Cap, n = 10). Cumulative FI was measured at 1, 2, 4, and 8 h postinjection. •, $P < 0.05$; ••, $P < 0.01$ *vs.* vehicle-saline-treated mice; *, $P < 0.05$ *vs.* vehicle-Ex4-treated mice; #, $P < 0.05$ *vs.* vehicle-PYY$^{3-36\text{NH2}}$-treated mice, +, $P < 0.05$ *vs.* mice treated with vehicle-Ex4 and PYY$^{3-36\text{NH2}}$ alone.
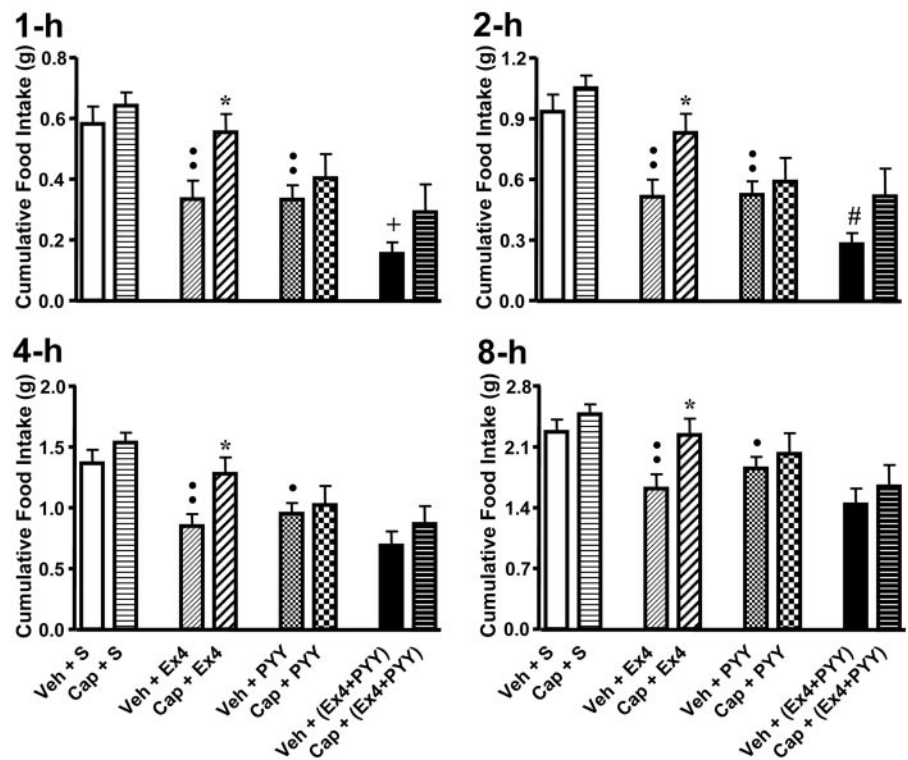
quires a functional Y2-R to decrease appetite, FI was measured in fasted mice that were systemically administered with vehicle or the specific Y2-R antagonist, BIIE0246, alone or in combination with Ex4 or PYY$^{3-36\text{NH2}}$. The appropriate dose of BIIE0246 was determined in pilot studies (data not shown). Treatment of fasted mice with BIIE0246 completely abrogated the effects of PYY$^{3-36\text{NH2}}$ on FI ($P < 0.001$), but did not prevent the inhibitory effects produced by Ex4 (Fig. 3B). When taken together, these studies provide further evidence that Ex4 and PYY$^{3-36\text{NH2}}$ reduce appetite through independent pathways.

To examine other potential mechanisms underlying the effects of Ex4 and PYY$^{3-36\text{NH2}}$ on FI, the rate of gastric emptying was first determined in mice administered saline, Ex4, or PYY$^{3-36\text{NH2}}$ alone or in combination (Fig. 4A). Mice injected with either Ex4 or PYY$^{3-36\text{NH2}}$ (0.06 and 3 μg, respectively) displayed a small but significant decrease in the rate of gastric emptying (by 6 ± 2 and 8 ± 2% of controls, respectively; $P < 0.01$–0.001), whereas the combination of peptides induced an even greater decrease (by 19 ± 3% of controls; $P < 0.001$). However, two-way ANOVA did not indicate a significant interaction between the peptides, suggesting that Ex4 and PYY$^{3-36\text{NH2}}$ produced an additive rather than a synergistic effect on gastric emptying.

Because Ex4 at high doses (0.3 and 3 μg) was observed to alter activity levels, possible adverse behavioral effects of the lower doses of Ex4 and/or PYY$^{3-36\text{NH2}}$ were also assessed. Mice peripherally treated with saline, Ex4 (0.06 μg), or PYY$^{3-36\text{NH2}}$ (3 μg) alone or in combination did not alter total locomotor activity (Fig. 4B). However, a significant reduction in activity was observed in mice injected with the high dose of Ex4 (3 μg) compared with the vehicle-treated mice ($P < 0.001$; positive control). Similarly, low doses of Ex4 and/or

PYY$^{3-36\text{NH2}}$ had no effect on CTA. However, aversion was induced in mice treated with Ex4 at 3 μg ($P < 0.001$; positive control) (Fig. 4C). When taken together, these findings indicate that the suppression of appetite produced by Ex4 and/or PYY$^{3-36\text{NH2}}$ may be partially attributed to delayed gastric emptying, but are not associated with significant behavioral changes.

Finally, to determine whether the acute effects of Ex4 and/or PYY$^{3-36\text{NH2}}$ on FI are sustained, mice were placed on a high-fat diet and peripherally injected twice a day with saline, Ex4, or PYY$^{3-36\text{NH2}}$ alone or in combination for 14 d. Body weight and 8-h FI were measured every 24 and 48 h, respectively. Chronic administration of Ex4 (0.06 μg) or PYY$^{3-36\text{NH2}}$ (3 μg) alone had no effect on 8-h FI compared with vehicle-treated animals. However, Ex4 and PYY$^{3-36\text{NH2}}$ in combination significantly decreased 8-h FI compared with mice given Ex4 or PYY$^{3-36\text{NH2}}$ alone (by 30 ± 7 to 44 ± 4%) on d 5, 9, 11, and 13 ($P < 0.01$–0.001) (Fig. 5A). Two-way ANOVA indicated a significant interaction between these peptides on d 1, 5, 7, 9, and 11 ($P < 0.05$–0.01), suggesting that the synergistic effect of Ex4 and PYY$^{3-36\text{NH2}}$ to suppress 8-h FI was not lost in the chronic setting. However, despite the effects on FI, there was no difference in body weight gain between the animals treated with Ex4 and/or PYY$^{3-36\text{NH2}}$ compared with controls (Fig. 5B). Body composition analysis further verified that there was no difference between the different groups of mice (data not shown).

Discussion

GLP-1 and PYY are cosecreted from the distal gut after nutrient ingestion. Although peripheral administration of GLP-1 or PYY$^{3-36\text{NH2}}$ has been found to suppress appetite in
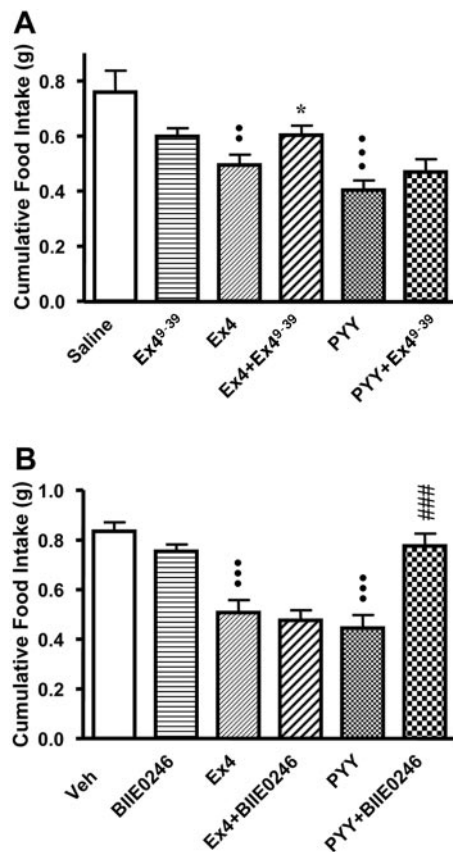
FIG. 3. Effects of selective antagonists on inhibitory effects of Ex4 and PYY$^{3-36NH2}$ on FI. Male C57BL/6 mice (7–10 wk; 20–25 g) fasted for 16–18 h were injected ip with saline (n = 10), the GLP-1R antagonist, Ex4$^{9-39}$ (5 μg), and/or Ex4 (0.06 μg) or PYY$^{3-36NH2}$ (3 μg) (n = 11 for each group) (A), and vehicle (50% DMSO) or the Y2-R antagonist, BIIE0246 (50 μg), and/or Ex4 (0.06 μg) or PYY$^{3-36NH2}$ (3 μg) (n = 7 for each group) (B). Cumulative FI was measured at 1 h postinjection. ••, $P < 0.01$; •••, $P < 0.001$ *vs.* saline or vehicle-treated mice; *, $P < 0.05$ *vs.* Ex4-treated mice; ###, $P < 0.001$ *vs.* PYY$^{3-36NH2}$-treated mice.

rodents and humans, it is not known whether these peptides interact to produce their anorexigenic effects. The results of this study have now demonstrated for the first time that systemically administered Ex4, a long acting GLP-1 analog, and PYY$^{3-36NH2}$ act synergistically to reduce FI through independent mechanisms.

Fasted control mice in this study ate approximately 6–7.5 g or 0.3 g per gram of body weight over a 24-h period, which is consistent with the results of other studies (47). The anorexigenic effects of peripheral Ex4 and PYY$^{3-36NH2}$ on fasted mice were found to be synergistic when administered acutely, by up to 67% for an 8-h period. This interaction was not lost during chronic studies, indicating the absence of tachyphylaxis to the acute effects of Ex4 and PYY$^{3-36NH2}$ on FI. Synergistic interactions between a number of other anorexigenic hormones have been reported. CCK is a short-term satiety factor that is released from the gut after nutrient ingestion, whereas leptin is a long-term regulator of appetite and body fat stores, the levels of which are proportional to fat mass. Coadministration of leptin and CCK-8 synergistically decreases FI in rodents both acutely and chronically (40,
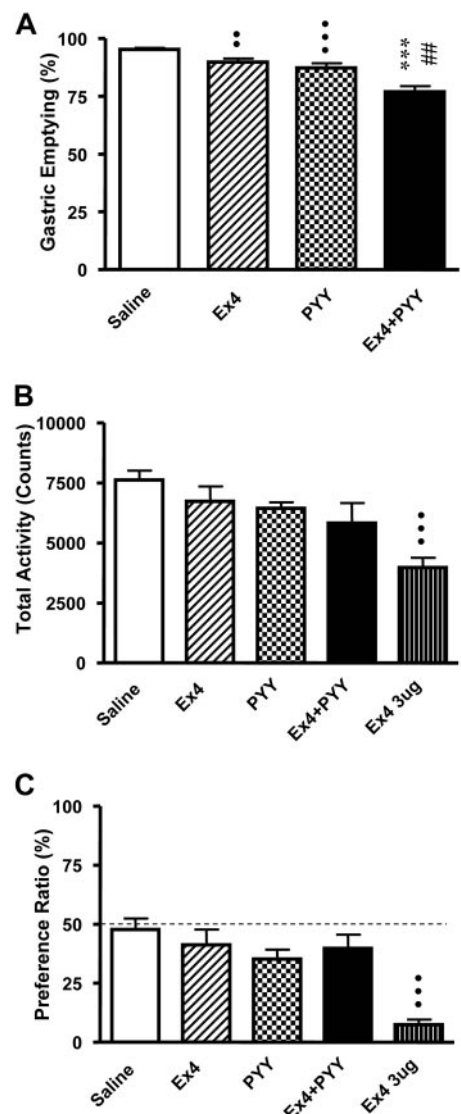

FIG. 4. Effects of Ex4 and PYY$^{3-36NH2}$ on gastric emptying, locomotor activity, and CTA. Male C57BL/6 mice (7–10 wk; 20–25 g) were injected ip with saline (n = 24), Ex4 (0.06 μg, n = 19), or PYY$^{3-36NH2}$ (3 μg, n = 16) alone or in combination (n = 13) (A). Fasted mice (16–18 h) were fed for 1 h, treated with peptide, and then euthanized 3 h postinjection for determination of gastric content. Gastric emptying (%) = [1 − (dry weight of food recovered from stomach/total FI)] × 100. B, Total locomotor activity was measured in mice fasted for 16–18 h. Mice were injected ip with saline (n = 9), Ex4 (0.06 μg, n = 5), or PYY$^{3-36NH2}$ (3 μg, n = 5) alone or in combination (Ex4 0.06 μg and PYY$^{3-36NH2}$ 3 μg, n = 5). Ex4 (3 μg, n = 5) was used as a positive control. C, Mice were injected ip with saline (n = 13), Ex4 (0.06 μg, n = 14), or PYY$^{3-36NH2}$ (3 μg, n = 14) alone or in combination (Ex4 0.06 μg and PYY$^{3-36NH2}$ 3 μg, n = 11). Ex4 (3 μg, n = 4) was used as a positive control. CTA was assessed and expressed as a preference ratio for a novel flavor when paired with each treatment (Ex4 and/or PYY$^{3-36NH2}$) [(intake of treatment-paired flavor/total intake) × 100%] (n = 4–14). *Dashed line* represents expected ratio for no effect of treatment (50%). ••, $P < 0.01$; •••, $P < 0.001$ *vs.* saline-treated mice; ***, $P < 0.001$ *vs.* Ex4-treated mice; ##, $P < 0.01$ *vs.* PYY$^{3-36NH2}$-treated mice.

48). Similarly, Bhavsar *et al.* (41) examined the interaction of CCK with another regulator of appetite, amylin, which is cosecreted with insulin from pancreatic B cells in response to
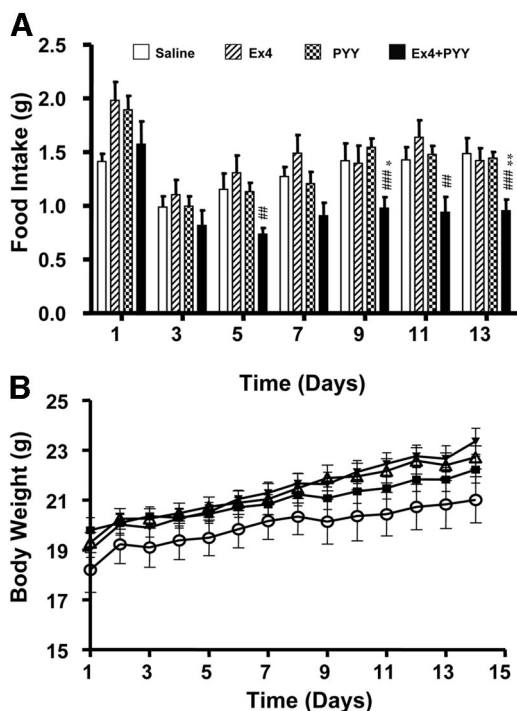
FIG. 5. Effects of chronic administration of Ex4 and PYY$^{3-36\text{NH2}}$ on FI and body weight. Male C57BL/6 mice (5 wk, 17–19 g) were placed on a high-fat diet and injected ip twice a day (10 μl/g mouse) with saline (□), Ex4 (0.06 μg ▨), or PYY$^{3-36\text{NH2}}$ (3 μg ▩) alone or in combination (■) for 14 d (n = 8 for each group). Eight-hour FI was measured every 48 h (A), and body weight was measured daily for each group (B) (saline ■, Ex4 △, PYY$^{3-36\text{NH2}}$ ▼, in combination ○). *, $P < 0.05$; **, $P < 0.01$ *vs.* Ex4-treated mice; ##, $P < 0.01$; ###, $P < 0.001$ *vs.* PYY$^{3-36\text{NH2}}$-treated mice.

nutrient stimuli. Systemic coadministration of these hormones in mice acutely reduced FI by up to 91%, an inhibition that was greater than that produced by amylin or CCK alone. Because secretion of GLP-1, PYY, and CCK, as well as of another intestinal satiety hormone, oxyntomodulin, is enhanced in the fed state, these findings suggest that the total gut satiety response to nutrient ingestion is mediated through the actions of multiple hormones acting in an integrated fashion.

The present study has also demonstrated that Ex4 and PYY$^{3-36\text{NH2}}$ produce their anorexigenic effects through independent mechanisms. Specifically, Ex4 decreased FI through a sensory neural mechanism, whereas PYY$^{3-36\text{NH2}}$ did not appear to require such pathways. Furthermore, the actions of these peptides were produced through activation of the GLP-1R and Y2-R, respectively, as demonstrated through the use of receptor-specific antagonists. Recently, mRNA transcripts for the GLP-1 and Y2-receptors have been localized in the rat nodose ganglion, which contains the cell bodies of afferent vagal neurons (35, 36). Systemic pretreatment of mice with capsaicin causes selective degeneration of small diameter unmyelinated sensory neurons (49), including the nodose ganglion (50) and the vagus nerve (36). Interestingly, capsaicin blocked the inhibition of appetite by Ex4, but had no such effect on PYY$^{3-36\text{NH2}}$-induced satiety. It is acknowledged that there are some side-effects associated with the use of capsaicin, including effects to decrease blood

pressure and body temperature, which are thought to be induced via peripheral actions (44). However, like the GLP-1R and Y2-R, capsaicin receptors are also centrally located in the NTS and AP in rats (52), areas that relay signals to the hypothalamus to regulate FI. It is currently unclear whether peripherally administered capsaicin affects these central regions but, based on the results of the present study, capsaicin treatment did not alter FI in control mice, suggesting that its effects on Ex4-induced FI were mediated via ablation of peripheral sensory afferent signals. Consistent with this notion, a number of studies have demonstrated that although peripheral Ex4 can induce c-*fos* in the AP and NTS (45), Ex4 can also directly activate vagal fibers (53). Other biological actions of GLP-1, such as the stimulation of insulin secretion, are also proposed to require a sensory afferent neural pathway, because a low dose of GLP-1 does not augment the insulin response to glucose in capsaicin-pretreated mice (54). Furthermore, capsaicin treatment completely abrogated the effects of CCK and leptin on FI (40), indicating that these peptides produce at least some of their anorexigenic actions through similar peripheral neural mechanisms. Nonetheless, these findings differ from a report demonstrating that PYY$^{3-36\text{NH2}}$ acts through the vagus to inhibit FI in rats (36). These contradictory results may occur consequent to species differences in the expression of the Y2-R in the nodose ganglion, as c-*fos* immunoreactivity is not observed in the NTS after peripheral administration of PYY$^{3-36\text{NH2}}$ in mice (55), but is induced in rats treated systemically with PYY$^{3-36\text{NH2}}$ (36).

The possibility that Ex4 and/or PYY$^{3-36\text{NH2}}$ may suppress FI by acting directly in the brain must be considered, as both of these peptides have been demonstrated to cross the BBB (38, 39). Baggio *et al.* (47) have shown that peripheral administration of Albugon, a GLP-1-albumin recombinant protein, suppresses FI in mice, thereby demonstrating that the satiating effects of GLP-1 can be mediated through the periphery. Furthermore, a long-acting analog of GLP-1 suppresses FI when injected peripherally into monosodium glutamate-treated rats that cannot respond to central administration of the peptide (57). Therefore, when taken together, these findings suggest that effects of Ex4 on FI in the present study are mediated peripherally. Nonetheless, it remains possible that some of the effects of higher doses of Ex4 on FI may be exerted through direct effects on GLP-1R expressed in the brain. Similarly, although numerous studies have found that PYY$^{3-36\text{NH2}}$ is effective at decreasing FI when given systemically (25–30), Nonaka *et al.* (39) have reported that PYY$^{3-36\text{NH2}}$ crosses the BBB by a nonsaturable process, perhaps thereby reaching the Y2-R in the arcuate nucleus (58). Because pretreatment with capsaicin did not prevent the effects of PYY$^{3-36\text{NH2}}$ on feeding, it cannot be excluded that the actions of PYY$^{3-36\text{NH2}}$ on satiety are mediated through direct modulation of Y2-receptors in the brain.

GLP-1 and PYY are cosecreted from the L cells of the distal colon along with other gut peptides that have been found to decrease FI, including oxyntomodulin (45, 46). Interestingly, previous studies have shown that oxyntomodulin requires the GLP-1R signaling pathway to produce its anorexigenic effects (45, 46). Similarly, administration of the CCK-A receptor antagonist, devazepide, with CCK and leptin com-

pletely prevents the inhibitory effects of CCK and leptin coinjection (40). However, the effects of Ex4 and PYY$^{3-36NH2}$ on FI have now been demonstrated to be mediated independently, because the GLP-1R antagonist, Ex4$^{9-39}$ did not block the inhibitory effects induced by PYY$^{3-36NH2}$, whereas the Y2-R antagonist BIIE0246 was unable to prevent the effects of Ex4.

The present study suggests that the inhibition of FI produced by Ex4 and PYY$^{3-36NH2}$ may be partially attributed to decreased gastric emptying. This is consistent with previous reports that peripheral administration of Ex4 or PYY$^{3-36NH2}$ alone in rodents and humans delays gastric emptying (9, 47, 59, 60), possibly through a vagal pathway. The present study also demonstrated that coadministration of Ex4 and PYY$^{3-36NH2}$ induced an even greater inhibition of gastric emptying than Ex4 or PYY$^{3-36NH2}$ alone. However, unlike their effects on FI, the combined effects of these peptides on gastric emptying were additive rather than synergistic. Furthermore, coinjection of CCK and leptin reduced FI but had no effect on gastric emptying (40). Hence, although gastric distension may induce satiety (61), the synergistic effects of Ex4 and PYY$^{3-36NH2}$ on FI may be produced through alternative mechanisms. One possible mechanism may be at the level of the arcuate nucleus, where signals from a variety of different peripheral and central inputs are integrated to modulate the balance between orexigenic (*e.g.* NPY and agouti-related protein) and anorexigenic (*e.g.* α-MSH) neuropeptides (33, 34). Further studies will clearly be necessary to elucidate the specific neural mechanisms by which the combination of Ex4 and PYY$^{3-36NH2}$ produce their synergistic effects to regulate ingestive behavior.

To determine whether the reduction of food consumption was secondary to nonspecific behavioral effects of the treatments in the present study, both taste aversion and locomotor activity were assessed. The present study demonstrated that mice treated with doses of Ex4 and/or PYY$^{3-36NH2}$ sufficient to decrease FI, did not display either taste aversion or altered motor activity; however, a very high dose of Ex4 caused a significant taste aversion and decreased total activity. Although adverse effects of Ex4 have not been extensively examined in rodents, considerable evidence exists for such effects of GLP-1. Central administration of GLP-1 in the lateral and third ventricle, but not in the PVN, induces taste aversion in rats (62–64). Furthermore, the mechanisms underlying taste aversion appear to be species-specific, as central administration of the toxin LiCl to rats produces CTA in a GLP-1-dependent manner; however, mice lacking the GLP-1R still display taste aversion after administration of LiCl (43). In addition, Ex4 has been shown to produce noxious responses in humans leading to nausea and vomiting (65). Previous studies have also demonstrated that peripheral administration of high doses of Ex4 caused a decrease in activity in rats (66), although central administration of GLP-1 in rodents had no such effect (5, 64). Finally, Tschop *et al.* (67) did not find an effect of PYY$^{3-36NH2}$ on taste aversion after either central or peripheral administration, nor was there any significant effect of peripheral PYY$^{3-36NH2}$ on locomotion in rodents. Thus, the adverse side effects of these particular peptides are both species- and dose-dependent and rely, at least in part, on the route of administration. However, based

on the results of the present study, it may be inferred that Ex4 (at 0.06 μg) and PYY$^{3-36NH2}$ (at 3 μg) most likely exerted specific effects on ingestive behavior, rather than inducing behavioral abnormalities.

One unexpected observation in these studies was the finding that the GLP-1R antagonist, Ex4$^{9-39}$ significantly reduced FI at the high dose (50 μg) (data not shown). These findings indicate that Ex4$^{9-39}$ may be a partial agonist of the GLP-1R, consistent with previous studies that examined cAMP responses to Ex4$^{9-39}$ *in vitro* and FI in rats after peripheral administration of Ex4$^{9-39}$ (3, 66). This may also explain the observation that Ex4$^{9-39}$ was unable to completely block the effects of Ex4 on FI, even when given at 83-fold greater amounts relative to Ex4 (5.0 *vs.* 0.06 μg, respectively).

Although Ex4 and PYY$^{3-36NH2}$ decreased 8-h FI, this did not translate into decreased body weight in chronic studies on mice eating a high-fat diet. Chronic peripheral administration of a long-acting analog of GLP-1 previously has been demonstrated to reduce both FI and body weight in nonobese, nondiabetic rats (57). However, the dose of peptide necessary to induce body weight loss was 200 μg/kg, whereas Ex4 was administered at approximately 3 μg/kg in the present study, a dose that was selected to minimize the possibility of side-effects such as nausea contributing to the reductions in FI. Furthermore, although chronic peripheral administration of PYY$^{3-36NH2}$ at a dose of 50 μg/kg decreases FI and body weight in normal rats (25), 2-fold higher amounts of PYY$^{3-36NH2}$ suppress FI but do not reduce body weight in chronic studies in wild-type mice (51). These latter findings are consistent with the results of the present study, in which approximately 170 μg/kg PYY$^{3-36NH2}$ was administered chronically to mice. Hence, in chronic feeding studies, both the dose of peptide being tested and the species being used may influence the ultimate effect on body weight. Finally, the injection schedule used in such studies (1000 and 1800 h in the present study) may contribute to any lack of effect on body weight, as animals are known to adaptively modulate their FI to maintain long-term body weight (56).

In conclusion, the present study has demonstrated that Ex4 and PYY$^{3-36NH2}$ (at the doses evaluated) act synergistically to reduce appetite without producing adverse behavioral effects. These peptides regulate FI through independent mechanisms, whereby Ex4 acts via a sensory afferent pathway involving the GLP-1R, but not the Y2-R, and PYY$^{3-36NH2}$ acts through an alternative pathway involving the Y2-R, but one that is independent of the GLP-1R. Although high doses of Ex4 are known to cause nausea and vomiting in humans, lower doses of Ex4 alone or in combination with PYY$^{3-36NH2}$ exerted only a small effect on gastric emptying, and neither peptide exhibited adverse behavioral actions. Therefore these findings suggest that the anorexigenic effects of low doses of Ex4 may be enhanced by coadministration of PYY$^{3-36NH2}$, without induction of major side effects. Collectively, this information will serve as basis for the development of a more effective treatment for obesity.

References

1. **Gale SM, Castracane VD, Mantzoros CS** 2004 Energy homeostasis, obesity and eating disorders: recent advances in endocrinology. J Nutr 134:295–298
2. **Crowley VE, Yeo GS, O'Rahilly S** 2002 Obesity therapy: altering the energy intake-and-expenditure balance sheet. Nat Rev Drug Discov 1:276–286
3. **Eng J, Kleinman WA, Singh L, Singh G, Raufman JP** 1992 Isolation and characterization of exendin-4, an exendin-3 analogue, from *Heloderma suspectum* venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. J Biol Chem 267:7402–7405
4. **Thorens B, Porret A, Bühler L, Deng S-P, Morel P, Widmann C** 1993 Cloning and functional expression of the human islet GLP-1 receptor: demonstration that exendin-4 is an agonist and exendin-(9–39) an antagonist of the receptor. Diabetes 42:1678–1682
5. **Tang-Christensen M, Larsen PJ, Göke R, Fink-Jensen A, Jessop DS, Moller M, Sheikh SP** 1996 Central administration of GLP-1-(7–36) amide inhibits food and water intake in rats. Am J Physiol Regul Integr Comp Physiol 271:R848–R856
6. **Van Dijk G, Thiele TE, Donahey JCK, Campfield LA, Smith FJ, Burn P, Bernstein IL, Woods SC, Seeley RJ** 1996 Central infusions of leptin and GLP-1-(7–36) amide differentially stimulate c-FLI in the rat brain. Am J Physiol Regul Integr Comp Physiol 271:R1096–R1100
7. **Flint A, Raben A, Astrup A, Holst JJ** 1998 Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. J Clin Invest 101:515–520
8. **Rodriquez de Fonseca F, Navarro M, Alvarez E, Roncero I, Chowen JA, Maestre O, Gomez R, Munoz RM, Eng J, Blazquez E** 2000 Peripheral versus central effects of glucagon-like peptide-1 receptor agonists on satiety and body weight loss in Zucker obese rats. Metabolism 49:709–717
9. **Edwards CM, Stanley SA, Davis R, Brynes AE, Frost GS, Seal LJ, Ghatei MA, Bloom SR** 2001 Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers. Am J Physiol Endocrinol Metab 281:E155–E161
10. **Drucker DJ** 2002 Biological actions and therapeutic potential of the glucagon-like peptides. Gastroenterology 122:531–544
11. **Meier JJ, Nauck MA** 2004 The potential role of glucagon-like peptide 1 in diabetes. Curr Opin Investig Drugs 5:402–410
12. **Zander M, Madsbad S, Madsen JL, Holst JJ** 2002 Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. Lancet 359:824–830
13. **Meier JJ, Gallwitz B, Schmidt WE, Nauck MA** 2002 Glucagon-like peptide 1 as a regulator of food intake and body weight: therapeutic perspectives. Eur J Pharmacol 440:269–279
14. **Bottcher G, Sjolund K, Ekblad E, Hakanson R, Schwartz TW, Sundler F** 1984 Coexistence of peptide YY and glicentin immunoreactivity in endocrine cells of the gut. Reg Pep 8:261–266
15. **Wettergren A, Maina P, Boesby S, Holst JJ** 1997 Glucagon-like peptide-1 7–36 amide and peptide YY have additive inhibitory effect on gastric acid secretion in man. Scand J Gastroenterol 32:552–555
16. **Eissele R, Göke R, Willemer S, Harthus HP, Vermeer H, Arnold R, Göke B** 1992 Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. Eur J Clin Invest 22:283–291
17. **Arantes RM, Nogueira AM** 1997 Distribution of enteroglucagon- and peptide YY-immunoreactive cells in the intestinal mucosa of germ-free and conventional mice. Cell Tissue Res 290:61–69
18. **Kreymann B, Ghatei MA, Williams G, Bloom SR** 1987 Glucagon-like peptide-1 7–36: a physiological incretin in man. Lancet 2:1300–1304
19. **Adrian TE, Ferri GL, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, Bloom SR** 1985 Human distribution and release of a putative new gut hormone, peptide YY. Gastroenterology 89:1070–1077
20. **Anini Y, Fu-Cheng XM, Cuber JC, Kervran A, Chariot J, Rozé C** 1999 Comparison of the postprandial release of peptide YY and proglucagon-derived peptides in the rat. Pflugers Arch 438:299–306
21. **Dumoulin V, Moro F, Barcelo A, Dakka T, Cuber JC** 1998 Peptide YY, glucagon-like peptide-1, and neurotensin responses to luminal factors in the isolated vascularly perfused rat ileum. Endocrinology 139:3780–3786
22. **Mentlein R, Gallwitz B, Schmidt WE** 1993 Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7–36)amide, peptide histidine methionine and is responsible for their degradation in human serum. Eur J Biochem 214:829–835
23. **Medeiros MD, Turner AJ** 1994 Processing and metabolism of peptide-YY: pivotal roles of dipeptidylpeptidase-IV, aminopeptidase-P, and endopeptidase-24.11. Endocrinology 134:2088–2094
24. **Nauck MA** 1998 Glucagon-like peptide 1 (GLP-1): a potent gut hormone with a possible therapeutic perspective. Acta Diabetol 35:117–129
25. **Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, Bloom SR** 2002 Gut hormone PYY(3–36) physiologically inhibits food intake. Nature 418:650–654
26. **Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, Ghatei MA, Bloom SR** 2003 Inhibition of food intake in obese subjects by peptide YY3–36. N Engl J Med 349:941–948
27. **Pittner RA, Moore CX, Bhavsar SP, Gedulin BR, Smith PA, Jodka CM, Parkes DG, Paterniti JR, Srivastava VP, Young AA** 2004 Effects of PYY[3–36] in rodent models of diabetes and obesity. Int J Obes Relat Metab Disord 28:963–971
28. **Adams SH, Won WB, Schonhoff SE, Leiter AB, Paterniti Jr JR** 2004 Effects of peptide YY[3–36] on short-term food intake in mice are not affected by prevailing plasma ghrelin levels. Endocrinology 145:4967–4975
29. **Chelikani PK, Haver AC, Reidelberger RD** 2005 Intravenous infusion of peptide YY(3–36) potently inhibits food intake in rats. Endocrinology 146:879–888
30. **Moran TH, Smedh U, Kinzig KP, Scott KA, Knipp S, Ladenheim EE** 2005 Peptide YY(3–36) inhibits gastric emptying and produces acute reductions in food intake in rhesus monkeys. Am J Physiol Regul Integr Comp Physiol 288:R384–R388
31. **Park A, Bloom SR** 2004 Peptides and obesity: the PYY3–36 story. Regul Pept 119:1–2
32. **Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CMB, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JPH, Smith DM, Ghatei MA, Herbert J, Bloom SR** 1996 A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 379:69–72
33. **Campos RV, Lee YC, Drucker DJ** 1994 Divergent tissue-specific and developmental expression of receptors for glucagon and glucagon-like peptide-1 in the mouse. Endocrinology 134:2156–2164
34. **Gustafson EL, Smith KE, Durkin MM, Walker MW, Gerald C, Weinshank R, Branchek TA** 1997 Distribution of the neuropeptide Y Y2 receptor mRNA in rat central nervous system. Brain Res Mol Brain Res 46:223–235
35. **Nakagawa A, Satake H, Nakabayashi H, Nishizawa M, Furuya K, Nakano S, Kigoshi T, Nakayama K, Uchida K** 2004 Receptor gene expression of glucagon-like peptide-1, but not glucose-dependent insulinotropic polypeptide, in rat nodose ganglion cells. Auton Neurosci 110:36–43
36. **Koda S, Date Y, Murakami N, Shimbara T, Hanada T, Toshinai K, Niiji A, Furuya M, Inomata N, Osuye K, Nakazato M** 2005 The role of the vagal nerve in peripheral PYY3–36-induced feeding reduction in rats. Endocrinology 146:2369–2375
37. **Kastin AJ, Akerstrom V, Pan W** 2002 Interactions of glucagon-like peptide-1 (GLP-1) with the blood-brain barrier. J Mol Neurosci 18:7–14
38. **Kastin AJ, Akerstrom V** 2003 Entry of exendin-4 into brain is rapid but may be limited at high doses. Int J Obes Relat Metab Disord 27:313–318
39. **Nonaka N, Shioda S, Niehoff ML, Banks WA** 2003 Characterization of blood-brain barrier permeability to PYY3–36 in the mouse. J Pharmacol Exp Ther 306:948–953
40. **Barrachina MD, Martinez V, Wang L, Wei JY, Tache Y** 1997 Synergistic interaction between leptin and cholecystokinin to reduce short-term food intake in lean mice. Proc Natl Acad Sci USA 94:10455–10460
41. **Bhavsar S, Watkins J, Young A** 1998 Synergy between amylin and cholecystokinin for inhibition of food intake in mice. Physiol Behav 64:557–561
42. **Doods H, Gaida W, Wieland HA, Dollinger H, Schnorrenberg G, Esser F, Engel W, Eberlein W, Rudolf K** 1999 BIIE0246: a selective and high affinity neuropeptide Y Y(2) receptor antagonist. Eur J Pharmacol 384:R3–R5
43. **Lachey JL, D'Alessio DA, Rinaman L, Elmquist JK, Drucker DJ, Seeley RJ** 2005 The role of central glucagon-like peptide-1 in mediating the effects of visceral illness: differential effects in rats and mice. Endocrinology 146:458–462
44. **Sasamura T, Kuraishi Y** 1999 Peripheral and central actions of capsaicin and VR1 receptor. Jpn J Pharmacol 80:275–280
45. **Baggio LL, Huang Q, Brown TJ, Drucker DJ** 2004 Oxyntomodulin and glucagon-like peptide-1 differentially regulate murine food intake and energy expenditure. Gastroenterology 127:546–558
46. **Dakin CL, Small CJ, Batterham RL, Neary NM, Cohen MA, Patterson M, Ghatei MA, Bloom SR** 2004 Peripheral oxyntomodulin reduces food intake and body weight gain in rats. Endocrinology 145:2687–2695
47. **Baggio LL, Huang Q, Brown TJ, Drucker DJ** 2004 A recombinant human glucagon-like peptide (GLP)-1-albumin protein (albugon) mimics peptidergic activation of GLP-1 receptor-dependent pathways coupled with satiety, gastrointestinal motility, and glucose homeostasis. Diabetes 53:2492–2500

48. **Matson CA, Reid DF, Cannon TA, Ritter RC** 2000 Cholecystokinin and leptin act synergistically to reduce body weight. Am J Physiol Regul Integr Comp Physiol 278:R882–R890

49. **Gamse R** 1982 Capsaicin and nociception in the rat and mouse. Possible role of substance P. Naunyn Schmiedebergs Arch Pharmacol 320:205–216

50. **Ward SM, Bayguinov J, Won KJ, Grundy D, Berthoud HR** 2003 Distribution of the vanilloid receptor (VR1) in the gastrointestinal tract. J Comp Neurol 465:121–135

51. **Challis BG, Coll AP, Yeo GS, Pinnock SB, Dickson SL, Thresher RR, Dixon J, Zahn D, Rochford JJ, White A, Oliver RL, Millington G, Aparicio SA, Colledge WH, Russ AP, Carlton MB, O'Rahilly S** 2004 Mice lacking pro-opiomelanocortin are sensitive to high-fat feeding but respond normally to the acute anorectic effects of peptide-YY(3–36). Proc Natl Acad Sci USA 101:4695–4700

52. **Szallasi A, Nilsson S, Farkas-Szallasi T, Blumberg PM, Hokfelt T, Lundberg JM** 1995 Vanilloid (capsaicin) receptors in the rat: distribution in the brain, regional differences in the spinal cord, axonal transport to the periphery, and depletion by systemic vanilloid treatment. Brain Res 703:175–183

53. **Nakabayashi H, Nishizawa M, Nakagawa A, Takeda R, Nijima A** 1996 Vagal hepatopancreatic reflex effect evoked by intraportal appearance of tGLP-1. Am J Physiol Endocrinol Metab 271:E808–E813

54. **Ahren B** 2004 Sensory nerves contribute to insulin secretion by glucagon-like peptide-1 in mice. Am J Physiol Regul Integr Comp Physiol 286:R269–R272

55. **Halatchev IG, Ellacott KL, Fan W, Cone RD** 2004 Peptide YY3–36 inhibits food intake in mice through a melanocortin-4 receptor-independent mechanism. Endocrinology 145:2585–2590

56. **Woods SC, Seeley RJ, Porte Jr D, Schwartz MW** 1998 Signals that regulate food intake and energy homeostasis. Science 280:1378–1383

57. **Larsen PJ, Fledelius C, Knudsen LB, Tang-Christensen M** 2001 Systemic administration of the long-acting GLP-1 derivative NN2211 induces lasting and reversible weight loss in both normal and obese rats. Diabetes 50:2530–2539

58. **Peruzzo B, Pastor FE, Blazquez JL, Schobitz K, Pelaez B, Amat P, Rodriguez EM** 2000 A second look at the barriers of the medial basal hypothalamus. Exp Brain Res 132:10–26

59. **Chelikani PK, Haver AC, Reidelberger RD** 2004 Comparison of the inhibitory effects of PYY(3–36) and PYY(1–36) on gastric emptying in rats. Am J Physiol Regul Integr Comp Physiol 287:R1064–R1070

60. **Savage AP, Adrian TE, Carolan G, Chatterjee VK, Bloom SR** 1987 Effects of peptide YY (PYY) on mouth to caecum intestinal transit time and on the rate of gastric emptying in healthy volunteers. Gut 28:166–170

61. **Hellstrom PM, Naslund E** 2001 Interactions between gastric emptying and satiety, with special reference to glucagon-like peptide-1. Physiol Behav 74:735–741

62. **Kinzig KP, D'Alessio DA, Seeley RJ** 2002 The diverse roles of specific GLP-1 receptors in the control of food intake and the response to visceral illness. J Neurosci 22:10470–10476

63. **Wang T, Edwards GL, Baile CA** 1998 Glucagon-like peptide-1 (7–36) amide administered into the third cerebroventricle inhibits water intake in rats. Proc Soc Exp Biol Med 219:85–91

64. **McMahon LR, Wellman PJ** 1998 PVN infusion of GLP-1-(7–36) amide suppresses feeding but does not induce aversion or alter locomotion in rats. Am J Physiol Regul Integr Comp Physiol 274:R23–R29

65. **Kolterman OG, Buse JB, Fineman MS, Gaines E, Heintz S, Bicsak TA, Taylor K, Kim D, Aisporna M, Wang Y, Baron AD** 2003 Synthetic exendin-4 (exenatide) significantly reduces postprandial and fasting plasma glucose in subjects with type 2 diabetes. J Clin Endocrinol Metab 88:3082–3089

66. **Peters CT, Choi Y-H, Brubaker PL, Anderson GH** 2001 GLP-1 receptor agonist and antagonist alter macronutrient selection in the rat. J Nutr 131:2164–2170

67. **Tschop M, Castaneda TR, Joost HG, Thone-Reineke C, Ortmann S, Klaus S, Hagan MM, Chandler PC, Oswald KD, Benoit SC, Seeley RJ, Kinzig KP, Moran TH, Becksickinger AG, Koglin N, Rodgers RJ, Blundell JE, Ishii Y, Beattie AH, Holch P, Allison DB, Raun K, Madsen K, Wulff BS, Stidsen CE, Birringer M, Kreuzer OJ, Schindler M, Arndt K, Rudolf K, Mark M, Deng XY, Whitcomb DC, Halem H, Taylor J, Dong J, Datta R, Culler M, Craney S, Flora D, Smiley D, Heiman ML** 2004 Physiology: does gut hormone PYY3–36 decrease food intake in rodents? Nature 430:1