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Involvement of Apolipoprotein A-IV and Cholecystokinin$_1$ Receptors in Exogenous Peptide YY$_{3–36}$-Induced Stimulation of Intestinal Feedback

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Peptide YY (PYY)$_{3–36}$, released by intestinal lipid elicits functional effects that comprise the intestinal feedback response to luminal nutrients, but the pathway of action is not fully characterized. The aim of the present study was to determine the role of the apolipoprotein (apo) A-IV-cholecystokinin (CCK)$_1$ receptor (CCK$_1$R) pathway in exogenous PYY$_{3–36}$-induced activation of the gut-brain axis and inhibition of gastric emptying and food intake. PYY$_{3–36}$ (5 μg/100 g ip) significantly inhibited gastric emptying of a chow meal in wild-type but not A-IV$^{-/-}$ mice andCCK$_1$R receptor blockade with devazepide (10 μg/100 g), abolished PYY$_{3–36}$-induced inhibition of gastric emptying. PYY$_{3–36}$-induced inhibition of food intake in both *ad libitum*-fed and 16-h fasted mice was unaltered in A-IV$^{-/-}$ mice, compared with wild-type controls, or by CCK$_1$R receptor blockade with devazepide. PYY$_{3–36}$ activated neurons in the midregion of the nucleus of the solitary tract (bregma −7.32 to −7.76 mm) in A-IV$^{+/+}$ mice; this was measured by immunohistochemical localization of Fos protein. PYY$_{3–36}$-induced Fos expression was significantly reduced by 65% in A-IV$^{+/+}$ mice pretreated systemically with the sensory neurotoxin capsaicin (5 mg/100 g), 78% by the CCK$_1$R antagonist, devazepide (10 μg/100 g), and 39% by the Y2R antagonist, BIIE0246 (200 and 600 μg/100 g) and decreased by 67% in apo A-IV$^{-/-}$ mice, compared with A-IV$^{+/+}$ controls. The data suggest a role for apo A-IV and the CCK$_1$R in PYY$_{3–36}$-induced activation of the vagal afferent pathway and inhibition of gastric emptying, but this is likely not the pathway mediating the effects of PYY$_{3–36}$ on food intake. (*Endocrinology* 148: 4695–4703, 2007)

PEPTIDE YY (PYY) is a 36-amino-acid peptide secreted by the L cells of the distal gut after nutrient intake (1). Postprandial release of PYY is proportional to caloric consumption and is directly influenced by meal composition, primarily by dietary fat as well as dietary protein and carbohydrate (2). Regulation of PYY release occurs through both direct and indirect mechanisms. The presence of lipid in the distal gut directly stimulates release of PYY. However, intraduodenal infusion of lipid increases plasma PYY, even before nutrients reach the distal gut, suggesting a potential neural or hormonal regulation of PYY release. Lipid-stimulated PYY release was inhibited by the cholecystokinin (CCK)$_1$ receptor (CCK$_1$R) antagonist, devazepide, demonstrating that CCK may serve as a foregut signal connecting fat in the proximal gut with the release of PYY in the distal gut (3, 4).

The predominant form of PYY in plasma is PYY$_{3–36}$ (5), which is relatively selective for the Y2 receptor and binds with less affinity to the Y1 and Y5 receptors (6). The Y2 receptor is localized within both the peripheral and central nervous systems. Peripherally, the Y2 receptor is localized to the nodose ganglion, which contains the cell bodies of vagal afferent neurons (7). Centrally, the receptor is localized to the arcuate nucleus of the hypothalamus and the nucleus of the solitary tract (NTS) (8). The Y2 receptor is also localized to the gastrointestinal tract throughout the small intestine and colon (9).

PYY$_{3–36}$ has a number of different effects after exogenous administration in rodents and humans including inhibition of food intake (10), inhibition of gastric emptying (11), gastric acid secretion (12), and intestinal motility (13). The Y2 receptor mediates the anorexigenic effects of PYY$_{3–36}$ (10, 14), whereas the Y1 and Y5 receptors act to stimulate feeding (15, 16). Peripheral PYY$_{3–36}$ administration has been shown to activate neurons in the NTS (17) and the arcuate nucleus of the hypothalamus (7). There is evidence that peripheral PYY$_{3–36}$ can cross the blood-brain barrier (18) and therefore could directly activate neurons in these regions. However, there is also evidence that activation of neurons in the central nervous system occurs via activation of the vagal afferent pathway (7, 19); thus, PYY$_{3–36}$ may alter activity of neurons in the central nervous system via activation of the vagal afferent pathway to inhibit food intake (7, 19), but the mechanism by which PYY$_{3–36}$ inhibits gastric emptying is unknown.

Long-chain triglyceride is the principal macronutrient that stimulates release of both CCK and PYY from intestinal endocrine cells. Lipid-induced activation of the vagal afferent pathway and intestinal feedback is partially mediated by CCK$_1$R on vagal afferent terminals (20, 21). Recently we demonstrated that lipid-induced activation of the vagal afferent pathway and stimulation of intestinal feedback of gastric function is mediated, at least in part, via apolipoprotein (apo) A-IV (22, 23). Apo A-IV is a protein secreted by the enterocyte of the proximal gut (24, 25) in response to active

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Abbreviations: AP, Area postrema; apo, apolipoprotein; CCK, cholecystokinin; CCKR, CCK receptor; NTS, nucleus of the solitary tract; PYY, peptide YY.

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lipid absorption and in association with chylomicrons (26, 27). Apo A-IV acts to inhibit gastric motor function via activation of CCK-responsive vagal afferent fibers and by a mechanism dependent on CCK_1Rs (22). In addition, lipid-induced activation of the vagal afferent pathway and lipid-induced inhibition of gastric function are markedly attenuated in apo A-IV null mice (23). Collectively, these data suggest that active lipid absorption results in apo A-IV release from enterocytes in the lamina propria and release of CCK from enteroendocrine cells in the intestinal epithelium followed by activation of vagal afferents via the CCK_1R. Interestingly, synthesis and secretion of apo A-IV in the jejunum is also stimulated by exogenous PYY via a pathway involving the vagus nerve (28, 29). Thus, in addition to lipid-induced release of PYY involving CCK_1Rs, it is possible that PYY-induced release of apo A-IV and subsequent activation of CCK_1Rs may be involved in mediating the actions of $PYY_{3–36}$ on intestinal feedback and food intake.

Therefore, the present study was undertaken to determine the role of apo A-IV and CCK_1Rs in $PYY_{3–36}$-induced activation of the vagal afferent pathway and in inhibition of gastric emptying and food intake. The specific aims were to determine: 1) the role of apo A-IV, CCK_1Rs, and capsaicin-sensitive afferents in $PYY_{3–36}$-induced activation of the vagal afferent pathway and 2) the role of the apo A-IV/CCK_1R pathway in $PYY_{3–36}$-induced inhibition of gastric emptying and food intake. To establish a role for apo A-IV in the gastrointestinal response to $PYY_{3–36}$, we used apo A-IV null mice and their wild-type counterparts.

Materials and Methods

Animals

Experiments were performed using male C57BL/6J mice (JAX West, University of California, Davis) and male apo A-IV knockout mice (hereafter referred to as apo A-IV$^{-/-}$ mice) (30). These mice were generated Dr. Jan Breslow (Rockefeller University, New York, NY) using homologous recombination in embryonic stem cells. Apo A-IV$^{-/-}$ mice are 98% congenic with C57BL/6J mice (hereafter referred to as wild-type or apo A-IV$^{+/+}$). Mice were of initial weight 18–20 g (6–10 wk of age) and were maintained on regular laboratory chow (Purina Laboratory, St. Louis, MO). Mice were fasted overnight but allowed water *ad libitum* before all experimental procedures. The institutional guidelines for care and use of laboratory animals were followed throughout the study.

Immunohistochemistry: c-fos protein expression in the NTS

This method has been described in detail previously (31). Briefly, 2 h after treatment (detailed below in *Effect of $PYY_{3–36}$ on Fos expression in NTS*), mice were anesthetized with sodium pentobarbital (50 mg/ml, 100 mg/kg ip, Western Medical Supply, Arcadia, CA) and transcardially perfused with 20 ml heparinized 0.9% saline (0.1 ml heparin/100 ml saline) followed by 25 ml of 4% paraformaldehyde (Sigma, St. Louis, MO). The brainstem was removed and postfixed in 4% paraformaldehyde for 1 h. Sections were cut at 100 μm using a vibratome. Sections were incubated for 1 h in goat serum-PBS (Chemicon, Temecula, CA), incubated in primary antibody (1:2000 rabbit anti-fos; Santa Cruz Biotechnology, Santa Cruz, CA) for 3 h, followed by incubation with the secondary antibody (1:200 biotinylated goat antirabbit; Vector Laboratories, Burlingame, CA) for 2 h. Tissue was incubated for 3 h in avidin biotin complex solution (standard Elite Vectastain avidin biotin complex kit, Vector Labs). Diaminobenzidine solution (Sigma) was added for a 5-min incubation and then 50 μl H_2O_2-PBS (0.1 ml 30% H_2O_2 and 10 ml PBS) were added to catalyze the diaminobenzidine reaction; the reaction was stopped with a PBS wash. Tissue was thoroughly washed between each incubation period.

Images were taken on a Provis (Olympus, Center Valley, PA) microscope and analyzed using Corel Paint Shop Pro, edition 7 (Corel, Eden Prairie, MN). The researcher was blinded to all treatments before image analysis. A stereotaxic mouse brain atlas was used to determine the location of the NTS in each section of tissue (32). A region of interest was drawn around the NTS and the area postrema (AP), and all activated neurons in the NTS region of interest were counted in both regions. Neurons were determined to be immunopositive (above threshold) by their color and size. Representative sections were chosen to represent regions of the NTS: caudal (bregma −8.00 to −7.92 mm, mid (−7.76 to −7.32 mm), and rostal (−7.08 to −6.48 mm). Three sections were chosen for each region for a total of nine sections per mouse. The numbers of labeled neurons per section were summed for each region for each mouse; this value was used in subsequent statistical analyses.

Gastric emptying of chow

After an overnight fast, apo A-IV$^{+/+}$ and apo A-IV$^{-/-}$ mice (n = 8 in each treatment group) were allowed to feed freely on regular laboratory chow. The chow was removed after 60 min and the mice received 0.1 ml of 0.9% saline, $PYY_{3–36}$ (5 μg/100 g ip; Bachem, Torrance, CA), or the Y_2 receptor antagonist, BIIE0246 (200 μg/100 g; Tocris Bioscience, Ellisville, MO) (33, 34). A group of mice were pretreated with the CCK_1R antagonist, devazepide (15 min pretreatment; 10 μg/100 g ip) (35) or vehicle followed by either 0.1 ml of 0.9% saline or $PYY_{3–36}$ ip (5 μg/100 g). Two hours after treatment, the mice were anesthetized with sodium pentobarbital (50 mg/ml, 10 mg/100 g ip; Western Medical Supply, Arcadia, CA) and their stomachs were isolated and removed. The weight of the full stomach and the weight of the emptied stomach were recorded. Stomach volume was calculated by subtracting the weight of the empty stomach from the weight of the full stomach.

Food intake

Age-matched (8 wk old) apo A-IV$^{+/+}$ and apo A-IV$^{-/-}$ mice were individually housed in wire-bottom cages for 1 wk before data collection. To acclimate the mice to ip injections, the mice were weighed and handled daily and their abdomens were massaged for 5 sec to stimulate the future injection site. Mice were either offered *ad libitum* food for 8 h a day beginning at the start of the dark cycle or were fed *ad libitum*. For fasted mice, food was removed at the end of the feeding period and the mice were fasted for 16 h. On treatment days, mice received ip treatment injections immediately before food was offered. Mice in the basal group received no treatment. After treatment, mice were then allowed to freely feed on regular laboratory chow in petri dishes. Because the effect of $PYY_{3–36}$ is rapid and short lived, total food intake was recorded at 1, 2, and 4 h after treatment (7, 17). Food intake was recorded by weighing the petri dishes and comparing the weight with the weight at $time_0$. The weight of the mice was recorded daily to adjust the food intake data for body weight.

Experimental protocols

Effect of $PYY_{3–36}$ on Fos expression in NTS. Apo A-IV$^{+/+}$ and apo A-IV$^{-/-}$ mice (n = 4–5/treatment group) were used for these experiments. Fasted mice were administered 0.1 ml of 0.9% saline or $PYY_{3–36}$ ip (1.67, 5, or 16.7 μg/100 g; Bachem, Torrance, CA) (17). An additional group of mice were treated with the selective nonpeptide Y_2 receptor antagonist, BIIE0246 (15 min pretreatment; 200 μg/100 g or 600 μg/100 g mouse; Tocris Bioscience) (33, 34) or vehicle (0.9% saline), followed by either 0.1 ml of 0.9% saline or $PYY_{3–36}$ ip (5 μg/100 g).

A further group of mice were pretreated with the chemical neurotoxin capsaicin which selectively ablates C-type unmyelinated sensory nerve fibers (35). Mice were anesthetized with halothane and administered a sc injection of capsaicin (5 mg/100 g; Sigma) or vehicle, followed by an ip injection of sodium pentobarbital (6.5 mg/100 g) to maintain anesthesia for 1 h. A second injection of capsaicin or vehicle was administered 2 d later following the same protocol. Capsaicin efficacy was determined using a corneal chemosensory reflex test, which consists of monitoring the wiping reflex after ocular administration of 0.1% NH_4OH solution (34). One week after the second capsaicin injection, mice were fasted overnight and treated with either 0.1 ml of saline or $PYY_{3–36}$ (5 μg/100 g ip).

An additional group of mice were pretreated with the CCK_1R antagonist, devazepide (15 min pretreatment; 10 μg/100 g ip) (36) or its vehicle followed by either 0.1 ml of 0.9% saline or $PYY_{3–36}$ ip (5 μg/100 g). CCK_1R antagonist, devazepide (kindly donated by Merck, Sharpe, and Dohme, Whitehouse Station, NJ) was dissolved in 0.1 ml dimethylsulfoxide followed by 0.1 ml Tween 80, and 0.8 ml physiological saline to a stock concentration of 200 μg/ml.

Effect of $PYY_{3–36}$ on food intake. In both *ad libitum*-fed mice and 18-h fasted mice, apo A-$IV^{+/+}$ and apo A-$IV^{-/-}$ mice (n = 10 mice/treatment group) received 0.1 ml of either 0.9% saline or $PYY_{3–36}$ (5 μg/100 g ip) (17). Additional groups of apo A-$IV^{+/+}$ mice were pretreated with the CCK_1R antagonist, devazepide (15 min before treatment injection; 10 μg/100 g ip) (36) or its vehicle followed by either 0.1 ml of saline or $PYY_{3–36}$ ip (5 μg/100 g). Because the effects of devazepide last approximately 2 h, total food intake was recorded only at 1 and 2 h after $PYY_{3–36}$ treatment.

Statistical analysis

Fos protein expression in the NTS and food intake. Significant differences between treatment groups were calculated using a one-way ANOVA followed by Bonferroni's multiple comparison test. $P < 0.05$ was taken as significantly different. All reported results are the number of Fos-positive neurons or grams of food consumed per gram of body weight ± SEM.

Gastric emptying of chow. Significant differences between treatment groups were calculated using a nonpaired *t* test. $P < 0.05$ was taken as significantly different. All reported results are the grams of food consumed ± SEM.

Results

$PYY_{3–36}$-induced activation of neurons in the NTS

The number of Fos-positive NTS neurons was analyzed with respect to region within the NTS (caudal, mid, and rostral). We tested the effect of increasing doses of $PYY_{3–36}$ (1.67 μg/100 g, 5 μg/100 g, and 16.65 μg/100 g ip) in C57B6 (apo A-$IV^{+/+}$) mice, on activation of the vagal afferent pathway by determining the number of Fos-expressing neurons in the NTS. There was no significant difference in expression of Fos protein in neurons in the mid-NTS between $PYY_{3–36}$ (1.67 μg/100 g ip) and saline (0.1 ml ip) (NS, n = 5 and 4 mice, respectively, Fig. 1). However, treatment with two higher doses of $PYY_{3–36}$ (5 μg/100 g and 16.65 μg/100 g ip) significantly increased the number of Fos-positive neurons in the mid-NTS compared with saline ($P < 0.001$, n = 5 and 4 mice, respectively, Fig. 1). There were no significant differences in Fos protein expression for any dose of $PYY_{3–36}$ in the caudal or rostral NTS in both apo A-$IV^{+/+}$ and apo A-$IV^{-/-}$ mice (Table 1). Similarly, there were no significant differences in Fos protein expression for all treatments within the AP (Table 1). Because administration of $PYY_{3–36}$ above the 5 μg/100 g threshold significantly increased Fos expression, we used 5 μg/100 g in all subsequent experiments (Fig. 1).

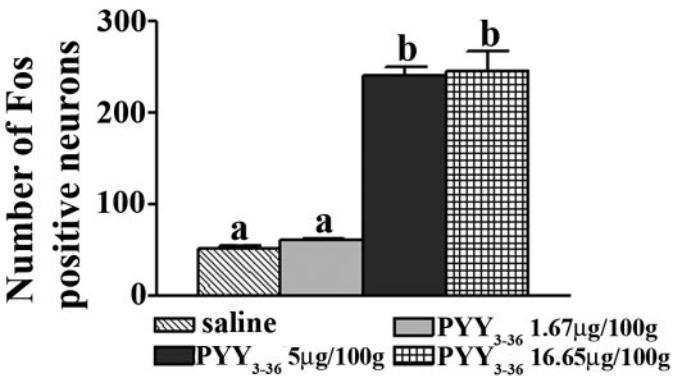

FIG. 1. $PYY_{3–36}$-induced activation of neurons in the NTS in response to increasing doses of peripherally administered $PYY_{3–36}$. Activation of Fos expression measured by immunocytochemistry in mid-NTS (bregma −7.76 to −7.32 mm) is significantly increased in response to the two highest doses of $PYY_{3–36}$ (n = 5, $P < 0.001$, 5 μg/100 g and 16.65 μg/100 g ip) but not the lowest dose of $PYY_{3–36}$ (NS, n = 5, 1.67 μg/100 g) or saline (NS, n = 4). *Letters* signify significant statistical differences between treatment groups.

Pretreatment with the CCK_1R antagonist, devazepide (15 min pretreatment; 10 μg/100 g ip) significantly reduced by 78% the Fos response to $PYY_{3–36}$ (5 μg/100 g ip) in the mid-NTS ($P < 0.001$, n = 5 wild-type mice in each group, Fig. 2A).

Fos expression in the mid-NTS in response to $PYY_{3–36}$ (5 μg/100 g ip) was significantly reduced by 65% in wild-type mice pretreated with the sensory neurotoxin capsaicin (10 d pretreatment; 5 mg/100 g ip), compared vehicle pretreatment ($P < 0.001$, n = 4 mice in each group, Fig. 2B).

As shown above, in wild-type (apo-$IV^{+/+}$) mice, treatment with $PYY_{3–36}$ (5 μg/100 g ip) significantly increased the number of Fos-positive neurons in the mid-NTS, compared with treatment with saline ($P < 0.001$, n = 5, Fig. 2A). Fos expression in the NTS in response to $PYY_{3–36}$ was significantly decreased by 67% in apo A-$IV^{-/-}$ mice, compared with apo A-$IV^{+/+}$ mice ($P < 0.001$, n = 5 mice, Fig. 2C).

In wild-type mice, administration of the Y2 receptor antagonist, BIIE0246 (15 min pretreatment; 200 μg/100 g or 600 μg/100 g ip) significantly inhibited the number of $PYY_{3–36}$-induced Fos-positive neurons by 39 and 40%, respectively, in the NTS, compared with saline treatment ($P < 0.001$, 175 ± 6 neurons, 163 ± 4 neurons, respectively, *vs.* 52 ± 3, n = 4–5 mice).

TABLE 1. Numbers of fos-positive neurons in the NTS, caudal NTS (bregma −8.00 to −7.92), rostral NTS (bregma −7.08 to −6.48), and AP (bregma −7.76 to −7.32)

	Caudal	Rostral	AP
Apo A-$IV^{+/+}$			
Saline	51 ± 8	55 ± 2	20 ± 3
$PYY_{3–36}$, 1.67 μg/100 g	51 ± 4	53 ± 1	16 ± 1
$PYY_{3–36}$, 5 μg/100 g	71 ± 5	60 ± 2	17 ± 2
$PYY_{3–36}$, 16.7 μg/100 g	56 ± 3	55 ± 3	14 ± 1
Capsaicin-V + saline	53 ± 3	67 ± 4	20 ± 3
Capsaicin-V + $PYY_{3–36}$	59 ± 5	69 ± 1	19 ± 3
Capsaicin + saline	63 ± 7	69 ± 5	18 ± 2
Capsaicin + $PYY_{3–36}$	68 ± 8	68 ± 1	17 ± 3
Devazepide + saline	54 ± 2	58 ± 4	18 ± 3
Devazepide + $PYY_{3–36}$	60 ± 5	59 ± 3	23 ± 2
Devazepide-V + $PYY_{3–36}$	64 ± 6	58 ± 1	19 ± 3
BIIE0246 + saline	57 ± 5	68 ± 4	19 ± 3
BIIE0246 + $PYY_{3–36}$	62 ± 6	63 ± 3	18 ± 3
Apo A-$IV^{-/-}$			
Saline	44 ± 3	48 ± 1	22 ± 2
$PYY_{3–36}$	66 ± 1	64 ± 2	24 ± 2

There was no significant difference in the number of fos-positive neurons in the NTS of apo A-IV wild-type and knockout mice treated with saline or $PYY_{3–36}$ for all three brain regions (caudal NTS, rostral NTS, and AP). In addition, there was no significant difference in the number of fos-positive neurons between apo A-IV wild-type and knockout mice for all treatments within all three brain regions.

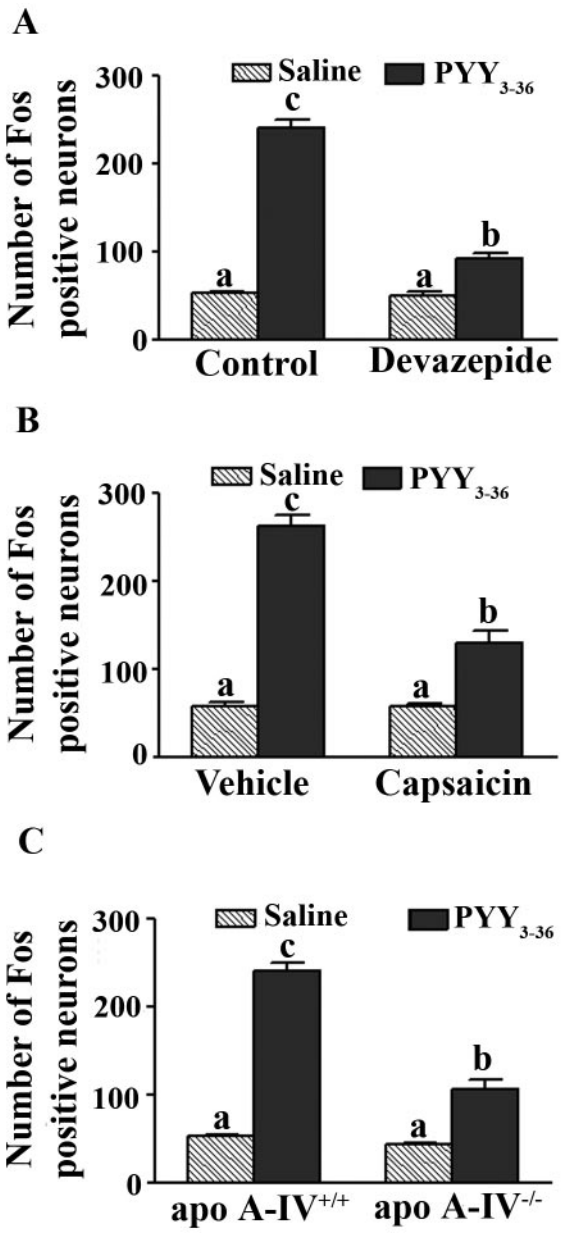

FIG. 2. $PYY_{3–36}$-induced activation of neurons in the NTS. Activation of Fos expression in the NTS in apo A-IV wild-type mice treated with IP saline (n = 4), $PYY_{3–36}$ (n = 5), devazepide + saline (n = 4), and devazepide + $PYY_{3–36}$ (n = 5) (A) or capsaicin vehicle + saline (n = 5), capsaicin vehicle + $PYY_{3–36}$ (n = 4), capsaicin + saline (n = 4), and capsaicin + $PYY_{3–36}$ (n = 4) (B). C, Mean data of $PYY_{3–36}$-induced activation of neurons in the NTS showing attenuation in apo A-IV knockout mice. Activation of Fos expression in the NTS in apo A-IV wild-type (data from Fig. 3 included for purposes of clarity) and knockout mice with ip saline (n = 4) and $PYY_{3–36}$ (n = 5). *Letters* signify significant statistical differences between treatment groups.

Effect of $PYY_{3–36}$ treatment on gastric emptying of chow

There was no significant difference in the amount of food in the stomach of the apo A-$IV^{+/+}$ and apo A-$IV^{-/-}$ mice at the end of the 60-min feeding period (NS, 0.4 ± 0.1 *vs.* 0.6 ± 0.1 g, n = 5 mice). Similarly, there was no significant difference in gastric emptying of a chow meal between apo A-$IV^{+/+}$ and apo A-$IV^{-/-}$ mice in response to saline (0.1 ml ip, NS, n = 8, Fig. 3A).

Administration of $PYY_{3–36}$ (5 μg/100 g ip) significantly inhibited gastric emptying in apo A-$IV^{+/+}$ mice, ($P < 0.001$, n = 8, Fig. 3A) but not in apo A-$IV^{-/-}$ mice (NS, n = 8, Fig. 3A). In apo A-$IV^{+/+}$ mice, inhibition of gastric emptying of chow in response to $PYY_{3–36}$ was significantly attenuated in mice pretreated with the CCK_1R antagonist, devazepide (15 min pretreatment; 10 μg/100 g ip) ($P < 0.0001$, n = 8 mice, Fig. 3B). Gastric emptying of chow in response to $PYY_{3–36}$ in apo A-$IV^{+/+}$ mice in the presence of devazepide was not significantly different from apo A-$IV^{+/+}$ mice treated with saline or apo A-$IV^{-/-}$ mice treated with saline or $PYY_{3–36}$ (NS, n = 8 mice, Fig. 3).

In wild-type mice, administration of the Y2 receptor antagonist alone, BIIE0246 (200 μg/100 g) significantly accelerated the rate of gastric emptying of chow, compared with saline (grams of chow remaining in stomach, control *vs.* BIIE0246: 0.30 ± 0.03 *vs.* 0.15 ± 0.015 g, respectively; $P < 0.01$, n = 8 mice).

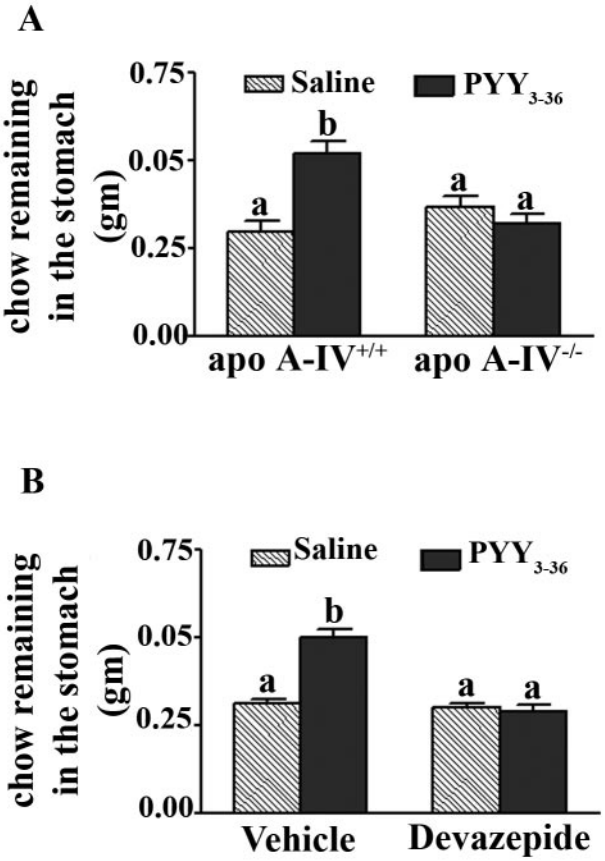

FIG. 3. Gastric emptying of chow was significantly inhibited in apo A-$IV^{+/+}$ mice treated with $PYY_{3–36}$, compared with apo A-$IV^{+/+}$ mice pretreated with the CCK_1R antagonist, devazepide (A; 15 min pretreatment; 100 μg/kg ip) with $PYY_{3–36}$ (5 μg/100 g ip) ($P < 0.0001$, n = 8 mice). Gastric emptying of chow is significantly slowed in response to $PYY_{3–36}$ in apo A-IV wild-type but not apo A-IV knockout mice after treatment with saline or $PYY_{3–36}$ (B; 5 μg/100 g, n = 8 mice in each group; $P < 0.001$, apo A-IV wild-type saline *vs.* $PYY_{3–36}$; $P < 0.001$ apo A-IV wild-type *vs.* knockout). Gastric emptying of chow is expressed as grams of food remaining in the stomach. *Letters* signify significant statistical differences between treatment groups.

Effect of $PYY_{3–36}$ treatment on food intake of chow in fasted mice

Both apo A-IV$^{+/+}$ and apo A-IV$^{-/-}$ mice gained weight during the test period (apo A-IV$^{+/+}$: 19.6 ± 0.3 *vs.* 20.6 ± 0.3 g, $P < 0.02$, n = 22; apo A-IV$^{-/-}$: 21.0 ± 0.2 *vs.* 22.6 ± 0.2 g, $P < 0.0001$, n = 10). Despite being age matched, the apo A-IV$^{-/-}$ mice weighed significantly more than apo A-IV$^{+/+}$ mice at the beginning of the test period (21.0 ± 0.2 *vs.* 19.6 ± 0.3 g, $P < 0.01$). Because of the difference in body weight, food intake data were corrected for body weight and expressed as grams of food consumed per gram of body weight.

There was no significant difference in food intake under basal conditions or in response to saline treatment (0.1 ml ip) between apo A-IV$^{+/+}$ and apo A-IV$^{-/-}$ mice at all time points (1, 2, and 4 h) (NS, n = 10 mice; Fig. 4). Administration of $PYY_{3–36}$ (5 μg/100 g ip) significantly inhibited food intake at 1, 2, and 4 h in both apo A-IV$^{+/+}$ and apo A-IV$^{-/-}$ mice, compared with basal (1 h: $P < 0.01$, $P < 0.001$, respectively; 2 h: $P < 0.01$, $P < 0.001$, respectively; 4 h: $P < 0.001$, $P < 0.01$, respectively; n = 10 mice, Fig. 4) or saline treatment (1 h: $P < 0.01$, $P < 0.05$, respectively; 2 h: $P < 0.05$; 4 h: $P < 0.01$, $P < 0.05$, respectively; n = 10 mice, Fig. 4). There was no significant difference in inhibition of food intake by $PYY_{3–36}$ between apo A-IV$^{+/+}$ and apo A-IV$^{-/-}$ mice at any time point (NS, n = 10 mice, Fig. 4).

In wild-type mice, $PYY_{3–36}$-induced inhibition of food intake was not significantly altered by pretreatment with the CCK_1R antagonist, devazepide (15 min pretreatment; 10 μg/100 g ip) or vehicle (NS, n = 6, Fig. 4A).

Effect of $PYY_{3–36}$ treatment on food intake of chow in ad libitum-fed mice

There was no significant difference in food intake under basal conditions or in response to saline treatment (0.1 ml ip) between apo A-IV$^{+/+}$ and apo A-IV$^{-/-}$ mice at all time points (1, 2, and 4 h) (NS, n = 10 mice, Fig. 5). Administration

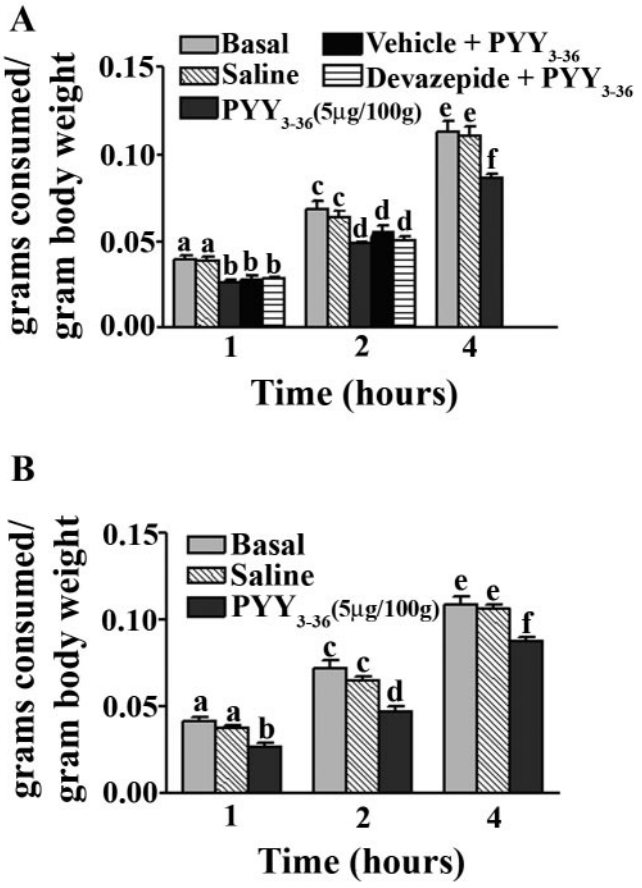

FIG. 4. Inhibition of food intake in response to $PYY_{3–36}$ in 16-h fasted apo A-IV wild-type (A) and apo A-IV knockout mice (B). $PYY_{3–36}$ (5 μg/100 g ip) significantly inhibited food intake at all time points in both apo A-IV$^{+/+}$ and apo A-IV$^{-/-}$ mice, compared with basal conditions (1 h: $P < 0.01$, $P < 0.001$, respectively; 2 h: $P < 0.01$, $P < 0.001$, respectively; 4 h: $P < 0.001$, $P < 0.01$, respectively; n = 10 mice) or saline treatment (1 h: $P < 0.01$, $P < 0.05$, respectively; 2 h: $P < 0.05$; 4 h: $P < 0.01$, $P < 0.05$, respectively; n = 10 mice). Devazepide (15 min pretreatment; 100 μg/kg ip) or vehicle pretreatment had no effect on PYY-induced inhibition of food intake in apo A-IV$^{+/+}$ mice (A) (NS, n = 6 mice). *Letters* signify significant statistical differences between treatment groups.

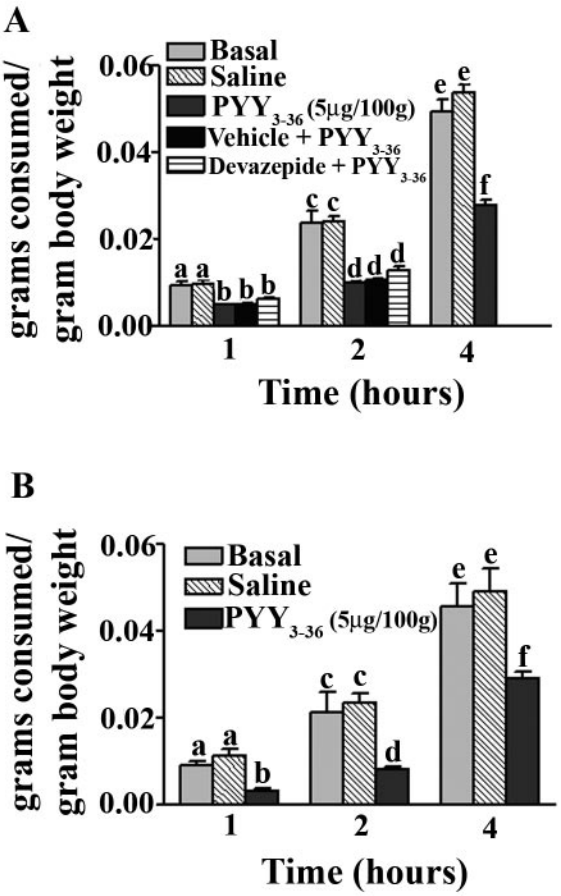

FIG. 5. Inhibition of food intake in response to $PYY_{3–36}$ in *ad libitum*-fed apo A-IV wild-type (A) and apo A-IV knockout mice (B). $PYY_{3–36}$ (5 μg/100 g ip) significantly inhibited food intake at all time points in both apo A-IV$^{+/+}$ and apo A-IV$^{-/-}$ mice, compared with basal conditions (1 h: $P < 0.05$, $P < 0.001$, respectively; 2 h: $P < 0.01$; 4 h: $P < 0.01$, $P < 0.05$, respectively; n = 10 mice) or saline treatment (1 h: $P < 0.01$, $P < 0.001$; 2 h: $P < 0.01$; 4 h: $P < 0.001$, $P < 0.01$, respectively; n = 10 mice). Devazepide (15 min pretreatment; 100 μg/kg ip), or vehicle pretreatment had no effect on PYY-induced inhibition of food intake in apo A-IV$^{+/+}$ mice (A) (NS, n = 10 mice). *Letters* signify significant statistical differences between treatment groups.

of $PYY_{3–36}$ (5 μg/100 g ip) significantly inhibited food intake at 1, 2, and 4 h in both apo A-IV$^{+/+}$ and apo A-IV$^{-/-}$ mice, compared with basal (1 h: $P < 0.05$, $P < 0.001$, respectively; 2 h: $P < 0.01$; 4 h: $P < 0.01$, $P < 0.05$, respectively; n = 10 mice, Fig. 5) or saline treatment (1 h: $P < 0.01$, $P < 0.001$; 2 h: $P < 0.01$; 4 h: $P < 0.001$, $P < 0.01$, respectively; n = 10 mice, Fig. 5). There was no significant difference in inhibition of food intake by $PYY_{3–36}$ between apo A-IV$^{+/+}$ and apo A-IV$^{-/-}$ mice at any time point (NS, n = 10 mice, Fig. 5).

In wild-type mice, $PYY_{3–36}$-induced inhibition of food intake was not significantly altered by pretreatment with the CCK_1R antagonist, devazepide (15 min pretreatment; 10 μg/100 g ip) or vehicle (NS, n = 10, Fig. 5A).

Discussion

The presence of lipid in the intestine activates intestinal feedback inhibition of gastric function and short-term food intake. $PYY_{3–36}$ has been shown to participate in intestinal feedback in response to intestinal lipid (10–13), yet the pathway and mechanism of action are not completely defined. In the present study, we have demonstrated that activation of the vagal afferent pathway in response to exogenous $PYY_{3–36}$, as determined by activation of neurons within the NTS, was significantly reduced in apo A-IV null mice, suggesting a role for apo A-IV in mediating the response to $PYY_{3–36}$. We previously demonstrated that both intestinal lipid and apo A-IV activate NTS neurons via a vagal afferent, CCK_1R-dependent pathway (22, 23, 37); thus, we determined the role of this pathway in mediating the NTS response to $PYY_{3–36}$. We observed that systemic capsaicin treatment or blockade of CCK_1Rs significantly reduced $PYY_{3–36}$-induced activation of NTS neurons, suggesting a requirement for apo A-IV and CCK_1Rs in the NTS response to $PYY_{3–36}$. These observations are consistent with $PYY_{3–36}$-induced stimulation of apo A-IV release, which then acts via release of CCK and activation of CCK_1R on vagal afferents nerve terminals in the gut wall, as previously demonstrated (Fig. 6) (22). Furthermore, inhibition of gastric emptying induced by $PYY_{3–36}$ was abolished in apo A-IV$^{-/-}$ mice, compared with the wild-type controls, and by CCK_1R blockade. These data suggest that $PYY_{3–36}$-induced activation of intestinal feedback inhibition of gastric emptying is mediated, at least in part, via a vagal reflex pathway mechanism involving apo A-IV and the CCK_1R (Fig. 6). This is the first demonstration of the involvement of apo A-IV and CCK_1Rs in functional response to exogenous $PYY_{3–36}$. We have previously shown that lipid in the proximal gut activates vagal afferents via an apo A-IV and CCK_1R-dependent pathway (23); the present data demonstrate that exogenous $PYY_{3–36}$ activates the vagal afferent pathway and inhibits gastric emptying via this same pathway. However, $PYY_{3–36}$-induced inhibition of food intake in both fed and fasted apo A-IV null mice, and in mice treated with the CCK_1R antagonist, devazepide, is unaltered, suggesting that $PYY_{3–36}$-induced inhibition of food intake does not require the apo A-IV/CCK_1R pathway. These findings provide further insight into our understanding of the relationship among apo A-IV, CCK_1Rs, and $PYY_{3–36}$ and their roles in activation of the gut-brain axis and the control of gastrointestinal function and food intake in response to dietary fat.

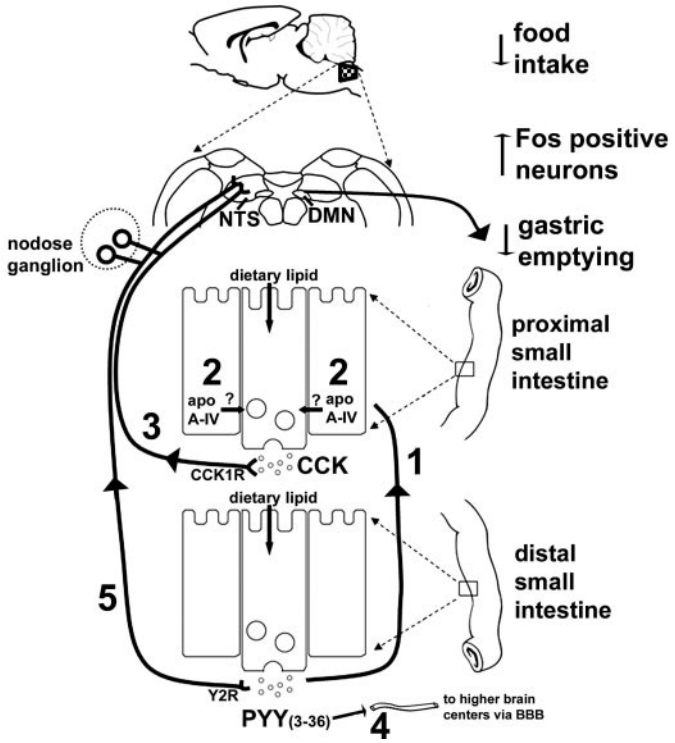

FIG. 6. It has previously been shown that PYY, released from endocrine cells in the distal small intestine, can stimulate the release of apo A-IV from the proximal gut. In the current study, we determined the role of this apo A-IV-CCK_1R pathway in mediating action of exogenous $PYY_{3–36}$, a ligand for the Y2 receptor. Our data support the model that PYY-induced release of apo AIV (1), which is thought to act on adjacent CCK-expressing endocrine cells to release CCK (2), in turn activates the CCK_1R via on vagal afferent nerve terminals (3) to increase expression of fos in neurons in the NTS and inhibit gastric emptying (22, 23). However, it is likely that the effect of $PYY_{3–36}$ on food intake was mediated via crossing the blood-brain barrier and a direct effect on neurons in the hypothalamus (4) (52). In addition, the Y2R is expressed by vagal afferents and $PYY_{3–36}$ could directly activate vagal afferents via this pathway (5) (7).

These findings are also consistent with an earlier observation that administration of PYY induces release of apo A-IV (28, 29). In that study, the pathway by which PYY induces release of apo A-IV was investigated and was shown, via complete vagal nerve transaction, to be mediated via the vagus nerve. However, the role of either the vagal afferent pathway or activation of the vagal efferent pathway (via an effect on parasympathetic preganglionic neurons) was not determined. Findings in the present study and another showing activation of the vagal afferent pathway by $PYY_{3–36}$ (7) suggest that release of apo A-IV might be mediated via a vagovagal reflex; $PYY_{3–36}$ is an agonist for the Y2 receptor located on vagal afferent neurons. It is also possible that $PYY_{3–36}$ may release apo A-IV from enterocytes via a direct humoral effect. Whatever the mechanism by which $PYY_{3–36}$ results in the release of apo A-IV, data from the present study suggest that it is important in mediating the functional responses at least to exogenous PYY. It remains to be determined how this pathway plays a role in mediating the responses to endogenous PYY released by dietary fat.

Protein expression of the immediate-early gene, c-*fos*, is used as an index of neuronal activation (31). Activation of neurons within the NTS, as the result of vagal input, can be measured by expression of the c-*fos* protein, Fos (38, 39). Exogenous peripheral administration of $PYY_{3–36}$ has been shown to significantly increase Fos protein expression in the NTS (17). In the present study, as originally demonstrated by Halatchev and Cone (17), we observed an increase in Fos-positive neurons in the midregion of the NTS but not in the more rostral or caudal NTS. This is the region in which vagal afferents from the proximal gut terminate (40), suggesting that activation of neurons in the NTS is likely via vagal afferents terminating in the proximal gastrointestinal tract. Activation of the NTS in response to peripheral $PYY_{3–36}$ was also significantly reduced in capsaicin-treated mice, suggesting the response is mediated, at least in part, by capsaicin-sensitive vagal afferents. This is consistent with published data showing expression of Y2 receptors by vagal afferents and activation of vagal afferents by $PYY_{3–36}$ in rats (7). The residual response of NTS neurons to $PYY_{3–36}$ after either capsaicin treatment, CCK_1R blockade, or in apo A-IV null mice, might be via a direct effect on NTS neurons because $PYY_{3–36}$ may have direct access to neurons within the brainstem (18). In addition, it is possible that the capsaicin-insensitive portion of the response of the NTS to $PYY_{3–36}$ could be mediated via central apo A-IV and CCK_1Rs (41, 42).

$PYY_{3–36}$ is relatively selective for the Y2 receptor (43). In the present study, there was only a 40% attenuation of the activation of neurons in the NTS after administration of either dose of theY2 receptor antagonist. BIIE0246 is specific antagonist and selective for Y2 receptors (33). It has been reported (referenced as a personal communication in Ref. 44) that this compound does not cross the blood-brain barrier. Thus, the residual response to $PYY_{3–36}$ in the NTS may be mediated via a direct effect of $PYY_{3–36}$ on neurons in the NTS or may be mediated by another PYY receptor subtype as suggested recently (45).

Activation of the vagal afferent pathway, via CCK_1R-dependent mechanisms, results in the activation of NTS neurons and reflex changes in gastrointestinal function (35, 37, 46, 47). It has previously been shown that $PYY_{3–36}$ inhibits gastric emptying (11). In the present study, we determined the contribution of the apo A-IV/CCK_1R pathway to $PYY_{3–36}$-induced inhibition of gastric emptying. We quantified gastric emptying of a chow meal in apo A-$IV^{+/+}$ and apo A-$IV^{-/-}$ mice in response to exogenous $PYY_{3–36}$ treatment and found that, in apo A-$IV^{-/-}$ mice, the inhibitory effect of $PYY_{3–36}$ on gastric emptying was abolished. In addition, pretreatment with the CCK_1R antagonist, devazepide, abolished the inhibitory effect of $PYY_{3–36}$ on gastric emptying in wild-type mice. These findings support a significant role of apo A-IV and CCK_1Rs in $PYY_{3–36}$-induced inhibition of gastric emptying. The data are consistent with the mechanism whereby $PYY_{3–36}$, released from the distal gut in response to a meal, stimulates the release of apo A-IV by the proximal gut (either via a neural or humoral pathway) (28), and in turn, apo A-IV activates the vagal afferent pathway and produces changes in gastric emptying via a CCK_1R-dependent pathway (Fig. 6). It is likely that $PYY_{3–36}$ may play a physiological role in the regulation of gastric emptying; administration of the Y2 receptor antagonist accelerated the rate of gastric emptying in the present study.

Exogenous ip $PYY_{3–36}$ is an effective inhibitor of food intake in rats, mice, monkeys, and humans (10, 48–51). Recent findings are contradictory regarding the role of the vagus nerve in $PYY_{3–36}$-induced inhibition of food intake. Koda *et al.* (7) demonstrated that $PYY_{3–36}$ acts through the vagus nerve to inhibit food intake in rats because the effects of peripheral $PYY_{3–36}$ were blocked by abdominal vagotomy. Conversely, in mice, total subdiaphragmatic vagotomy not only did not block the effect of $PYY_{3–36}$ on food intake but also prolonged its action (17). When mice were pretreated with the systemic neurotoxin capsaicin, the inhibitory effect of peripheral $PYY_{3–36}$ on food intake was not altered (34). Whether this discrepancy in the role of the vagus nerve and capsaicin-sensitive afferents is due to species differences between rat and mouse or methodological difference, remains to be determined. Recent studies provide evidence that peripheral $PYY_{3–36}$ inhibits food intake via a direct effect on neurons in the arcuate nucleus of the hypothalamus. For example, $PYY_{3–36}$ can cross the blood-brain barrier via a nonsaturable process (52). High levels of Y2 mRNA expression, moderate to high densities of $PYY_{3–36}$ binding, and activation of Y2 receptors by agonist-stimulated binding of [^{35}S]GTPγ have been detected in rat hypothalamus (43, 53, 54). Central administration of $PYY_{3–36}$ or the Y2 receptor agonist *N*-acetyl (Leu^{28}, Leu^{31}) NPY (24–36) into the arcuate nucleus of the hypothalamus dose-dependently inhibits food intake in rats (10). In addition, central administration of the Y2 receptor antagonist BIIE0246 in the hypothalamic arcuate nucleus significantly attenuated inhibition of food intake by peripheral $PYY_{3–36}$ (14). Taken together, these data suggest that $PYY_{3–36}$ can inhibit food intake by directly acting on Y2 receptors in the arcuate nucleus.

Data obtained in the present study suggest it is unlikely that the vagal, apo A-IV-CCK_1R pathways plays a role in the ability of $PYY_{3–36}$ to inhibit food intake. We quantified food intake of a chow meal in both *ad libitum*-fed and fasted mice in response to exogenous $PYY_{3–36}$ treatment and found that $PYY_{3–36}$ produces a reproducible decrease in food intake in both apo A-$IV^{+/+}$ and apo A-$IV^{-/-}$ mice. $PYY_{3–36}$ also significantly inhibited food intake at all time points in apo wild-type mice pretreated with the CCK_1R antagonist, devazepide, which is able to cross the blood-brain barrier. Therefore, it does not appear that interaction of $PYY_{3–36}$ with either apo A-IV or peripheral or central CCK_1Rs is essential for $PYY_{3–36}$-induced inhibition of food intake. This is consistent with other data in mice that the vagal pathway is not important for the action of $PYY_{3–36}$ to inhibit food intake (17, 34). It is interesting to note that $PYY_{3–36}$-induced inhibition of food intake was more potent in *ad libitum*-fed mice *vs.* fasted mice (Figs. 4 and 5). This is perhaps due to the fact that fasted mice have to overcome a strong orexigenic drive when food is offered or that other endogenous factors act synergistically with $PYY_{3–36}$ to inhibit food intake (34, 55).

In summary, the present data provide evidence that $PYY_{3–36}$-induced activation of the vagal reflex pathway and inhibition of gastric emptying release is dependent on apo A-IV and CCK_1Rs. In addition, our data suggest that $PYY_{3–36}$-induced inhibition of food intake does not occur through

an interaction with apo A-IV or the CCK_1R. These findings provide further insight into the mechanism by which $PYY_{3–36}$ activated the gut-brain axis and regulates postprandial gastrointestinal function and food intake.

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