

PYY stimulates synthesis and secretion of intestinal apolipoprotein AIV without affecting mRNA expression

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Kalogeris, Theodore J., Xiaofa Qin, William Y. Chey, and Patrick Tso. PYY stimulates synthesis and secretion of intestinal apolipoprotein AIV without affecting mRNA expression. *Am. J. Physiol.* 275 (*Gastrointest. Liver Physiol.* 38): G668–G674, 1998.—We tested whether exogenous peptide YY (PYY) can stimulate synthesis and lymphatic secretion of intestinal apolipoprotein AIV (apo AIV). Rats with mesenteric lymph fistulas and right atrial cannulas were given continuous intravenous infusions of control vehicle or PYY at 25, 50, 75, 100, or 200 pmol·kg⁻¹·h⁻¹. PYY (75–200 pmol·kg⁻¹·h⁻¹) stimulated lymphatic apo AIV output from 1.5- to 3.5-fold higher than basal output. In separate experiments, PYY (100 pmol·kg⁻¹·h⁻¹) produced a 60% increase in jejunal mucosal apo AIV synthesis but had no effect on mucosal apo AIV mRNA levels at doses up to 200 pmol·kg⁻¹·h⁻¹. Finally, exogenous PYY infusion (100 pmol·kg⁻¹·h⁻¹) produced a plasma PYY increment of 30 pM compared with an increment of 18.7 pM in response to ileal infusion of lipid. These results support the hypothesis that PYY may be an endocrine mediator of the effects of distal gut lipid on production and release of intestinal apo AIV, likely via a posttranscriptional mechanism of action.

ileal brake; lipid; nutrient absorption; chylomicrons; gastrointestinal hormones

APOLIPOPROTEIN AIV (apo AIV) is a major glycoprotein component of intestinally synthesized and secreted triglyceride-rich lipoproteins. Apo AIV has several proposed roles: modulation of plasma cholesterol and lipoprotein metabolism (4, 9, 12), control of food intake (11), and upper gastrointestinal function (29, 30). Most recently, it was shown that apo AIV may play a protective role with respect to atherogenesis (7, 10). Thus it is important to understand the mechanisms responsible for control of synthesis and secretion of apo AIV. These mechanisms are incompletely understood.

Expression, synthesis, and secretion of intestinal apo AIV are stimulated by dietary fat (2, 13, 15, 20, 23, 24, 33). However, it is not clear how apo AIV is stimulated by lipid: either fat has a direct effect or some other signal released in response to lipid might act as a mediator, or both of these mechanisms could play a role. Dietary lipid stimulates neural reflexes (31) and the release of several gastrointestinal peptide hormones known to influence gut motility, digestion, metabolism, and feeding behavior (3, 19, 28). Thus, in view of the recently proposed roles of apo AIV in the inte-

grated control of both digestive function and feeding behavior, it is important to determine whether lipid-induced gut neural and/or humoral signals might be involved in mediating the effect of dietary fat on apo AIV.

In a recent report we demonstrated that physiological amounts of lipid delivered to the ileum are capable of stimulating the synthesis of apo AIV in a proximal jejunal Thiry-Vella loop (24). Use of the Thiry-Vella loop precluded a direct role for jejunal lipid in the apo AIV response, and therefore the results suggest the existence of a lipid-responsive signal, arising from the distal intestine, which acts to stimulate synthesis of apo AIV in the proximal intestine (23, 24). The proposed signal might be neural, hormonal, or a combination of these two. Because we found this signal to arise preferentially from the distal intestine (24) and because of the recently described inhibitory actions of apo AIV on gastric emptying and acid secretion (29, 30), we proposed that stimulation of apo AIV release may be a new effect of the “ileal brake” (27) or that apo AIV itself may be a component of the ileal brake (24). In addition, other findings, by both our laboratories (33) and others (17, 18, 25), suggest that the ileal brake may play a role in the normal control of gut function, rather than only being operative under abnormal conditions such as malabsorption. Thus it is possible that ileal signals play a role in the AIV response to a meal.

The ileal brake is thought to be mediated by one or more peptide hormones from endocrine cells in the distal intestinal mucosa (28). At present the most likely hormonal candidate for mediator of the ileal brake is peptide tyrosine-tyrosine (PYY) (28), although PYY appears unlikely to be the only factor (32). Because one of these factors could be apo AIV (29, 30), it is important to determine whether there is an association between lipid-induced PYY release and release of apo AIV.

The purposes of this study were to determine 1) the effect of exogenous PYY on mucosal expression, synthesis, and lymphatic secretion of apo AIV, and 2) whether plasma levels of PYY produced by ileal lipid infusion are sufficient to elicit stimulation of apo AIV. The results of these studies suggest that PYY may mediate at least a part of the response of intestinal apo AIV to dietary fat and does so via a posttranscriptional mechanism.

METHODS

All experiments used male Sprague-Dawley rats (300–325 g; Harlan Sprague Dawley, Indianapolis, IN), housed at 26°C on a 12:12-h light-dark cycle, with lights on at 0600. The rats were allowed free access to a standard diet (Teklad Rodent Chow, Harlan) and water.

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Surgical preparation. Surgery and experiments were conducted 1–2 wk after rats were delivered. They were deprived of food for 24 h and then anesthetized with halothane. For lymph transport studies, the right atrium was cannulated via the external jugular vein as previously described (33). Atrial cannulas were externalized at the back of the neck, filled with polyvinylpyrrolidone (2.2 g/ml), and sealed. Rats were then laparotomized, and the superior mesenteric lymph duct was cannulated with a vinyl tube (medical grade; 0.50 mm ID and 0.80 mm OD, Dural Plastics and Engineering, Dural, Australia); the tube was fixed in place with a drop of cyanoacrylate glue (Krazy Glue, Jadov & Sons, New York, NY) and externalized through a stab wound in the right flank. A second cannula (Silastic medical grade 602–205, 0.75 mm ID and 1.6 mm OD, Dow Corning Medical Products, Midland, MI) was passed through the fundus of the stomach, extended 2 cm into the duodenum, and then secured in place with a purse-string suture and a drop of cyanoacrylate glue. For apo AIV synthesis studies, rats were equipped with right atrial catheter cannulas only. For measurement of plasma apo AIV levels, rats (without lymph fistulas) were equipped with both right atrial and left external carotid cannulas as previously described (33). For ileal lipid infusion studies, a vinyl cannula (as above for the lymph cannula) was inserted through a stab wound in the ileum (about 20 cm proximal to the ileocecal junction) and secured in place with a purse-string suture and a drop of cyanoacrylate glue. This cannula was exteriorized out the right flank of the animal. After surgery, rats were placed in restraint cages in a temperature-regulated chamber at 30°C and allowed to recover for 24 h. During recovery they received continuous duodenal or ileal infusions of a glucose-saline solution (145 mM NaCl, 4 mM KCl and 0.28 M glucose) at 3 ml/h (duodenal infusion) or 0.85 ml/h (ileal infusion). The rationale for the lower ileal infusion rate was described previously (24). The purpose of duodenal infusions in the lymph transport studies was to ensure that lymph flow would be sufficient to allow measurement of apo AIV output in the presence or absence of intravenous PYY infusion.

Infusate preparation: effect of exogenous PYY on plasma PYY levels and on lymph output, plasma levels, and jejunal mucosal synthesis and mRNA expression of apo AIV. Rat PYY was purchased from Peninsula Laboratories (Belmont, CA) and dissolved in infusion vehicle (0.15 M NaCl plus 0.5% BSA). Stocks of this preparation were aliquoted and frozen at –80°C. All experiments were conducted using freshly thawed stock, diluted in infusion vehicle.

Effect of ileal lipid infusion on plasma PYY levels. Lipid emulsions were prepared containing 20 μmol 1-monoolein, 40 μmol oleic acid, 2.21 μmol phosphatidylcholine, and 16.15 μmol sodium taurocholate, sonicated in 0.85 ml PBS (pH 6.4). These fatty acid-monoglyceride emulsions were infused at 0.85 ml/h. This dose of monoglyceride and fatty acid is equivalent to a triolein dose of 20 $\mu\text{mol}/\text{h}$. Rationale for this approach was described previously (24).

Effect of PYY infusion on lymphatic apo AIV output. After rats had recovered from surgery, lymph was collected for 30 min before beginning intravenous infusions. Then they were intravenously infused for 30 min with infusion vehicle (0.15 M NaCl plus 0.5% BSA, 1.74 ml/h) and a basal lymph sample was collected. Vehicle infusion was either continued (control group) or was switched to vehicle plus PYY, and infusions were continued for 6 h. PYY doses tested were 0, 25, 50, 75, 100, and 200 $\text{pmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$. Lymph samples were collected every 30 min into ice-chilled glass tubes. Aliquots of lymph were taken for assay of apo AIV. At the end of the infusion period, rats were anesthetized using halothane and eutha-

nized by pneumothorax. Each rat in the study was tested once with either vehicle or a single dose of PYY.

Lymph apo AIV was measured using an electroimmunoassay (EIA) as previously described (15). Gels for EIA were made of 1.2% Indubiose agarose and 2% dextran in Tris-Veronal buffer containing 1% goat anti-rat apo AIV antiserum. Samples were subjected to electrophoresis at 3.5 V/cm for 6 h at 20°C. Immunoprecipitates were stained, and the areas under the immunoprecipitate rockets were measured using a computer-controlled digitizing system (Sigma Scan, Jandel Scientific, Sausalito, CA). The lymphatic output of apo AIV at each time point was calculated by multiplying assayed concentrations in each lymph sample by the lymph flow rate measured at that time point.

Effect of PYY infusion on plasma levels of apo AIV. Rats were given continuous intravenous infusions of either vehicle or PYY (100 $\text{pmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) via the right atrial cannula for 4 h. Hourly blood samples (0.05 ml) were taken from the left carotid cannula for measurement of plasma apo AIV levels. Plasma samples were assayed for AIV as described above.

Effect of PYY on jejunal biosynthesis of apo AIV. Because lymph transport studies showed maximal apo AIV output by 4 h after the start of PYY infusion, subsequent measurements of apo AIV synthesis were made after 4 h infusions of either vehicle or vehicle plus PYY (100 $\text{pmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$). Apo AIV synthesis was measured as previously described (23). Briefly, at the conclusion of the infusion period, rats were anesthetized and a 10-cm segment of proximal jejunum (proximal end about 3–5 cm distal to the ligament of Treitz) was isolated by ligatures. The loop was incubated for 10 min with luminally infused [^3H]leucine (11.1 $\times 10^6$ Bq) in saline and then removed and washed with ice-cold PBS containing leucine (20 mM). The washed segment was placed over ice and cut open, and then the mucosa was scraped off with a glass slide. The mucosa was homogenized in 1.5 ml PBS containing 1% Triton X-100, 2 mM leucine, 1 mM phenylmethylsulfonyl fluoride, 40 μg chymostatin, 160 μg leupeptin, and 6 μg pepstatin A. The homogenate was centrifuged at 100,000 g for 60 min in a Beckman 50.3 Ti rotor. The cytosolic supernatant was then subjected to precipitation using 10% TCA and assayed for protein concentration using the Bradford procedure (Bio-Rad, Hercules, CA). Total mucosal protein synthesis was expressed as the ratio of TCA-precipitable radioactivity to protein content. A separate aliquot was also subjected to specific immunoprecipitation of apo AIV, using a polyclonal goat anti-rat apo AIV antiserum described previously (15). Immunoprecipitated apo AIV was resolved using SDS-PAGE followed by autoradiography. The radioactive band corresponding to apo AIV was removed from the gel, solubilized using Solvable Tissue and Gel Solubilizer (Dupont-NEN Research Products, Boston, MA), and the radioactivity was measured. Synthesis of apo AIV was expressed as the amount of immunoprecipitable radioactivity as a percentage of total TCA-precipitable radioactivity.

Effect of PYY on jejunal apo AIV mRNA levels. We examined the effect of PYY (0, 100, and 200 $\text{pmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) on jejunal mucosal apo AIV mRNA levels using the same infusion protocol that was used in the synthesis studies. At the conclusion of the infusion period, jejunal mucosa was obtained as described previously and total RNA was isolated using the RNeasy RNA isolation kit (Qiagen, Valencia, CA), using the manufacturer's suggested protocol. Isolated RNA was stored at –80°C until analysis. Apo AIV mRNA was detected as previously described (24) after slot blotting using a [^{32}P]dCTP-labeled, rat intestinal apo AIV cDNA probe (13) (kindly provided by Dr. Jeffrey Gordon, Washington University, St. Louis, MO). Preliminary studies using Northern

Table 1. Intestinal lymph flow during 6 h continuous right atrial infusion of vehicle or PYY

	0 min	60 min	120 min	180 min	240 min	300 min	360 min
Vehicle	3.0 ± 0.11	3.2 ± 0.16	3.2 ± 0.08	3.8 ± 0.14	3.2 ± 0.12	3.6 ± 0.09	3.3 ± 0.10
PYY (100 pmol · mg ⁻¹ · h ⁻¹)	3.1 ± 0.13	2.9 ± 0.12	3.0 ± 0.12	3.4 ± 0.12	3.3 ± 0.17	3.4 ± 0.07	3.6 ± 0.08

Values are means ± SE in ml/h; *n* = 5 vehicle (see text) and 6 peptide YY (PYY) rats (same animals whose apolipoprotein AIV outputs are shown in Fig. 1). Lymph was sampled every 30 min; for simplicity, data shown are lymph flow rates determined every 60 min (i.e., every other sampling).

blotting established that the detected apo AIV transcript migrated as a single, 1.7-kb species. Blots were stripped and reprobed for 18S ribosomal RNA (Ambion, Austin, TX). After autoradiography, relative levels of apo AIV mRNA in control (vehicle) vs. PYY-treated rats were determined by densitometric analysis, normalized to the 18S signal.

Comparison of plasma PYY levels after ileal lipid infusion and exogenous PYY infusion. In one experiment, rats equipped with ileal cannulas were infused with either lipid emulsion (20 μmol/h monoolein plus 40 μmol/h oleic acid), or control glucose saline for 8 h. They were then anesthetized with halothane, and blood was obtained by cardiac puncture and placed into heparinized tubes. Aprotinin (500 U/ml) was added to the serum, and the serum was extracted using Sep-Pak C₁₈ cartridges as described previously (19). PYY levels were then determined using a sensitive and specific PYY RIA as previously described (19).

In a second experiment rats equipped with right atrial catheters were infused with either vehicle (0.15 M NaCl plus 0.5% BSA) or vehicle plus PYY (100 pmol · kg⁻¹ · h⁻¹) for 4 h, after which blood was collected, processed, and assayed for PYY levels as previously described.

Statistics. Data were analyzed using the SYSTAT software package (37). Time-dependent data on lymph output and plasma levels of apo AIV were analyzed using one-way repeated measures ANOVA with post hoc comparisons using a multiple linear general model. The effects of exogenous PYY on apo AIV synthesis were analyzed by *t*-test, and mRNA levels were analyzed by one-way ANOVA. Increments in plasma PYY levels produced by ileal lipid infusion and exogenous PYY infusion were compared by *t*-test. Differences were considered significant if *P* < 0.05.

RESULTS

Effect of PYY infusion on lymph flow rates in lymph fistula rats. Basal lymph flow was similar among all groups, ranging from 2.8 ± 0.11 to 3.2 ± 0.15 ml/h; there was no significant effect of either saline or PYY at any dose on intestinal lymph flow (Table 1); over the period of PYY infusion and over all treatment groups, lymph flow rates ranged from 2.7 ± 0.4 to 3.1 ± 0.5 ml/h.

Effect of PYY infusion on lymph apo AIV output in lymph-fistula rats. Exogenous PYY produced a dose-dependent increase in lymph apo AIV output (Fig. 1). As described previously, this could not be explained by changes in lymph flow rate. Infusion of PYY at 25 pmol · kg⁻¹ · h⁻¹ did not elicit AIV output that was significantly different from vehicle infusion at any time during the 6-h infusion period. The next highest dose of PYY (50 pmol · kg⁻¹ · h⁻¹) produced a transient increase in AIV output between 90 and 150 min; however, this increase was not significantly different from output during vehicle infusion at the same time points. After 150 min, AIV output was also not significantly different from outputs during vehicle infusion. There was consid-

erably more variability between rats at this dose of PYY, with one animal showing a clear elevation in AIV output at all time points and the others showing little response. These observations suggest that under the conditions of our study, 50 pmol · kg⁻¹ · h⁻¹ may be close to the threshold dose for stimulation of apo AIV. The next highest dose of PYY, 75 pmol · kg⁻¹ · h⁻¹, produced a

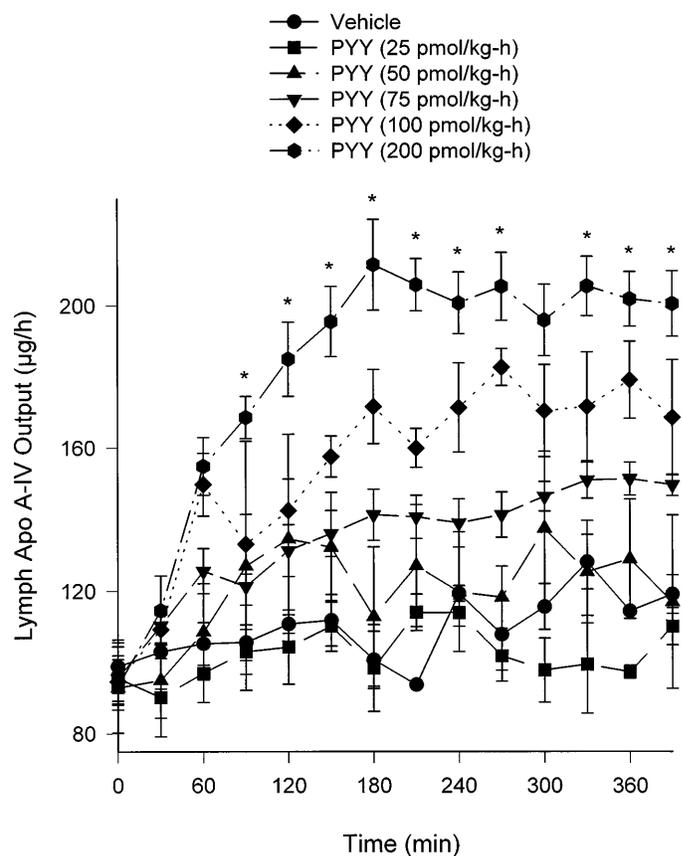


Fig. 1. Lymph output of apolipoprotein AIV (apo AIV) during continuous intravenous infusion of graded doses of peptide YY (PYY). Rats equipped with mesenteric lymph duct fistulas and right atrial catheters received infusions of vehicle (0.15 M NaCl plus 0.5% BSA) or vehicle plus PYY at the indicated doses, beginning at time 0 and continuing throughout the experimental period. Lymph samples were collected every 30 min and were assayed for apo AIV as described in METHODS. Values are means ± SE for *n* = 5 (vehicle), *n* = 5 (25 pmol · kg⁻¹ · h⁻¹), *n* = 6 (50 pmol · kg⁻¹ · h⁻¹), *n* = 9 (75 pmol · kg⁻¹ · h⁻¹), *n* = 6 (100 pmol · kg⁻¹ · h⁻¹), and *n* = 8 (200 pmol · kg⁻¹ · h⁻¹) rats. In response to PYY at 75, 100, and 200 pmol · kg⁻¹ · h⁻¹, apo AIV output was significantly different from that at time 0 at every time point from 30 min to the end of the experimental period (*P* < 0.05) and significantly different from output during vehicle infusion at every time point (*P* < 0.01) except at 0 and 30 min. *Significantly elevated output in response to PYY at 200 pmol · kg⁻¹ · h⁻¹ compared with that in response to 100 pmol · kg⁻¹ · h⁻¹ at a given time point (*P* < 0.05).

clearer increase in lymph output, with the output from 2 to 6 h being significantly elevated compared with the vehicle-infused control group. PYY at $100 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ produced an immediate elevation in apo AIV output, ranging from 1.7- to 2.2-fold higher than during vehicle infusion. At this dose apo AIV output appeared to reach a relative plateau by 3.5–4 h and remained elevated for the duration of the infusion period. The highest dose, $200 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, produced a further elevation in lymphatic apo AIV output, ranging from 2.5- to 3.5-fold higher than control output. From 1 to 6 h, output in response to this highest dose was significantly higher than that in response to $100 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (with the exception of the output at 300 min).

Effect of PYY infusion on plasma apo AIV levels. In vehicle and PYY-infused groups, basal levels of plasma apo AIV were 48.5 ± 2.78 and $51.6 \mu\text{g/ml}$, respectively (not significantly different). Infusion of PYY at $100 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ produced a 30% increase, to $67.3 \pm 3.69 \mu\text{g/ml}$ by 1 h; this increase was significant ($P = 0.042$). Plasma apo AIV levels remained significantly elevated at levels ranging from 25 to 29% higher than control levels for the duration of the 4-h infusion period (Fig. 2).

Effect of PYY infusion on jejunal apo AIV biosynthesis. Because results of the lymph output studies indicated attainment of maximum lymphatic output of apo AIV after 4 h of PYY ($100 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) infusion, we examined jejunal apo AIV synthesis under these conditions. Total protein synthesis, assessed by the specific

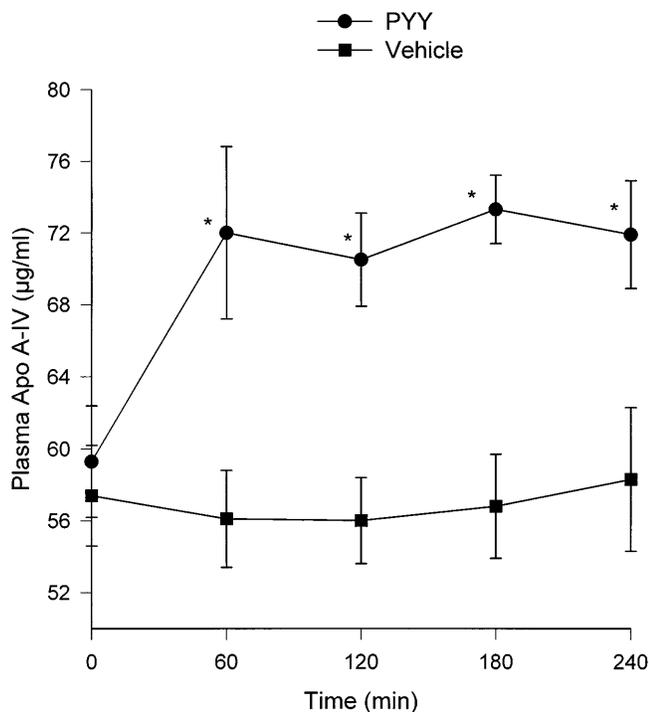


Fig. 2. Effect of exogenous PYY on plasma apo AIV levels. Rats equipped with right atrial and left carotid cannulas were given continuous intravenous infusion of either vehicle (same as Fig. 1) or PYY ($100 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) for 4 h through their right atrial catheter. Plasma samples were obtained at the indicated time points for measurement of apo AIV levels. Values are means \pm SE for $n = 6$ (vehicle) and $n = 7$ (PYY) rats. *Significant difference between vehicle and PYY-infused groups ($P < 0.01$) at a given time point.

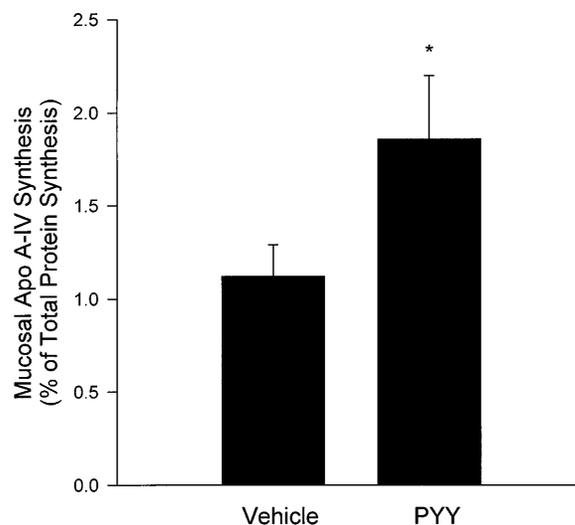


Fig. 3. Effect of exogenous PYY on jejunal mucosal apo AIV synthesis. Rats equipped with right atrial infusion catheters were given continuous intravenous infusion of either vehicle (same as Fig. 1) or PYY at $100 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 4 h. Mucosal apo AIV synthesis was then measured as described in METHODS. Values are means \pm SE for 6 rats each for the vehicle and PYY-infused groups. *Significantly different from vehicle-infused condition ($P < 0.05$).

activity of mucosal cytosolic supernatants, was unaffected by PYY infusion: specific activity of vehicle and PYY-infused mucosal cytosolic supernatants was 1.42 ± 0.10 and $1.51 \pm 0.17 \text{ dpm/ng}$, respectively ($P = 0.25$). Compared with vehicle infusion, PYY produced significant increases in apo AIV synthesis, ranging from 1.6- to 2.1-fold higher than in response to vehicle infusion (Fig. 3).

Effect of PYY on jejunal apo AIV mRNA levels. To determine whether PYY-elicited increases in jejunal apo AIV synthesis could be explained by increased apo AIV gene expression, we examined the effect of PYY infusion on AIV message abundance. We tested PYY at both 100 and $200 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, finding no effect of PYY on apo AIV mRNA levels at either dose, compared with vehicle infusion (Fig. 4).

Comparison of plasma PYY levels in response to ileal lipid infusion and exogenous PYY administration. In an effort to determine whether plasma levels of PYY produced by ileal lipid infusion are sufficient to produce the effects documented above, we measured these levels after intravenous infusion of PYY at $100 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ or after infusion of lipid into the distal intestine. In response to an 8-h ileal infusion of glucose saline solution or lipid ($20 \mu\text{mol/h}$ glycerol monooleate plus $40 \mu\text{mol/h}$ oleic acid) plasma PYY levels were $30.9 \pm 2.0 \text{ pM}$ ($n = 5$) and $60.9 \pm 5.03 \text{ pM}$ ($n = 3$), respectively, with the plasma PYY increment being 30 pM. PYY vehicle infusion produced plasma PYY levels of $18.4 \pm 0.94 \text{ pM}$ ($n = 4$); PYY infusion ($100 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) produced plasma PYY levels of $37.1 \pm 1.49 \text{ pM}$ ($n = 5$), with the increment being 18.7 pM.

DISCUSSION

The present study addresses the hypothesis (23) that the effects of intestinal lipid on the synthesis and

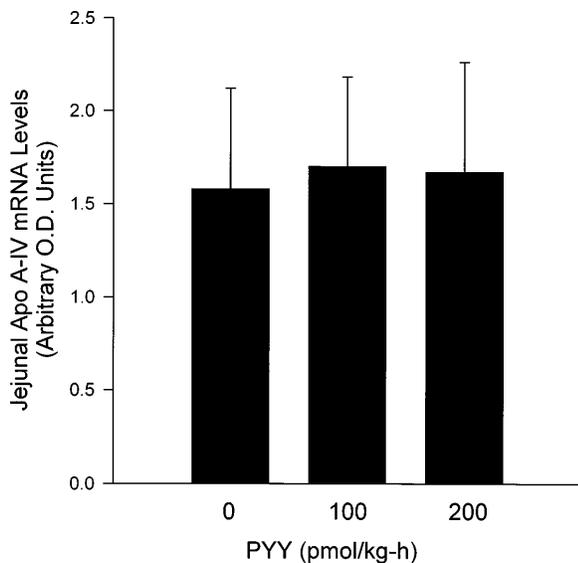


Fig. 4. Effect of exogenous PYY on jejunal mucosal apo AIV mRNA levels. Rats equipped as described in Fig. 2 were given intravenous infusions of either vehicle (0 PYY dose) or PYY at the indicated doses for 4 h, after which total RNA was isolated and apo AIV mRNA was measured by slot blotting as described in METHODS. Blots were then stripped and reprobbed for 18S ribosomal RNA. Hybridized signals for apo AIV on autoradiograms were scanned densitometrically and normalized to the respective 18S signal. Values are means \pm SE for $n = 6$ (vehicle), $n = 5$ (100 pmol·kg⁻¹·h⁻¹ PYY), and $n = 6$ (200 pmol·kg⁻¹·h⁻¹ PYY) rats.

secretion of apo AIV may at least in part be indirect and mediated by lipid-elicited systemic signals. Initial studies addressing this question ruled out a role for capsaicin-sensitive afferent nerves (22); other preliminary work suggests that vagal signals are not necessary for stimulation of lymphatic AIV output in response to proximal intestinal delivery of triglyceride emulsion (21). The present study was prompted by 1) recent findings that apo AIV has enterogastrone-like effects similar to PYY (29, 30) and 2) stimulation of proximal intestinal synthesis of apo AIV by lipid delivered to the distal gut (24). These observations suggest a relationship between apo AIV and the endocrine-mediated ileal brake, that collection of ileal lipid-stimulated upper gastrointestinal inhibitory effects that have been ascribed in part to the actions of PYY (28).

The purpose of the present study was to examine the hypothesis that the distal gut peptide hormone PYY might play a role in the control of intestinal apo AIV by fat. Our results indicate that exogenous PYY stimulates both jejunal mucosal synthesis and lymphatic output and plasma levels of apo AIV without affecting AIV mRNA expression. Moreover, the increment in plasma PYY levels produced by a dose of exogenous PYY that stimulates apo AIV is similar to that produced by ileal lipid infusion, suggesting that lipid-induced PYY release may be sufficient to produce the effects of ileal lipid on apo AIV. Thus the effect of intestinal lipid on apo AIV may in part be mediated by PYY. This constitutes the first evidence that an intestinal apolipoprotein may be controlled by a gastrointestinal hormone. Although novel, this mechanism is never-

theless consistent with numerous prior observations on the effects of intestinal lipid on upper gastrointestinal function and ingestive behavior, as well as recent findings that apo AIV itself may play a role as an enterogastrone.

Among the candidates for endocrine mediator of the ileal brake, only PYY shows increases in plasma levels sufficient to produce ileal brake effects in response to luminal delivery of lipid (28). Moreover, recent studies in dogs provide direct evidence that PYY is a likely hormonal mediator of the ileal brake (26). However, there is also evidence that PYY may not be the only mediator of the ileal brake: intestinal fat infusion elicits a greater suppression of pentagastrin-stimulated acid secretion than does PYY (32), suggesting that the enterogastrone effect of fat is mediated by more than one factor. In view of the recent findings that apo AIV has enterogastrone-like actions similar to those of PYY (29, 30), it is reasonable to propose that another effect of PYY might be stimulation of apo AIV, which may in turn contribute to the enterogastrone effect of intestinal fat.

Current evidence indicates that among the major intestinally synthesized apolipoproteins (AIV, AI, and B48), only apo AIV is significantly stimulated by dietary lipid. Nevertheless, in three of the PYY-infused rats (100 pmol·kg⁻¹·h⁻¹) whose results are shown in Fig. 1, we examined whether PYY could stimulate lymphatic secretion of apo AI. Compared with a basal apo AI output of 102.3 ± 20.3 (SD) μ g/h, there was no effect of PYY infusion on apo AI output (97.3 ± 13.6 – 116 ± 18.5 μ g/h over the course of the 6-h infusion period). These results suggest that the stimulatory effects of PYY on apo AIV were specific to this apolipoprotein.

Because the increment in plasma PYY levels produced by a dose of exogenous PYY that stimulated apo AIV synthesis and secretion was less than the plasma increment produced by ileal lipid infusion, our data support the hypothesis that PYY may be a physiological apo AIV-stimulatory hormone. Further work will be required to determine the role of endogenous PYY in mediating the effect of intestinal (especially ileal) lipid on jejunal apo AIV synthesis.

Mechanisms for the effects of PYY on gastrointestinal function vary with the effect tested. The inhibitory effects of PYY on intestinal water and electrolyte secretion (34) appear to be mediated by specific PYY binding sites located in the intestinal mucosa (36). Moreover, it was recently reported that a subclone of the hBRIE 380 (rat intestinal epithelial hybrid) cell line increases intestinal fatty acid binding protein expression when incubated with PYY (14). These findings support the notion of a direct effect of PYY on intestinal epithelium. On the other hand, inhibition of gastric motility (6) and gastric acid secretion (32) (i.e., ileal brake effects) by PYY appear to depend on central, vagally mediated mechanisms. These latter findings, coupled with the demonstration of saturable binding of circulating PYY in the dorsal vagal complex (16), have led to the proposal that PYY, released from the distal gut in response to lipid, acts as an endocrine neuromodu-

lator of vagally mediated gut function (16). At present, it is uncertain whether PYY affects apo AIV directly or indirectly or whether both types of mechanisms might be involved.

Previously available evidence has supported the idea that the effect of lipid on intestinal apo AIV synthesis is manifested at the level of transcription (2, 13, 24). The present results suggest that fat-elicited PYY release may stimulate apo AIV synthesis by a posttranscriptional mechanism. It is unknown whether this is at the level of translation or posttranslational fate of synthesized apo AIV; further work is needed to address this issue. However, our results raise the possibility that control of apo AIV by dietary lipid may be quite complex, with rapid onset of synthesis and secretion early during feeding mediated by a posttranscriptional mechanism (possibly involving PYY), as well as an increase in transcription that is slower in onset, but capable of maintaining sustained output of apo AIV. Recently we showed (33) that in rats given a gastric bolus of 0.1 g of triglyceride, jejunal apo AIV synthesis and lymphatic secretion were significantly elevated above basal levels within 15 and 30 min, respectively, after the load. This time interval is too short to be easily explained by increases in AIV mRNA levels, because the latter was previously shown to be significantly elevated no sooner than 2–4 h after a fat load (2, 13). Thus there is already evidence that a lipid-stimulated, rapid response mechanism for apo AIV synthesis and release may exist. It is currently unknown whether such a mechanism necessarily involves PYY as a mediator; this question requires further study.

The fact that PYY stimulated synthesis and secretion of apo AIV even in the absence of ongoing lipid absorption raises the question of the form of AIV that was transported. The conditions of our PYY infusion studies make it unlikely that apo AIV was secreted on triglyceride-rich lipoproteins, i.e., chylomicrons or very low-density lipoprotein; the alternative possibilities are the high-density lipoprotein and lipoprotein-free fractions of lymph (8). The present results do not distinguish between these possibilities. However, our findings underscore the importance in future studies of examining the relationship between the form of apo AIV released from the intestine and any subsequent physiological effects ascribed to apo AIV on its entry into the circulation.

In summary, we demonstrate for the first time that physiological doses of exogenous PYY stimulate mucosal synthesis and lymphatic output of intestinal apo AIV, without affecting AIV mRNA levels, in the rat. Taken together with our previous findings (24, 33) these results support the hypothesis that PYY is a lipid-elicited signal arising from the distal intestine, which stimulates synthesis and secretion of apo AIV; further work is necessary to determine whether this mechanism is involved in the integrative response of apo AIV to dietary fat.

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