Luminal peptide YY-releasing factors in the isolated vascularly perfused rat colon

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Abstract

Peptide YY (PYY) is produced in endocrine L cells primarily localized in the distal bowel. These open-type L cells make contact with the intestinal chyme which may thus affect their secretory activity. The aim of the present study was to examine a large variety of luminal compounds found in colonic contents for their potential as PYY-releasing factors, using the isolated vascularly perfused rat colon. The release of PYY into the portal effluent was measured by a specific RIA. Luminal administration of 5 mM glucose or 0.5% (w/v) starch for 30 min did not induce significant release of PYY. Oleic acid (10 and 100 mM) also did not significantly increase PYY secretion. A pharmacological concentration of glucose (250 mM) and a mixture of amino acids (total concentration 250 mM) both induced PYY secretion (200% of basal). Pectin, a polygalacturonic acid, evoked dose-dependent secretion of PYY-like immunoreactivity over the range 0.1–0.5% (w/v). The maximal response was observed after infusion of 0.5% pectin which induced a prompt and sustained release of PYY (300% of basal). Galacturonic acid itself (5%) produced marked PYY secretion. Gum arabic (0.5%) induced a gradual increase in portal PYY concentration (maximal response 250% of the basal value) whereas cellulose (0.5%) did not elicit PYY secretion. Luminal n-butyrate over the range 0.5–5 mM produced a dose-dependent release of PYY (maximal response 300% of the basal value with 5 mM n-butyrate). Increasing the concentration of n-butyrate to 100 mM provoked a gradual decrease in PYY secretion. Propionate was a less potent stimulant than n-butyrate, and acetate did not increase PYY secretion above the basal value. At a concentration of 2 or 20 mM, taurocholate, cholate and deoxycholate brought about PYY secretion while hyodeoxycholate was without effect.

In conclusion, glucose and amino acids may mediate PYY release but only when they are present at high supraphysiological concentrations in the colon while oleic acid does not produce any PYY secretion. Physiological concentrations of fibers (pectin, gum arabic), short-chain fatty acids (n-butyrate, propionate) and bile salts (taurocholate, cholate, deoxycholate) are all potent stimulants of PYY release. Whether the release of PYY by luminal factors is coupled to water and electrolyte transfer via a local/paracrine pathway remains an open question which requires additional work with the isolated vascularly perfused colon preparation.

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Introduction

Peptide YY (PYY) is a 36-amino acid polypeptide hormone that is found in mucosal endocrine L cells of the distal ileum, colon and rectum (Lundberg et al. 1982, Böttcher et al. 1984, Adrian et al. 1985a, 1987, Greeley et al. 1987). It has a variety of biological effects on the gastrointestinal tract and pancreas, including inhibition of gastric and pancreatic secretion (Tatemoto 1982, Adrian et al. 1985b, Pappas et al. 1985), gastric emptying (Suzuki et al. 1983, Pappas et al. 1986) and intestinal motility (Al-Saffar et al. 1985). PYY also increases net intestinal absorption of water and electrolytes (Okuno et al. 1992, Bilchik et al. 1993).

Although the L cells are located in the terminal bowel, circulating levels of PYY rise within 15–30 min of food intake in several species (Adrian et al. 1985a, Taylor 1985, Jin et al. 1993) or in response to intraduodenal administration of sodium oleate in dogs (Greeley et al. 1989b). These observations suggest that, after an oral meal, PYY release may be induced in part by hormonal and/or neural pathways originating from the proximal intestine. These putative signals have not yet been determined but recent studies indicate that PYY secretion may be controlled, in part, by cholecystokinin in dogs (Greeley et al. 1989b, Kuvshinoff et al. 1990) and by the gastric inhibitory polypeptide in rats (Brubaker et al. 1991, Plaisancié et al. 1995).
Since PYY is produced in open-type L cells of the terminal bowel, unabsorbed nutrients, fibers and products of bacterial metabolism in the colon, such as secondary bile acids and short-chain fatty acids (SCFAs), are potential stimulants of PYY release. This hypothesis is supported by the observation that an amino acid mixture, liver extracts, fatty acids and glucose all induce PYY secretion when administered directly into the colon in conscious dogs (Greeley et al. 1989a). In man, luminal perfusion of deoxycholate into the caecum, the transverse colon or the sigmoid colon also evokes an increase in plasma PYY concentration (Adrian et al. 1993). In addition, plasma PYY levels are significantly elevated in patients suffering from malabsorption (Adrian et al. 1986).

The present study was undertaken to investigate in detail the influence of luminal compounds (nutrients, fibers, bile acids and SCFAs) on PYY release. For this purpose, isolated vascorally perfused rat colon was used. In this model, polarized endocrine cells are exposed to well-defined luminal factors which make contact with the endocrine L cells.

Materials and Methods

Materials

BSA was purchased from Biovalori (Cassens, France). Azonutril 25, a mixture of amino acids, was obtained from Laboratoires Roger Bellon (Neuilly-sur-Seine, France). This solution consists of 3-4% (w/v) isoleucine, 9.3% leucine, 8.5% lysine, 6.3% methionine, 8.3% phenylalanine, 3.4% threonine, 1.7% tryptophan, 8.4% valine, 2.7% aspartic acid, 3.4% glutamic acid, 6.4% alanine, 16.8% arginine, 1% cysteine, 6% glycine, 3.4% histidine, 5.4% proline, 0.9% serine, 0.2% tyrosine, 2% citrulline and 1.5% ornithine. The total amino acid content was 14.8 g/100 ml of mixture. The following reagents were purchased from Sigma (Saint-Quentin-Fallavier, France): cellulose, corn starch, gum arabic, pectin from apple, galacturonic acid, acetate, propionate, n-butrate, taurocholic acid, deoxycholic acid, cholic acid and hyodeoxycholic acid. Oleic acid was purchased from Merck (Darmstadt, Germany). 125I was supplied as Na125I by Amersham (Les Ulis, France).

Surgical preparation

The operative procedure for the preparation of an isolated vascorally perfused rat colon has recently been described in detail (Plaisancié et al. 1995). Briefly, male Wistar rats (250–350 g) purchased from Le Centre d’Eléveage Dépré (Saint Douichard, France) were anesthetized with pentobarbital sodium (50 mg/kg, ip.). The proximal and transverse colons were freed of their visceral fixations. A Silastic cannula was inserted and tied in the proximal colon at the caecal junction and another one was introduced into the transverse colon, 8 cm distal to the first one, and tied. The loop was then flushed out with prewarmed isotonic saline. A metal cannula (0.6 mm internal diameter, 0.8 mm outer diameter) and a Silastic one (0.5 mm internal diameter, 0.94 mm outer diameter) were then quickly inserted into the superior mesenteric artery and portal vein respectively. The arterial perfusion was started immediately at a rate of 2.5 ml/min with a Krebs–Henseleit buffer (pH 7.4) containing 25% washed bovine erythrocytes, 3% BSA, 5 mm glucose and 1% (v/v) Azonutril. The perfusion pressure, continuously recorded with a mercury manometer, ranged from 55 to 75 mmHg. The preparation was removed and transferred to a plastic box filled with isotonic saline. Venous blood effluent was collected as 2 min fractions in tubes containing 200 µl 300 mM EDTA. The supernatant was rapidly separated from erythrocytes by centrifugation and frozen at −20 °C for subsequent determination of PYY-like immunoreactivity (PYY-LI).

Experimental design

The experiments consisted of a 20 min control basal period, during which isotonic saline was infused into the lumen at a rate of 250 µl/min. This was followed by a 30 min period of stimulation of PYY release and a subsequent 10 min control period. Each luminal compound was administered first as a bolus of 2 ml followed by a slow infusion of 250 µl/min for 29 min. The lumen was then flushed out with air followed by an infusion of isotonic saline at a rate of 250 µl/min. Cellulose was administered as a bolus of 2 ml which was kept in the loop for 30 min because its poor solubility did not allow slow infusion. The pH of each infused compound was adjusted to 7–7.5, and the osmolality was increased when required to 300 mosmol/kg H2O by addition of appropriate amounts of sodium chloride. The amino acid mixture, Azonutril 25, was diluted fivefold in water to obtain a final osmolality of 300 mosmol/kg H2O. Oleic acid (10 and 100 mM) was infused as a soap after adjustment of the pH of the solution to 7.8 with NaOH.

Radioimmunoassay

The PYY assay was performed as recently described (Plaisancié et al. 1995). Briefly, antiserum A4D was raised against synthetic porcine PYY; it exhibited less than 0.1% crossreactivity with bovine pancreatic polypeptide and neuropeptide Y (Bosshard et al. 1989). Synthetic porcine PYY was iodinated with carrier-free Na125I using the chloramine–T method and purified by reverse-phase HPLC (C18 Bondapak column). The minimum detectable concentration of PYY and the half-maximal inhibitory dose were 1 and 7 fmol/tube respectively (Plaisancié et al. 1995).
**Figure 1** Release of PYY-LI in the portal effluent of the isolated vascularly perfused rat colon on luminal administration of 250 mM glucose (n=8), a mixture of amino acids (n=5) or 100 mM oleic acid (n=3). Results are means ± s.E.

**Figure 2** Effect of luminal administration of pectin, gum arabic and cellulose on PYY secretion (means ± s.E). Pectin and gum arabic were administered first as a bolus followed by slow infusion. Cellulose was administered as a bolus as described in Materials and Methods. Six preparations were used for each set of experiments.

**Calculations and statistics**

Data in all figures are presented as the mean ± s.e. and are expressed as fmol per time unit. For the kinetic studies, paired Student’s t-test was used. For statistical treatment of integrated responses, one-way ANOVA was used, followed by Student’s t-test for unpaired values. The integrated responses of immunoreactive material released by a given stimulus were calculated by subtraction of the basal immunoreactivity produced during a given period from the immunoreactivity released upon stimulation during the same period.
Results

Effects of nutrients on PYY release

Luminal infusion of glucose (250 mM) in the isolated rat preparation induced an increase in PYY-LI in the portal supernatant (maximal value of 35 ± 5 fmol/2 min at 28 min from a basal value of 16 ± 1 fmol/2 min; n=8, P<0.05). Portal PYY levels rapidly returned to a near-basal value during the subsequent control period (Fig. 1). In contrast, plasma PYY-LI was unaltered during luminal infusion of 5 mM glucose or 0.5% starch (data not shown). Luminal administration of the amino acid mixture (total concentration 250 mM) produced a rise in portal PYY-LI (maximal value of 43 ± 5 fmol/2 min from a basal value of 21 ± 2 fmol/2 min; n=5, P<0.05) (Fig. 1). Oleic acid (10 or 100 mM) did not significantly stimulate the release of PYY; the integrated responses of PYY were 45 ± 21 (n=6, P≥0.05) and 79 ± 30 (n=3, P>0.05) fmol/30 min with 10 and 100 mM oleic acid respectively.

Effects of fibers on PYY release

Gum arabic at 0.5% (w/v) elicited a gradual release of PYY in the portal effluent with a maximal effect observed at the end of the infusion period (51 ± 6 fmol/2 min from a basal level of 20 ± 2 fmol/2 min; n=6, P<0.05) (Fig. 2). Cellulose at 0.5% (w/v) only induced a modest rise in portal PYY-LI at the end of the period of stimulation (P>0.05) (Fig. 2). In contrast, 0.5% pectin produced a prompt and sustained secretion of PYY reaching a plateau value of 73 ± 18 fmol/2 min at 12 min from a basal level of 26 ± 4 fmol/2 min (n=6, P<0.05) (Fig. 2). As shown in Fig. 3, the pectin induced a dose-dependent release of PYY over the range 0.1–0.5%. The integrated responses of PYY were 198 ± 28 (n=6), 306 ± 45 (n=6) and 666 ± 108 (n=6) fmol/30 min with 0.1, 0.25 and 0.5% pectin solution respectively. The first significant response was obtained with 0.25% pectin (P<0.05). Higher concentrations of pectin were not used because of the high viscosity of the resulting mixtures. Galacturonic acid (0.5 and 5% solutions) produced a dose-dependent secretion of PYY (integrated response of 192 ± 31 fmol/30 min (n=6, P>0.05) with 0.5% galacturonic acid and 498 ± 67 fmol/30 min (n=5, P<0.05) with 5% galacturonic acid) (Fig. 3).

Effects of SCFAs on PYY release

Luminal administration of n-butyrate (5 mM) provoked a sustained release of PYY (plateau value of 82 ± 15 fmol/2 min at 12 min from a basal level of 27 ± 6 fmol/2 min; n=10, P<0.05) (Fig. 4). In contrast, neither 5 mM propionate nor 5 mM acetate produced a significant rise in portal PYY (Fig. 4). The n-butyrate-induced PYY release was dose-dependent over the range 0.5–5 mm (Fig. 5). Increasing the concentration of n-butyrate to 100 mM decreased the integrated PYY response. When 20 mM propionate was infused, secretion of PYY was observed with a maximal value of 56 ± 14 fmol/2 min at 16 min from a basal level of 23 ± 3 fmol/2 min (n=6, P<0.05). Then, portal PYY-LI decreased until the end of the propionate infusion (data not shown). At a concentration of 100 mM, propionate did not elicit any significant release of PYY (data not shown). Similarly, 20 and 100 mM acetate was without effect (data not shown).
**Effects of bile acids on PYY secretion**

Luminal administration of 2 mM taurocholate induced PYY release (maximal value of 59 ± 9 fmol/2 min at 12 min from a basal level of 32 ± 4 fmol/2 min; n=6, P<0.05) (Fig. 6). On luminal administration of 20 mM taurocholate, portal PYY immunoreactivity progressively rose to a peak of 90 ± 21 fmol/2 min at 18 min from a basal level of 28 ± 6 fmol/2 min (n=6, P<0.05) (Fig. 6). At a concentration of 2 mM, cholate provoked a gradual secretion of PYY in portal effluent with a maximal effect observed at the end of the infusion period (84 ± 14 fmol/2 min from a basal value of 25 ± 4 fmol/2 min; n=5, P<0.05). In contrast, 20 mM cholate produced a prompt and sustained secretion of PYY reaching a plateau level of 110 ± 17 fmol/2 min at 6 min from a basal value of 30 ± 7 fmol/2 min (n=6, P<0.05). When deoxycholate (2 mM) was infused into the lumen, portal PYY-LI rapidly reached a plateau value of 50 ± 14 fmol/2 min at 6 min from a basal level of 19 ± 5 fmol/2 min (n=8, P<0.05) (Fig. 7). When 20 mM deoxycholate was administered, the release of PYY was more pronounced (Fig. 7). In contrast, hyodeoxycholate (2 or 20 mM) failed to induce a significant rise in portal PYY-LI (Fig. 7). The integrated responses to PYY observed upon luminal administration of various bile salts are shown in Fig. 8.

**Discussion**

The greatest concentrations of PYY-LI are found in open-ended L cells localized in the distal bowel. However, circulating PYY concentrations increase significantly within 15–30 min of an oral meal, rise progressively over 2 h and remain elevated during the experimental period in both humans and dogs (Taylor 1985, Greely et al. 1989b). These findings suggest the existence of two mechanisms for PYY secretion. The early PYY release may be activated from the proximal bowel by hormonal and/or neural mediators. Luminal contents reaching the distal part of the gut may also contribute to the sustained stimulation of endocrine L cells. Indeed, glucose infusion into the colonic lumen of anesthetized rat or into the isolated porcine ileum evoked PYY secretion (Orskov et al. 1987, Fu-Cheng et al. 1995). In the present study, intracolonic administration of 250 mM glucose induced PYY release. However, the lack of PYY response upon luminal administration of 5 mM glucose, which reproduces the upper physiological concentration of glucose in the colon (Ferraris et al. 1990),
indicates that glucose is probably not a physiological stimulant of PYY secretion in the rat colon.

In dogs, infusion of amino acids into the colon elicited PYY release (Greeley et al. 1989a, Zhang et al. 1993). Similarly, Fu-Cheng et al. (1995) demonstrated that intracolonic administration of amino acids induced PYY secretion in vivo. Luminal infusion of a mixture of amino acids evoked a small but significant increase in plasma PYY level in the isolated vascularly perfused rat colon. Taken together, these data suggest that amino acids are capable of releasing colonic PYY. The physiological meaning of this result is not clear because amino acids are normally absorbed in the proximal and mid intestine. Therefore, they may not contribute to the secretory activity of colonic L cells under physiological conditions. However, in gastrointestinal diseases such as malabsorption, the colonic concentration of nutrients increases dramatically and might induce PYY release. In support of this view, Adrian et al. (1986) showed that PYY concentrations were significantly elevated in patients suffering from malabsorption.

Plasma PYY levels increased in response to sodium oleate infusion (100 ml/l) into the colon of conscious dogs 1 h after the start of oleate administration (Greeley et al. 1989a). In rats, colonic instillation of oleic acid (43 g/l emulsified in 1% Tween 80) also produced PYY release (Fu-Cheng et al. 1995). In contrast, in the isolated vascularly perfused rabbit colon, there was no significant difference between the integrated responses of PYY release upon infusion of oleic acid in graded concentrations (0-22–22 mm) and the PYY responses obtained upon administration of the vehicle alone (10 mm deoxycholic acid).
(Ballantyne et al. 1989). In addition, the present study indicated that the perfusion of sodium oleate (10 or 100 mM) into the lumen of isolated rat colon did not bring about PYY secretion. Taken together, these data suggest that oleic-acid-stimulated PYY release requires an indirect mechanism that is absent in the isolated perfused preparation. Interestingly, sodium oleate (10^{-4} M) increased PYY secretion in fetal rat intestinal cultures (Brubaker et al. 1991). These findings are in contrast with our results. It is likely that these discrepancies are related to the different models used. Differences in secretory mechanisms between fetal and adult enteroendocrine cells may also be the reason.

As very small amounts of nutrients reach the colonic lumen under physiological conditions, the release of PYY from the distal bowel may involve other luminal factors. The present study demonstrated for the first time that luminal perfusion of pectin, a polygalacturonic acid, induces immediate PYY secretion. We also tested the effect of galacturonic acid as a potential PYY secretagogue and demonstrated that this compound is a potent stimulant of PYY release. We also showed that PYY was released by gum arabic, another soluble fiber which is a branched polymer of galactose, rhamnose, arabinose and glucuronic acid. In contrast, cellulose had no effect on basal PYY release. These data suggest that some soluble fibers can elicit PYY secretion. Supporting this hypothesis, a recent experiment showed that, after small bowel resection, the level of plasma enteroglucagon, a peptide co-produced with PYY in L cells (Böttcher et al. 1984, Nilsson et al. 1991), was increased more in rats fed on a pectin-containing elemental diet than in rats fed on a fiber-free elemental diet (Bamba et al. 1994).

Longo et al. (1991) showed that 10 mM SCFAs (acetate, acetoacetate or n-butyrate) stimulated to a similar extent the secretion of PYY in isolated rabbit distal colon. Whereas 5 mM n-butyrate was a strong stimulant of PYY release in the present study, neither 5 mM acetate nor 5 mM propionate modified the basal level of PYY. Of these two SCFAs, when the concentration was increased to 20 mM, only propionate provoked a weak secretion of PYY. SCFAs are the major anions of colonic contents. Total concentrations of SCFAs in the large bowel are in the range 60–150 mM/l, with acetate, propionate and n-butyrate in greatest amounts and in a molar ratio of 60:20:20 (Clausen & Mortensen 1994). The concentrations of SCFAs used here are therefore in the physiological range. In the present study, n-butyrate (0.5–5 mM) produced a dose-dependent release of PYY. The highest concentrations of n-butyrate (10, 20 and 100 mM) resulted in a significant diminution of the PYY response. This is consistent with a previous study by Longo et al. (1991) which demonstrated a maximal effect of n-butyrate at a concentration of 10 mM. These observations suggest that luminal infusion of 5–10 mM n-butyrate induces the release of an inhibitory factor of PYY secretion. Somostatin appears to be a good candidate for the mediation of this effect since it occurs in mucosal endocrine cells throughout the distal bowel and it abolishes the release of PYY induced by oral glucose in patients with dumping syndrome (Adrian et al. 1985c). Additional experiments
with the isolated vascularity perfused rat colon are required to validate this hypothesis.

A significant secretion of PYY was observed when deoxycholate was introduced into the human sigmoid colon at a dose of 3-3 mm which is the concentration found in human stool (Adrian et al. 1993). In rabbits, deoxycholate induced a marked release of PYY from the isolated perfused left colon (Ballantyne et al. 1989), and luminal infusion of bile increased PYY release in the isolated terminal ileum (Armstrong et al. 1993). Similarly, the present study showed a pronounced rise in PYY levels when deoxycholate (2 and 20 mm) was administered to the lumen. Since the concentration of bile acids in the colon is about 2 mm (Dietschy 1968), the deoxycholate-induced release of PYY is probably of physiological relevance. Together, these data suggest that the control of PYY secretion involves bile salts, particularly deoxycholate. The effects of other bile salts on PYY release were also investigated in the present study. Taurocholate and cholate elicited PYY secretion. In contrast, hyodeoxycholate, which is a major bile salt in the rat colonic lumen (Sacquet et al. 1979), did not significantly modify basal PYY release. All bile salts are therefore not equally potent in triggering PYY secretion.

In conclusion, this study shows that the release of PYY in the isolated rat colon may be induced by factors that are found in the colonic lumen such as fibers (pectin, gum arabic), SCFAs (n-butyrate, propionate) and bile salts (taurocholate, cholate, deoxycholate). In contrast, classical nutrients (glucose, amino acids, fatty acids) are not likely to be major secretagogues. Whether luminal PYY-releasing factors also modulate fluid and electrolyte secretion via a paracrine pathway remains an open question which requires additional work with the isolated perfused intestine model.

References


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