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ORIGINAL ARTICLE

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Mechanisms of peptide YY release induced by an intraduodenal meal in rats: neural regulation by proximal gut

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Abstract Peptide YY (PYY) release in anaesthetized rats was studied during the 2 h following the intraduodenal administration of a semi-liquid meal of 21 kJ. Surgical and pharmacological manipulations were performed in order to analyse the mechanisms of PYY release. Postprandial PYY release was suppressed or strongly decreased by caecocolonectomy, truncal vagotomy, tetrodotoxin, hexamethonium, sensory denervation by perivagal capsaicin, and by the NO-synthase inhibitor L-*N*-arginine methyl ester, while atropine, adrenergic blockers, antagonists of type-A or type-B cholecystokinin (CCK) receptors or bombesin receptors had no effect. Comparing the digestive transit of the semi-liquid meal with the amount of PYY contained in the small bowel wall showed that nutrients had not reached the area rich in cells containing PYY by 30 min, the time at which there was a large PYY release in plasma. By 120 min, the meal front had travelled 72% of the small intestine length, just beginning to reach the PYY-rich part of the ileum. We conclude that the main postprandial PYY release studied in this model comes from ileal and colonic L-cells indirectly stimulated through a neural mechanism originating in the proximal gut and involving sensory vagal fibres, nicotinic synapses and NO release, while CCK and bombesin do not seem to be physiologically involved.

Key words Hormone release · Duodenum · Sensory nerves · Nitric oxide · Antagonists and inhibitors

Introduction

Peptide YY (PYY) is a 36-amino-acid regulatory peptide mainly synthesized by, and released after meals from, endocrine L-cells of the terminal small bowel, caecum, colon and rectum in various species [1, 3, 13, 19, 25, 26]. Intravenous (i.v.) infusion of exogenous PYY results in a potent inhibition of stimulated gastric acid [2, 6, 17] and pancreatic exocrine secretion [2, 18, 23, 29, 32], of intestinal secretion [7], and of gastrointestinal motility [46, 47] in animals and humans; PYY might physiologically function as an "ileal brake".

We previously found that circulating PYY levels significantly increased within 15–30 min, and peaked at 60 min, after the intraduodenal infusion of a semi-liquid meal in anaesthetized and conscious rats [12]. Within this time, however, meal components did not come into contact with the lumen of the distal bowel (caecum, colon and rectum), where PYY cells are mainly located, suggesting that PYY release from the terminal bowel is triggered by neural and/or humoral pathways originating in the proximal bowel [12, 19].

Such a mechanism has been previously demonstrated to occur in the dog, in which PYY release involved both direct stimulation of the endocrine cells by the luminal contents of the distal digestive tract and indirect stimulation originating in the proximal bowel and transmitted to endocrine L-cells through neural and humoral (cholecystotikin [CCK]) pathways [3, 14, 15, 25]. A triggering role of the proximal jejunum of the rat has been suggested [38], but not analysed in detail.

At the cellular level, PYY can be released from L-cells through both cAMP-dependent and calcium-dependent pathways, while PYY synthesis depends only upon cAMP [9].

The aim of the present work was to analyse the mechanisms of PYY release produced by an intraduodenal meal in the rat, by using various pharmacological and surgical manipulations in order to identify the pathways and neurotransmitters involved in PYY release.

Materials and methods

Male Wistar rats weighing 280–320 g were obtained from Iffa-Credo (Les Oncins, F-69210 L'Arbresle). They were fasted for

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18 h before the experiments, with free access to water, and anaesthetized with ethylurethane (1.125 g/kg, i.m.).

Duodenal catheter for meal administration

After median laparotomy, a 10-cm piece of Silastic tubing (1.02 × 2.16 mm, ID × OD) was inserted through the forestomach, passed into the proximal duodenum through the pylorus, and secured by a ligature over the pylorus and by a purse-string suture on the forestomach. The catheter was exteriorized through the laparotomy incision and the abdomen was closed.

Arterial catheter for blood sampling

A short polyethylene catheter (Clay Adams PE 50, 0.58 × 0.965 mm, ID × OD, length 15 mm) continued by a Silastic catheter (0.64 × 1.19 mm, ID × OD, length 15 cm) was inserted into a carotid artery and secured by a double ligature around the polyethylene part of the catheter. The rats immediately received 250 IU/kg heparin, injected through the carotid catheter as soon as it was secured. The catheter was then filled with saline and clamped. Blood samples (0.6 or 1 ml under stimulated or basal conditions, respectively) were collected in Eppendorf 1.5-ml centrifuge tubes containing 2 mg EDTA and 500 kIU aprotinine (50 µl Trasylol at 10,000 kIU/ml) per ml whole blood, after having discarded the first drops, corresponding to the catheter dead space. Blood samples were collected before the meal, and 15, 30, 60 and 120 min thereafter. To compensate for blood loss, an equal volume of Hæmaccel (Behring, Marburg, Germany) was injected through the carotid catheter after each blood sampling. After 3 min centrifugation at 7,000 g (Biofuge 13, Heraeus, Les Ulis, France), the plasma samples were collected and stored at –20°C until the PYY assay.

Truncal vagotomy

In 12 rats, cervical bilateral truncal vagotomy was carried out 30–60 min before administration of the intraduodenal meal.

Perivagal capsaicin pretreatment

Between 12 and 15 days before the PYY release experiment, a 1% solution of capsaicin in 90% olive oil plus 10% Tween 80 was applied for 30 min to each vagus nerve in eight rats anaesthetized with ketamine (100 mg/kg, i.p.) plus atropine sulphate (1 mg/kg, i.p.). Sham rats (n = 8) were similarly treated with the capsaicin vehicle [33]. Then, 10 days later, the effectiveness of the destruction of vagal afferent fibres by capsaicin was checked by a test of the effect of CCK on satiety, in which food intake was measured for 1 h after the i.p. administration of 40 µg/kg sulphated CKK octapeptide (CCK 8). We checked that the CCK-induced decrease of food intake was suppressed in capsaicin-treated rats, but remained present in sham-treated rats [42].

Caecocolonectomy

To examine the role of the caecum and colon, the plasma PYY in rats submitted to an acute total caecocolonectomy, carried out when the duodenal catheter was inserted (i.e. about 2 h before giving the meal) was measured. In these rats, a delay of at least 1 h was maintained between the caecocolonectomy and the insertion of the carotid catheter, in order to allow a proper haemostasis of the caecocolonic area before heparin was given to the rats for blood sampling.

Intraduodenal meal

The meal consisted of 3 ml of a semi-liquid diet, containing 21 kJ, provided as 57% carbohydrate (Caloreen: small glucose polymers), 13% lipid (Liprocil: medium-chain triglycerides), and 30% lactoserum protein, (courtesy of Clintec Nutrition Clinique, Sèvres, France). The meal was administered over 3 min to normal rats (controls), to surgically treated rats (vagotomized or caecocolonectomized) and to rats treated with pharmacological agents. Activated charcoal (Norit A, Aldrich, USA, 20 mg/3 ml) was added as a non-absorbable marker. Occasionally, 7% phenol red was also added to the meal as a marker of the liquid phase. Controls were given 3 ml of 154 mM saline intraduodenally.

Pharmacological treatments

Before administration of the meal eight groups of rats received one of the following treatments: tetrodotoxin (TTX), 5 µg/kg, i.v.; the CCK_A and CCK_B receptor antagonists L 364,718, 0.5 mg/kg i.p., and L 365,260, 1 mg/kg i.p. (courtesy of Dr. P. S. Anderson, Merck, USA); the bombesin antagonists BIM-26,226 [(D-pentafluoro-Phe_6,D-Ala_{11})BN(6–13)methyl ester, courtesy of Drs. J. Blumberg, Ipsen-Biotech, France, and P. Eden, Biomeasure, Canada], 50 µg/kg i.v., and JMV 641 {(DPhe-Glu-Trp-Ala-Val-Gly-His-NH-CH[CH_2-$CH(CH_3)_2$]-(CHOH)-$(CH_2)_3$-CH_3 courtesy of J. Martinez, CNRS URA 1845, Montpellier, France}, 100 nmol $kg^{-1}\cdot h^{-1}$; the NO synthase inhibitor L-*N*-arginine methyl ester (L-NAME), 13.5 mg/kg i.v.; an antiadrenergic mixture of idazoxan, 0.3 mg/kg plus prazosin, 0.5 mg/kg plus propranolol, 1 mg/kg i.v., 10 min before the meal; the ganglionic blocker hexamethonium, as an i.v. bolus (6.7 mg/kg), 1 h before the meal, followed by an i.v. infusion (6.7 mg $kg^{-1}\cdot h^{-1}$) until the end of the experiment; atropine, 75 µg/kg plus 75 µg $kg^{-1}\cdot h^{-1}$, i.v. (same schedule as hexamethonium). These doses were chosen according to previous experiments from our laboratory or from others: TTX [30], L365,260 [24], BIM 26,226 [10], JMV 641 [28] and L-NAME [21].

In a group of 12 rats, PYY release was measured for 2 h after the i.v. injection of 2 deoxy-D-glucose (225 mg/kg), a centrally acting vagal stimulant.

Measurement of PYY-like immunoreactivity in gastrointestinal tissues

Four rats weighing 280–320 g were fasted for 18 h, with free access to water, sacrificed by a blow on the neck and exsanguinated by aortic puncture. The whole digestive tract was excised and partitioned as follows: stomach, duodenum (from the pylorus to the Treitz ligament), jejuno-ileum (which was divided into ten equal segments of about 10 cm each), caecum, colon (which was divided in two equal segments) and rectum (2 cm proximal to the anus). Tissue samples were boiled in 5 vol of acetic acid 0.5 M for 5 min, then cooled in an ice-cold bath and 5 ml trifluoroacetic acid (4%) was added, according to Reinshagen et al. [34]. The mixture was homogenized for 2 min at 20,000 rpm (Ultra-Turrax, Janke and Kunkel, Staufen, Germany) and centrifuged at 3000 *g* for 30 min at 4°C. The supernatants were separated and stored at –20°C until PYY contents were assayed.

Intestinal transit of the meal

At the end of the PYY release experiments (120 min after administering the meal) the rats were sacrificed by an overdose of anaesthetic. The whole small bowel was withdrawn and laid out. The position of the charcoal-coloured meal front was determined and expressed as the percentage of the total small bowel length (total length of the small bowel: about 100 cm).

In a control group, the intestinal transit of the liquid phase of the meal was compared to the transit of the solid phase by using both phenol red and charcoal as markers. The presence of phenol

red in the ileal segment proximal to the charcoal-coloured meal front and its absence in the distal ileal segment were checked. For that purpose, the distal ileal segment and the proximal one (of the same length) were rinsed with 0.5 ml distilled water/cm intestine, the ileal contents were alkalinized by adding 0.1 ml NaOH 0.25 M/ml, centrifuged 15 min at 3000 *g* and the optical density was measured at 540 nm. No significant amount of phenol red was detected distal to the charcoal-marked front of the meal. Since the transit rates of the two markers were identical, only charcoal was routinely added to the meal.

In a separate experiment, the intestinal transit of the intraduodenal meal was also measured 4, 15 and 30 min after the meal, to determine at which time the meal had reached the L-cell-rich zone of the small intestine.

Intravenous infusion of CCK and bombesin

To investigate whether CCK and bombesin would release PYY in rats, sulphated CCK 8 and bombesin (purchased from Neosystem, Strasbourg, France) were intravenously infused in increasing doses, each for 40 min: 40, 430 and 1340 pmol $kg^{-1} \cdot h^{-1}$ for CCK 8, and 1, 3.3 and 10 nmol $kg^{-1} \cdot h^{-1}$ for bombesin. These doses were chosen as follows: the largest doses of CCK 8 and bombesin maximally stimulated pancreatic exocrine secretion in anaesthetized rats ([27] and unpublished data from our laboratory), while the smallest dose of CCK 8 mimicked the upper postprandial CCK blood level in rats [20]. Blood samples for PYY determination were collected before beginning the infusions, and at the end of each 40-min infusion period.

PYY radioimmunoassay

Plasma PYY was measured by a radioimmunoassay developed in our laboratory, and described in detail in [12]. Briefly, we used the antibody A4D (courtesy of J.C. Cuber, INSERM U 45, Lyon, France) raised in the rabbit against synthetic porcine PYY. Antibody A4D cross-reacts 100% with porcine $PYY_{1–36}$ and $PYY_{3–36}$ and less than 0.1% with neuropeptide Y (NPY) and pancreatic polypeptide. The tracer was porcine [^{125}I-Tyr1] monoiodo-PYY (courtesy of T. Voisin, INSERM U 410, Paris, France), the standard was synthetic porcine PYY (Neosystem). The minimum detectable amount of PYY was 2 pg, the intra-assay coefficient of variation, 8.9%, and the interassay coefficient of variation, 7.5–13.0% for values ranging between 30 and 1000 pg/ml. Recovery of PYY (250 pg/ml) added to plasma was 92%. Standard curves run using Haemaccel and hormone-free plasma were not significantly different, indicating that rat plasma did not interfere in the assay.

Tissue PYY

Samples of the supernatants from tissue extracts were assayed in duplicate at four dilutions (1/1 to 1/1000) to fit in the standard assay curve. Results were expressed as nanogram PYY contained in each intestinal segment analysed.

Expression of results

Data are expressed as means ± SEM. PYY concentrations are given in pg/ml. Integrated increases of plasma PYY over basal values during the 120 min after the meal (area under the curve) were calculated and expressed as ng $ml^{-1} \cdot 120$ min.

Statistical analysis of the data was performed by ANOVA followed by a Dunnett's test when several groups were compared, by the Student's *t*-test when only two groups were compared, or by the non-parametric Mann-Whitney test when the variance of the groups compared was too different to allow a proper use of ANOVA. Differences at $P<0.05$ were considered significant.

Results

Effect of caecocolonectomy on basal and meal-stimulated PYY

In control rats, the plasma PYY concentration after the intraduodenal meal increased from 27 ± 4 pg/ml to a peak of 395 ± 45 pg/ml (i. e. about 15-fold the basal level) at 60 min, and still remained at sevenfold the basal level (199 ± 21 pg/ml) at 120 min (Fig. 1A).

After caecocolonectomy, plasma PYY increased from 15 ± 2 pg/ml to a peak of 122 ± 26 pg/ml, 60 min after the intraduodenal meal (Fig. 1A). The PYY response to the intraduodenal meal was clearly decreased, producing an integrated 120-min increase of 10.0 ± 1.8 ng ml^{-1} 120 min^{-1}, as compared to 30.1 ± 3.4 ng ml^{-1} 120 min in control rats, i.e. a 67% decrease ($P<0.001$ versus control rats) (Fig. 1B).

In control rats receiving 3 ml saline in the duodenum instead of the meal, very little PYY was released (1.4 ± 0.6 ng ml^{-1} 120 min) (Fig. 1).

Effect of neural antagonists on meal-stimulated PYY release

In rats treated with TTX, plasma PYY increased from 15 ± 3 pg/ml to a peak of 178 ± 42 pg/ml, 30 min after

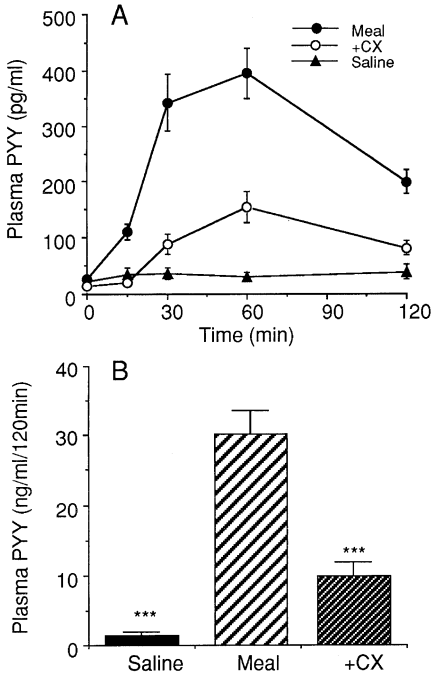

Fig. 1A, B Peptide YY (*PYY*) release by an intraduodenal meal (3 ml of a semi-liquid diet containg 21 kJ provided as 57% carbohydrate, 13% lipid and 30% protein) in normal rats (*Meal*, $n = 18$ rats) and in caecocolonectomized rats (*+CX*, $n = 9$). In a third group of 5 rats, saline was administered instead of the meal as a control (*Saline*). **A** Time course of plasma PYY. **B** The 120 min-integrated PYY responses over the basal level. Mean ± SEM; *** $P<0.001$ versus Meal

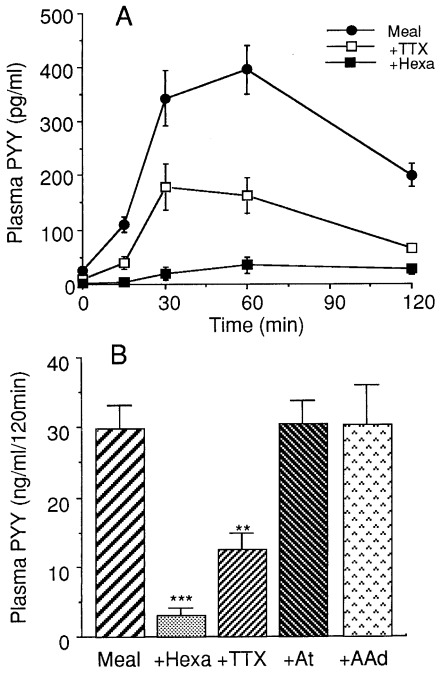

Fig. 2A, B Effect of neural antagonists on meal-stimulated plasma PYY. **A** Time course of plasma PYY. **B** Integrated responses over the basal level in rats given the intraduodenal meal alone (*Meal*, $n = 18$), after hexamethonium (*+Hexa*, $n = 6$), after tetrodotoxin (*+TTX*, $n = 5$), after atropine (*+At*, $n = 5$), and after a mixture of α1, α2 and β adrenoceptor antagonists (*+AAd*, $n = 6$). Mean ± SEM; ** $P<0.01$, *** $P<0.001$ versus Meal

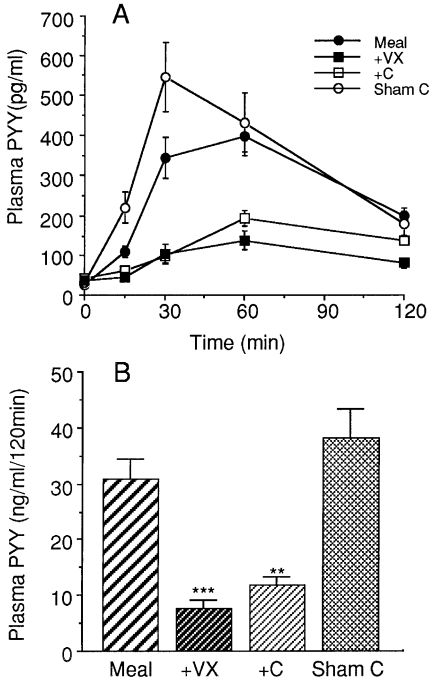

Fig. 3A, B Effects of vagotomy and of perivagal capsaicin treatment on meal-stimulated plasma PYY. **A** Time course of plasma PYY. **B** Integrated responses over the basal level in normal rats given the intraduodenal meal (*Meal*, $n = 18$), in vagotomized rats (*+VX*, $n = 12$), in rats treated by perivagal capsaicin (*+C*, $n = 6$), and in rats treated by perivagal capsaicin vehicle (*Sham C*, $n = 6$). Mean ± SEM; ** $P<0.01$, *** $P<0.001$ versus Meal

the meal, and then progressively decreased to 66 ± 5 pg/ml at 120 min (Fig. 2A). The integrated response was 12.8 ± 2.3 ng·ml^{-1} 120 min in TTX-treated rats, i.e. a 58% decrease versus control rats ($P<0.01$, Fig. 2B).

In hexamethonium-treated rats, a very weak increase of plasma PYY was observed after the meal (Fig. 2A). The integrated 120-min response was 2.7 ± 2.3 ng ml^{-1} 120 min, representing a 91% decrease ($P<0.01$) as compared to control rats (Fig. 2B).

Atropine, as well as the antiadrenergic mixture, did not modify the basal or meal-stimulated PYY release. The integrated responses were 30.9 ± 3.5 ng ml^{-1} 120 min with atropine and 30.8 ±5.7 ng ml^{-1} 120 min with the antiadrenergic mixture, neither being significantly different from the value of 30.1 ± 3.4 ng ml^{-1} 120 min observed for controls (Fig. 2B).

Effects of vagal nerve manipulations on PYY release

Basal plasma PYY was not significantly changed in vagotomized rats (37 ± 7 pg/ml), in perivagal capsaicin-pretreated rats (41 ± 2 pg/ml), and in sham perivagal capsaicin-pretreated rats (30 ± 5 pg/ml).

In vagotomized rats, the meal-induced PYY release was clearly decreased, peaking at 138 ± 24 pg/ml at 60 min after the meal, and decreasing to 81 ± 12 pg/ml after 120 min (Fig. 3A). The integrated response to the meal in vagotomized rats (7.4 ± 1.5 ng ml^{-1} 120 min) was decreased by 75% ($P<0.001$) as compared to that in control rats (Fig. 3B).

In rats submitted to perivagal capsaicin pretreatment, PYY release in response to the meal was close to that of vagotomized rats; the timecourse was similar (Fig. 3A), and the integrated response (11.4 ± 1.5 ng ml^{-1} 120 min) decreased by 70% ($P<0.01$) compared to the sham perivagal capsaicin-pretreated group (Fig. 3B).

To check the participation of efferent vagal fibres in meal-induced PYY release, the centrally acting vagal stimulant 2-deoxy-D-glucose (225 mg/kg) was administered to rats given intraduodenal saline. No significant increase of plasma PYY was observed after administration of 2-deoxy-D-glucose (not shown).

Effects of antagonists of CCK, bombesin and NO synthase on meal-induced PYY release

The CCK_A antagonist L 364,718, the CCK_B antagonist L 365,260 and the bombesin antagonists BIM-26,226 and JMV 641 did not significantly modify the meal-induced PYY release with respect to control experiments. The integrated responses were, respectively: 30.2 ± 4.9, 33.3 ± 6.4, 27.2 ± 5.7 and 37.6 ± 6.5 ng ml^{-1} 120 min (Fig. 4B).

After application of the NO synthase inhibitor L-NAME, the meal-induced PYY release was clearly

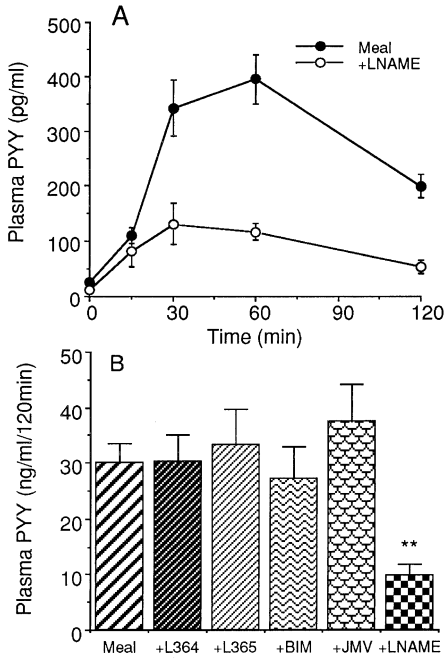

Fig. 4A, B Effect of antagonists of CCK_A, CCK_B and bombesin receptors and of a nitric oxide synthase inhibitor on meal-stimulated plasma PYY. **A** Time course of plasma PYY. **B** Integrated responses over the basal level in rats given the intraduodenal meal alone (*Meal*, n = 18), or the intraduodenal meal after L 364,718 (+*L364*, n = 6), after L 365,260 (+*L365*, n = 6), after BIM-26,226 (+*BIM*, n = 6), after JMV 641 (+*JMV*, n = 6) and after L-*N*-arginine methyl ester (+*LNAME*, n = 5). Mean ± SEM; *** P<0.01 versus Meal

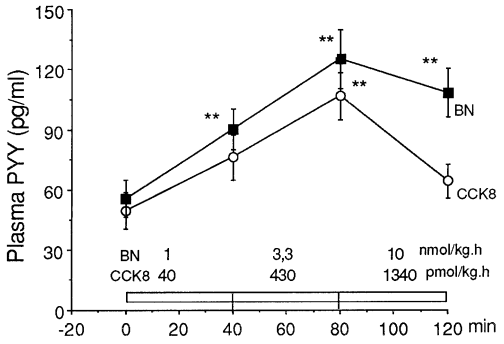

Fig. 5 Variations of plasma PYY in response to i.v. infusions of increasing doses of cholecystokinin octapeptide (*CCK 8*) or bombesin (*BN*), each for 40 min. Plasma PYY was measured before infusions, and at the end of each infusion period. Mean ± SEM, n = 6 animals per dose. ** P <0.01 versus basal level

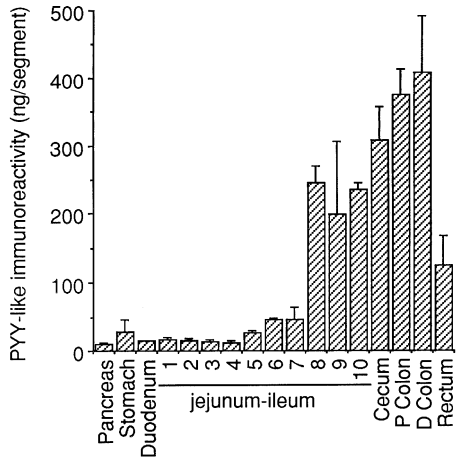

Fig. 6 Distribution of PYY-like immunoreactivity in the rat gastro-intestinal tract. The jejunum-ileum was divided in 10 equal segments, the colon was divided in proximal colon (*P colon*) and distal colon (*D colon*), n = 4

smaller than that after the meal alone (Fig. 4A). Starting from a basal level of 15 ± 4 pg/ml, plasma PYY peaked at 131 ± 38 pg/ml at 30 min and then decreased to 54 ± 12 pg/ml at 120 min (Fig. 4A). The integrated response (9.8 ± 2.1 ng ml^{-1} 120 min) was 57% smaller than the meal-induced response measured in controls (P<0.005, Fig. 4B).

Effects of CCK 8 and bombesin infusions on PYY release

CCK 8 increased plasma PYY in a dose-related manner from 50 ± 9 pg/ml before infusions to 76 ± 11 pg/ml after 40 pmol kg^{-1} h^{-1} and 106 ± 12 pg/ml after 430 pmol kg^{-1} h^{-1} (P<0.01). The dose 1340 pmol kg^{-1} h^{-1} was less active than 430 pmol kg^{-1} h^{-1}, and PYY increase was not significant after this dose of CCK (Fig. 5).

Bombesin was a little more active than CCK 8, with a very close profile and a maximally active dose about sevenfold higher than that of CCK 8 (Fig. 5).

PYY-like immunoreactivity in the gastrointestinal tissues

PYY-like immunoreactivity of all analysed tissues and intestinal segments is reported in Fig. 6. The proximal gastrointestinal tract, from the stomach to 70% of the jejuno-ileum, contained very little PYY. Then, PYY contents increased abruptly from the distal 30% of the jejuno-ileum to the rectum. Ranked as decreasing contents, the order of PYY-rich segments was colon > caecum > distal ileum > rectum.

Effect of the different treatments on intestinal transit of the intraduodenal meal

In control rats given the intraduodenal meal, the meal front had reached 29 ± 1.6% of the small bowel length after 4 min, 47 ± 1.3 after 15 min, 62 ± 3.2% after 30 min and 71 ± 0.2% after 120 min. Thus, after 120 min, the meal front just began to reach the PYY-rich part of the ileum (Fig. 6). Expressed another way, after 30 min, at which time the plasma PYY was submaximally increased, nutrients of the meal had come into contact with an amount of mucosa containing only 11% of

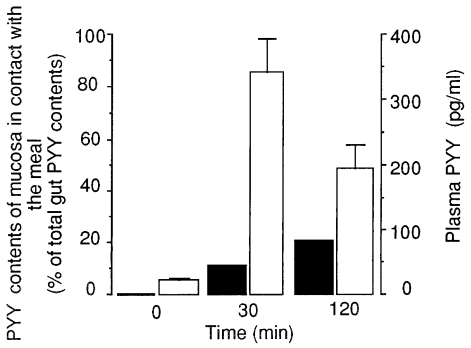

Fig. 7 Plasma PYY (*open columns*) and PYY contents of the intestinal segments in contact with the meal nutrients (*dark columns*) before the meal (0) and 30 and 60 min after administration of the meal. PYY contents were expressed as percentage of PYY contained in the whole gut, from duodenum to rectum. $n = 6$ (plasma PYY) and 4 (PYY contents of the gut)

the total PYY contents of the whole gut from the duodenum to the rectum (Fig. 7). After 120 min, the mucosa that had been reached by meal nutrients contained only 20% of the total PYY gut contents. The relationship between plasma PYY and the PYY contents of the mucosa in contact with the meal is shown in Fig. 7, demonstrating that the peak plasma PYY occurred at a time when nutrients were in contact with PYY-poor parts of the gut.

Intraluminal transit of the meal in the different treatment groups, expressed as the percentage of small bowel length reached by the meal front after 120 min, is reported in Table 1. In no case did the meal front reach the ileo-caecal valve. Only the rats receiving hexamethonium had a significantly slower transit (56 ± 7.5%) than controls. The transit was significantly faster in some groups, but never reached more than 90% of the total small bowel length. In these groups with faster transit PYY plasma release was never significantly larger than in controls.

Discussion

The technical conditions used in the present work were appropriate to study the early postprandial release of PYY, but not the late phase of PYY release, related to the presence of nutrients in the colon, since we stopped plasma PYY determinations 120 min after the meal, at which time nutrients had not reached the ileo-cecal valve. A double mechanism of PYY release (early indirect and late direct) has been clearly evidenced to occur in the dog [14] and has been suggested to occur in the rat [38]. Previous results from our laboratory added to the information on this double mechanism, since PYY release produced by intraduodenal oleic acid was suppressed by hexamethonium, while PYY release produced by intracolonic oleic acid was not [12].

Since PYY release after the intraduodenal meal was strongly decreased by TTX and hexamethonium, signal transmission is likely to be neuronal. The effect of TTX, however, was only partial; this may be due to an insufficient dose, since the in vivo toxicity of TTX does not allow injection of completely active doses of this substance, which would seriously affect vital functions. Hexamethonium nearly totally suppressed PYY release, indicating that at least one nicotinic synapse was involved in the transmission pathway to L-cells. Hexamethonium also produced a significant decrease of intestinal transit (56 versus 72% of total small intestine length) which may have participated in the reduction of PYY release.

The comparison of plasma PYY and cumulated PYY-like immunoreactivity of intestinal segments reached by the meal 30 and 120 min after intraduodenal administration (Fig. 7) shows that after 30 min, at which time plasma PYY submaximally increased, only 11% of PYY-producing cells of the gut could have been directly stimulated by the luminal contents. This percentage increased to 20% at 120 min, at which time plasma PYY had decreased to about 50% of its peak level. These results reinforce the concept that early release of PYY is triggered by the arrival of nutrients in the proximal small bowel, which sends PYY-releasing signals to the distal, PYY-rich digestive tract. It is unknown whether the proximal

Table 1 Percentage of the small bowel length reached by the meal front after 120 min

	Treatment	Meal	+Cx	+Hexa	+TTX	+At	+AAd	+Vx
% of small	Mean	72.2	71.3	56.3*	71.9	85.8*	90.2**	65.1
Bowel length	SEM	2.4	3.8	7.5	7.4	3.3	3.0	2.7

	Treatment	+C	+Sham C	+L 364	+L 365	+BIM	+LNAME
% of small	Mean	83.9*	86.4*	85.0*	83.7*	73.7	77.5
Bowel length	SEM	4.1	0.5	3.4	3.9	3.1	1.6

The meal was administered alone (meal) or after one of the following treatments: Cx: colonectomy, Hexa: hexamethonium (nicotinic antagonist), TTX: tetrodotoxin (sodium channel blocker), At: atropine (muscarinic antagonist), AAd: propranolol+prazosin+idazoxan (antiadrenergic mixture), Vx: vagotomy, C: perivagal capsaicin, Sham C: sham perivagal capsaicin, L 364: L 364,718 (CCKA antagonist), L 365: L 365,260 (CCKB antagonist), BIM: BIM 26,226 (bombesin antagonist), L-NAME: L-N arginine methyl ester (NO-synthase inhibitor). See "Methods" for doses and details. * $P<0.05$, ** $P<0.01$ versus meal alone.

site of action to stimulate distal release of PYY is located in the duodenum, jejunum, proximal ileum or all three.

Acute caecocolonectomy decreased by 67% the 120-min postprandial PYY release in our experiments. This suggests that caecocolonic L-cells participate in this phase of PYY release, and is in accordance with the complete suppression of a PYY response to a meal after ileocolonectomy in dogs [13], and with the data showing the presence of L-cells in the distal ileum, caecum, colon and rectum (the present work and [1, 5, 13, 19, 26]). The role of the colon in postprandial PYY release has been challenged [40] on the basis of chronic experiments in which basal PYY was not affected 10 days after total colonectomy, while postprandial PYY release was 50% greater than in sham-operated rats. However, these effects may have been due in part to an adaptative phenomenon, since in another study basal plasma PYY was elevated 1–12 weeks after subtotal colonectomy in rats [45]. In addition, the strain of rats used by these authors may have had an effect, since PYY concentrations were unusually high in the duodenum and jejunum of their rats (respectively 50 and 66% of the colonic concentration), while most studies report values varying between trace amounts [13] to 0.6% (duodenum) and 5% (jejunum) [26], or 6% for the proximal third of the small intestine [19], which quite agree with the data we report herein.

Inhibition of postprandial PYY release by vagotomy and by perivagal capsaicin strongly suggests that afferent vagal fibres are involved. No evidence was found for the participation of efferent vagal fibres, since no PYY release was observed following 2-deoxyglucose administration. This was also observed to occur in the dog: 2-deoxyglucose [39] and insulin [43] did not release PYY, while electrical stimulation of the peripheral ends of cut vagus nerves released PYY [48]. This last result may have been due to antidromic activation of sensory fibres by electrical stimulation. Splanchnic efferent fibres might also be involved, since in the dog splanchnic electrical stimulation released more PYY than did vagal stimulation [48], whereas in the pig splanchnic stimulation released less PYY than did vagal stimulation [41]. Thus, efferent fibres implicated in PYY release might be mainly splanchnic in the dog and in the rat, and mainly vagal in the pig.

Whatever the fibre pathway, the peripheral transmitter is non-cholinergic and non-adrenergic in the rat, since atropine and adrenolytics had no effect, and this transmitter may be, at least in part, nitric oxide, since an inhibitor of NO-synthase was efficient in reducing postprandial PYY release. Since we used a comparatively large dose of L-NAME, non-specific anticholinergic side-effects of L-NAME may have occurred. This, however, should not seriously influence the results, since PYY release was not changed by the potent anticholinergic drug atropine. In dogs, a peripheral non-cholinergic non-adrenergic mechanism was evidenced [48], but the participation of a nitrergic system has not been investigated.

In dogs, a complex relationship between CCK and PYY has been documented: exogenous CCK releases PYY [14, 25]; oleate and fat meals release both CCK and PYY [23, 25], while a CCK_A antagonist suppresses food-induced PYY release [25]. Conversely, exogenous PYY decreases CCK release [18, 23], thus suggesting a possible control loop between CCK and PYY after meals. In vitro, CCK did not release PYY from cultured rat fetal intestinal cells [9]. We demonstrate here that supraphysiological CCK doses can release PYY in the rat, but this is unlikely to participate in postprandial PYY release since CCK_A and CCK_B antagonists did not modify postprandial PYY release. Mechanisms implicated in the effect of such large doses of CCK could differ from those occuring at physiological doses. It has been clearly evidenced that CCK 8 stimulates pancreatic exocrine secretion via CCK_A receptors of vagal afferent neurons, while doses producing supraphysiological plasma CCK levels act on intrapancreatic neurons and on pancreatic acini [20].

In a rat model, using pancreatic juice diversion in the absence of intraluminal nutrients, Guan et al. [16] found increased CCK and PYY levels, and a CCK_A-antagonist-suppressed PYY increase. However, under conditions of elevation of plasma CCK by soybean trypsin inhibitor or by infusion of exogenous cerulein, no PYY was released. Therefore, the authors suggested that CCK receptors alone were not sufficient to modify PYY release.

In chronic experiments, PYY and PYY mRNA increased in the intestine and the plasma following a 70% small intestinal resection. This increase was reinforced by a CCK_A antagonist, suggesting an inhibitory function of CCK receptors on PYY synthesis [22].

In vitro, bombesin released PYY from cultured rat fetal intestinal cells [9], from dog cultured colonic cells [4] and from vascularly perfused isolated rat [31] and pig [41] colon. In vivo in dogs, an intravenous infusion of exogenous bombesin increased plasma PYY [11]. In previous experiments performed using rats, infusing 250 pmol $kg^{-1} \cdot h^{-1}$ of bombesin did not change plasma PYY levels [16], while the large dose of 10,000 pmol kg^{-1} h^{-1} increased plasma PYY (basal × 2.75) in about the same proportion as in our conditions [44]. In our hands the maximally effective dose of bombesin was 3,300 pmol $kg^{-1} \cdot h^{-1}$, and this released about 30% of the postprandial PYY peak level. However, the bombesin antagonists BIM-26,226 and JMV 641 did not change postprandial PYY release, suggesting that endogenous bombesin did not participate in the physiological postprandial PYY release.

Besides PYY, the endocrine L-cells synthesize and release proglucagon-derived peptides (PGDPs). In a recent work Roberge et al. [37] reported that large doses of exogenous gastrin-releasing peptide (GRP) released a little PGDP, while intraduodenal corn oil was a more potent releaser. These data agree with our results dealing with PYY. In contrast, infusion of the GRP antagonist BW10 completely abrogated the PGDPs response to duodenal fat, suggesting a GRP-dependent mechanism for PGDPs

release. Several differences could account for this discrepancy: first PYY andPGDPs, although colocalized in the same endocrine L-cells, could be released and regulated by different mechanisms. Second the stimuli of peptide release were different, since Roberge et al. used pure corn oil in a ligated duodenal loop in which distension and ligation could both produce conditions different from those in these experiments. Although the bombesin antagonists used in the present study were different from BW10 used in [37], they were probably used in efficient doses. BIM 26,226 was injected at half the dose that totally inhibited the pancreatic exocrine response to 2 nmol kg^{-1} h^{-1} bombesin in the rat [10], and one might object that this dose might have been insufficient. However, identical results were obtained with JMV 641, which was infused at a dose suppressing totally the pancreatic response to the maximal dose of 10 nmol kg^{-1} h^{-1} bombesin in the rat [28].

Other peptides, such as glucose-dependent insulinotropic peptide and calcitonin gene-related peptide, have been proposed to act as intermediates in the release of enteroglucagon maturation products [8, 35, 36] and of PYY [9, 31], but we presently have no data allowing a discussion of the possible role of these peptides in our model.

In conclusion, PYY release observed to occur in the rat during the 2 h following an intraduodenal meal is essentially due to the indirect activation of ileal and colonic L-cells through neural mechanisms involving afferent vagal fibres and a reflex pathway including nicotinic synapses and NO release. Whether other peptides and neurotransmitters are also involved in this complex mechanism remains to be investigated.

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