Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 4471–4475, July 1982
Physiological Sciences

Localization of peptide YY (PYY) in gastrointestinal endocrine cells and effects on intestinal blood flow and motility

(distal intestine/pancreatic polypeptides)

J. M. LUNDBERG*†, K. TATEMOTO‡, L. TERENIUS§, P. M. HELLSTRÖM*, V. MUTT‡, T. HÖKFELT¶, AND B. HAMBERGER‖

Departments of *Pharmacology, ‡Biochemistry, and ¶Histology, Karolinska Institutet, Stockholm, Sweden; ‖Department of Surgery, Karolinska Hospital, Stockholm, Sweden; and §Department of Pharmacology, Uppsala University, Uppsala, Sweden

Communicated by Rolf Luft, March 29, 1982

ABSTRACT **In immunohistochemical studies using antisera to peptide YY (PYY), a 36 amino acid polypeptide isolated from porcine duodenum, it was found that PYY-like immunoreactivity occurred mainly in endocrine cells of the gastrointestinal mucosa. PYY-immunoreactive cells were particularly abundant in the distal intestine and have been observed in five species, including man. By radioimmunoassay it was found that, in the rat, the amount of PYY immunoreactivity was about 100-fold higher in the colon than in the duodenum. The chromatographic profiles of PYY immunoreactivity from the rat colon and porcine PYY on a SP-Sephadex ion exchanger were similar. Furthermore, serial dilutions of extracts from the rat colon and porcine PYY had parallel displacement curves in radioimmunoassay. Close intraarterial administration of PYY in cats caused an intestinal vasoconstriction and an inhibition of jejunal and colonic motility. Simultaneously there was a rise in systemic arterial blood pressure. These effects of PYY were also observed after pretreatment with adrenergic blocking agents. It is concluded that PYY is a gastrointestinal peptide that is present mainly in endocrine cells of distal intestine and that has effects on both intestinal motility and the cardiovascular system.**

Pancreatic polypeptides (PPs) of 36 amino acids have been isolated from the pancreata of several species (1–3). Immunohistochemical studies suggest that a PP-like substance is also present in neurons (4, 5). Recently a peptide with structural similarities to PP, peptide YY [PYY, the peptide (P) having NH_2-terminal tyrosine (Y) and COOH-terminal tyrosine (Y) amide], has been isolated from porcine duodenum by making use of an assay for its COOH-terminal tyrosine amide structure (6, 7). In the present communication we report on the cellular localization of PYY, measurements and characterization using radioimmunoassay, and some of its effects on gastrointestinal functions. The results indicate that PYY is localized in gut endocrine cells of several species, including man, particularly in middle and distal intestine. Furthermore, PYY has a vasoconstrictor action and inhibits jejunal and colonic motility. We therefore propose that PYY is a gastrointestinal peptide with paracrine or hormonal actions.

MATERIALS AND METHODS

PYY isolated from porcine duodenum was used (6). Antisera to PYY were produced in rabbits by using a direct immunization procedure. PYY (50–100 μg) was emulsified with Freund's adjuvant and injected intradermally at multiple sites. Booster doses were given subcutaneously at 2-week intervals for 2 months.

PYY was labeled with ^{125}I to a high specific activity (about 200 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) by using the chloramine-T method (8). The peptide was separated from free iodine by chromatography on a small Sephadex G-25 column in 50% (vol/vol) acetic acid. The eluate was lyophilized and the labeled peptide was stored in aliquots in distilled water at −20°C. For studying antibody specificity, antiserum at 1:2,000 final dilution and 6,000 cpm of labeled PYY in 0.22 ml of buffer (0.1% gelatin/0.1% Triton X-100/0.01% bovine serum albumin in sodium phosphate buffer, pH 7.5) were incubated at 4°C overnight. The incubation was terminated by the addition of activated charcoal [200 μl of a suspension of 500 mg of active charcoal (Sigma), and 50 mg of dextran (D-70, Pharmacia) in 200 ml of 0.01 M sodium phosphate buffer, pH 7.4]. A 300-μl aliquot of the supernatant was measured for antibody-bound peptide. After correction for blanks, inhibition curves for different unlabeled peptides were calculated. The antiserum used (69D) does not crossreact to any major extent with avian PP, neurotensin, gastric inhibitory peptide (Fig. 1), glucagon, enkephalin, somatostatin, cholecystokinin, or vasoactive intestinal polypeptide. A small crossreaction (<0.1%) was found towards bovine PP (Fig. 1).

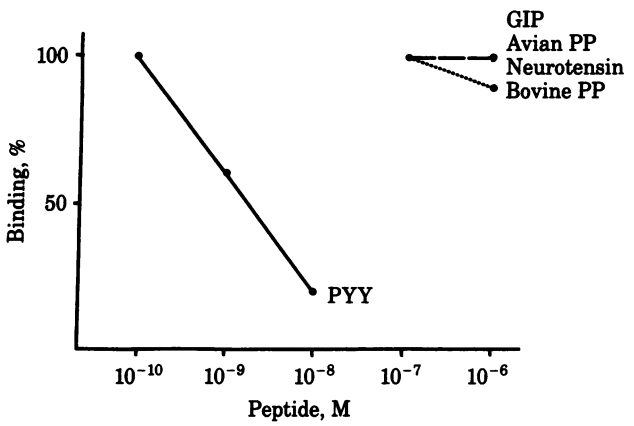

FIG. 1. Characteristics of the PYY antibody as indicated by inhibition of ^{125}I-labeled PYY binding by increasing concentrations of porcine PYY, bovine PP, avian PP, gastric inhibitory peptide (GIP), and neurotensin.

Some rat tissues (whole brain, duodenum, jejunum, ileum, colon, and pancreas) (about 300 mg of each) were thoroughly cut and immediately heated at 95°C in 6 ml of 1 M acetic acid for 15 min. After cooling, the samples were homogenized in a

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: PP, pancreatic polypeptide; PYY, the peptide (P) having NH_2-terminal tyrosine (Y) and COOH-terminal tyrosine (Y) amide.

† To whom reprint requests should be addressed at: Dept. of Pharmacology, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden.

Teflon/glass homogenizer. The homogenate was heated at 95°C for a further 5 min, cooled on ice, and centrifuged at 4°C. A 1-ml aliquot of the supernatant was added to 1 ml of pyridine/formate buffer, pH 3 (0.018 M pyridine/0.1 M formic acid). The sample was applied on a small (1 ml) SP-Sephadex C-25 (Pharmacia) ion-exchange column equilibrated in the same buffer. The column was then subjected to stepwise elution with pyridine/formate buffers (I, twice with 2 ml of 0.018 M pyridine/0.1 M formic acid, pH 3.01; II, 4 ml of 0.1 M pyridine/0.1 M formic acid, pH 4.32; III, 4 ml of 0.35 M pyridine/0.35 M formic acid, pH 4.35; IV, 4 ml of 1.0 M pyridine/1.0 M formic acid, pH 4.3; V, 4 ml of 1.6 M pyridine/1.6 M formic acid, pH 4.23). The elution patterns of porcine PYY and radioimmunoactive material from rat colon were then compared. For studies on

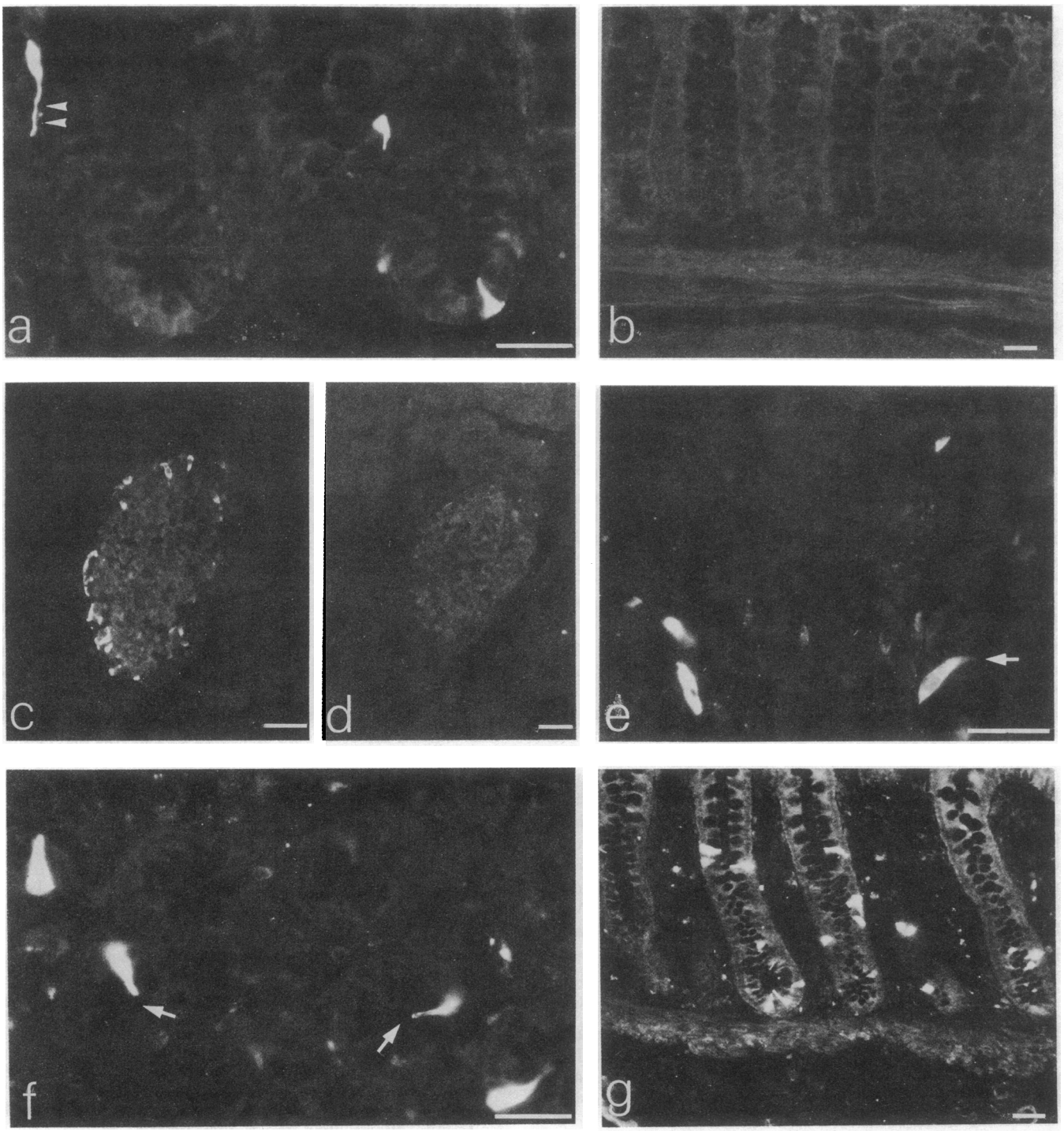

FIG. 2. Immunofluorescence micrographs of the rat colon (*a*, *b*), rat pancreas (*c*, *d*), and pig duodenum (*e*) and human colon (*f*, *g*) after incubation with PYY antiserum (*a*, *c*, *e*, *f*, *g*) and control serum (PYY antiserum preabsorbed with PYY) (*b*, *d*). PYY-like immunoreactivity is seen in endocrine cells in the mucosal epithelium of the intestine with an apical portion towards the lumen (arrows in *e* and *f*). Occasionally a long process was observed extending from the basal portion of the PYY immunoreactive cells (arrowheads in *a*). PYY immunoreactivity is also seen in some cells lying in the periphery of the islets of Langerhans of the rat pancreas (*c*). After incubation with control serum no fluorescent cells are seen (*b*, *d*). Bars indicate 50 μm.

tissue distribution of PYY, fraction IV was sampled and lyophilized. Serial dilutions of aliquots were added to the immunoassay system. Antibody displacement caused by the extract was then compared with the standard. The present isolation procedure had a recovery of PYY added to a cerebellar extract of about 65%.

Immunohistochemistry according to the indirect immunofluorescence methods of Coons and collaborators was performed on various parts of the intestine, pancreas, and brain, on sympathetic ganglia and the vas deferens as well as on the pituitary, thyroid, and adrenal glands of four rats and four guinea pigs. Furthermore, the duodenum, ileum, and ascending colon were studied in three cats and three pigs and the colon and pancreas in human patients. Human tissues were obtained from normal areas removed at tumor surgery on the areas. The present study was approved by the ethical committee at the Karolinska Hospital (81:204). After rinsing in ice-cold saline for 15 min, tissues were fixed by immersion in a mixture of 0.25% parabenzoquinone (Sigma) and 5% formalin at 4°C in phosphate buffer (9). After rinsing in sucrose and cryostat sectioning, the tissues were processed as described (9). Briefly, the sections were incubated in a humid atmosphere at 4°C for 14–20 hr with PYY antiserum (dilution 1:100), rinsed, incubated at 37°C for 30 min with fluorescein isothiocyanate-conjugated sheep antiserum to rabbit IgG (DAKO, Copenhagen), rinsed, mounted, and examined with a Zeiss fluorescence microscope. For controls the antibodies were preabsorbed with PYY, avian PP, bovine PP, neurotensin, gastric inhibitory peptide, or glucagon at 0.25 or 25 nmol of peptide per 200 μl of antiserum diluted 1:100.

In vivo experiments were performed in six cats anesthetized with chloralose (50 mg/kg) and urethane (100 mg/kg). Intestinal blood flow was recorded via a polyethylene catheter in the superior mesenteric vein connected to a closed silicone-filled drop chamber. Jejunal and colonic motility were studied by volume recording devices. Flaccid balloons permitting registration of complete relaxation of the intestine were placed 25 cm oral to the ileocecal valve and in the proximal colon. The balloons were connected to water reservoirs of wide dimensions that were suspended in weight recorders (10). Intestinal blood flow and motility as well as systemic arterial blood pressure and heart rate were recorded on a Grass polygraph 7B. An arterial catheter was also introduced into the superior mesenteric artery via the branch forming a connection with the inferior mesenteric artery. To test the experimental setup, acetylcholine (5 nmol/kg per min) was locally infused at the beginning of each experiment. This produced a marked intestinal vasodilatation as well as contraction of both the jejunum and the proximal colon (10). At the end of the experiments, Evans blue was injected via the arterial catheter to indicate the extension of the vascular bed reached by the infusion. This caused staining from jejunum to midcolon. The adrenergic blocking agents guanethidine (Ismelin, CIBA; 3 mg/kg) and phentolamine (Regitine, CIBA; 3 mg/kg) were administered as slow intravenous infusions 30–60 min prior to local intraarterial infusions of PYY. PYY (25–150 pmol/kg) was dissolved in 0.9% NaCl and infused at a rate of 100 μl/min.

RESULTS

PYY immunoreactivity was localized to a population of endocrine cells in the intestinal mucosa (Fig. 2 *a*, *e–g*). The cells had a characteristic appearance with an apical portion reaching the lumen, occasionally with a process at the basal region (Fig. 2 *a*, *e–g*), sometimes as long as 25–30 μm (Fig. 2*a*). PYY immunoreactive cells were rare or absent in the stomach and duodenum, whereas many cells were observed in distal small intestine and in colon and rectum. This distribution was similar in rat (Fig. 2*a*) and guinea pig. PYY cells were also present in cat, pig (Fig. 2*e*), and man (Fig. 2 *f* and *g*). Preabsorption with PYY but not other peptides abolished the immunostaining (Fig. 2*b*). PYY immunoreactivity was also found in a population of cells in the periphery of the islets of Langerhans in the rat pancreas (Fig. 2*c*). The PYY immunoreactivity in their cells was not affected by preabsorption with 25 nmol of avian or bovine PP, whereas 0.25 nmol PYY abolished the immunostaining (Fig. 2*d*). Some PYY cells were also seen in the human pancreas. No PYY immunoreactivity was found in the pituitary, thyroid, or adrenal glands or in brain, sympathetic ganglia, or vas deferens of the rat or guinea pig. By radioimmunoassay it was found that high concentrations of PYY immunoreactivity were present in the rat colon (about 1 nmol/g of tissue) (Table 1). Considerable amounts were also found in the ileum and pancreas. The PYY levels in the proximal small intestine as well as in the brain were

Table 1. Distribution of PYY-like immunoreactivity in rat tissues

Tissue	PYY, pmol/g tissue
Duodenum	<12, <12, <12, 17
Jejunum	<30, <30, 40
Ileum	68, 203, 351, 378
Colon	710, 851, 967, 1,215, 1,312
Pancreas	68, 132, 192, 259, 270
Whole brain	<20, <20, <20
Plasma	<5, <5, <5

The values represent means of duplicate or triplicate determinations from individual rats.

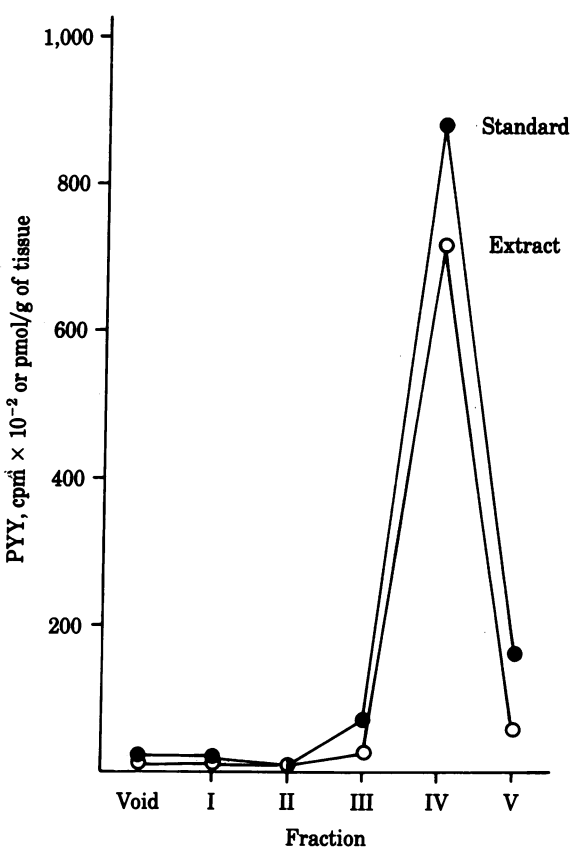

FIG. 3. Chromatographic profile of porcine PYY (●) or an extract from rat colon (○) on a SP-Sephadex ion exchanger. For definitions of fractions see *Materials and Methods*.

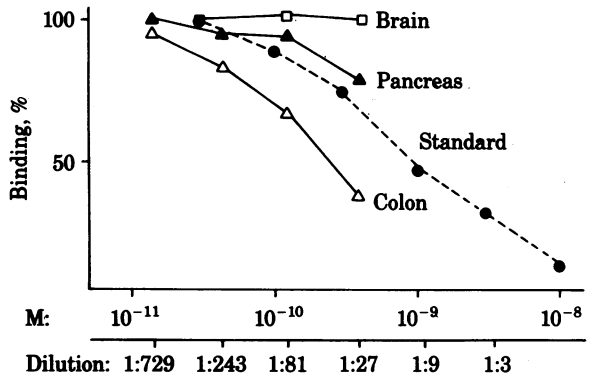

FIG. 4. Parallel displacement by porcine PYY and serial dilutions of extracts from rat colon and pancreas in radioimmunoassay.

close to or below detection limit. No PYY immunoreactivity was found in rat plasma. The chromatographic profiles of extracts from rat colon and porcine PYY on a SP-Sephadex ion-exchange column were similar (Fig. 3). Furthermore, serial dilutions of extracts from rat colon had parallel displacement curves with porcine PYY in radioimmunoassay (Fig. 4).

After close intraarterial administration of PYY at a dose of 25–150 pmol/kg per min to normal or to guanethidine- and phentolamine-pretreated cats, a dose-dependent intestinal vasoconstriction lasting for 1–2 min was recorded; it was followed by an escape response, which began during the infusion (Fig. 5). At the highest doses, the vasoconstriction was often accompanied by a parallel increase in systemic arterial blood pressure of a longer duration. The heart rate was usually somewhat reduced during the vasopressor response. During local intraarterial PYY infusion both jejunal and colonic motility were inhibited in the same doses that caused vasoconstriction. The inhibition of motility was most pronounced in the colon, where it lasted for up to 1 hr after cessation of infusion (Fig. 5).

DISCUSSION

The present data show that PYY, a recently isolated peptide, is present in a population of endocrine cells in the gastrointestinal mucosa of some species, including man. The cells are particularly abundant in the distal intestine. This intestinal distribution pattern is different from the distribution of most peptides, including PP, in endocrine cells of the gut (11). A small population of cells in the islets of Langerhans of the pancreas also seemed to contain PYY immunoreactive peptide. It cannot be definitely excluded, however, that this finding is due to the small crossreactivity of the PYY antisera with PP, especially because the structure of rat PP is not known. When administered locally *in vivo*, PYY caused a marked vasoconstriction and inhibition of colonic motility. The increase in systemic arterial blood pressure was of longer duration than the intestinal vasoconstriction, indicating that the vasoconstrictor response also involved other vascular beds. The intestinal vascular response to PYY is similar to the effects of sympathetic nerve stimulation, in which the vasoconstriction undergoes an autoregulatory escape during continuous stimulation (12). This effect has been attributed to a redistribution of blood flow from the mucosa to deeper intestinal wall layers (12). The effects of PYY on blood flow and motility were not secondary to release of norepinephrine, because adrenergic blocking agents did not inhibit the response. This is supported by the finding that PYY has a strong vasoconstrictor action in the submandibular salivary gland and in sympathectomized animals as well (unpublished results).

The localization of PYY in mucosal endocrine cells with an apical end reaching the lumen suggests that intraluminal contents can trigger the release of PYY and that PYY may have a paracrine or hormonal action. A paracrine action is further suggested by the fact that the PYY cells sometimes have long processes resembling those of somatostatin-immunoreactive cells in the stomach (13). The present findings suggest that a local PYY release would lead to, for example, a decrease in blood supply and colonic smooth muscle tone. Thus, PYY has actions opposite to those of local vasodilating and smooth muscle contracting factors present in the intestinal mucosa, such as vasoactive intestinal polypeptide (14, 15) and substance P (16–18). In fact, in the submandibular salivary gland, PYY inhibits the atropine-resistant vasodilatation induced by parasympathetic nerve stimulation as well as the blood flow increase induced by vasoactive intestinal polypeptide infusions (unpublished findings). The present data of a major occurrence of PYY immunoreactive cells in the distal intestine and the recent finding that PYY has an inhibitory action on exocrine secretion from the pancreas (7) is interesting in view of the earlier findings on the presence of inhibitory factor(s) of pancreatic exocrine secretion in extracts from ileal and colonic mucosa (19).

Although PYY (6, 7) and avian PP (2) have many structural similarities, they have different cellular localizations as revealed by immunohistochemistry. Thus, whereas PYY seems to be localized exclusively in endocrine cells, as shown in this paper, intestinal avian PP immunoreactivity seems to be present only in nerves (4). Furthermore, these peptides also differ in their effects on blood vessels: PYY but not avian PP causes a profound vasoconstriction (20).

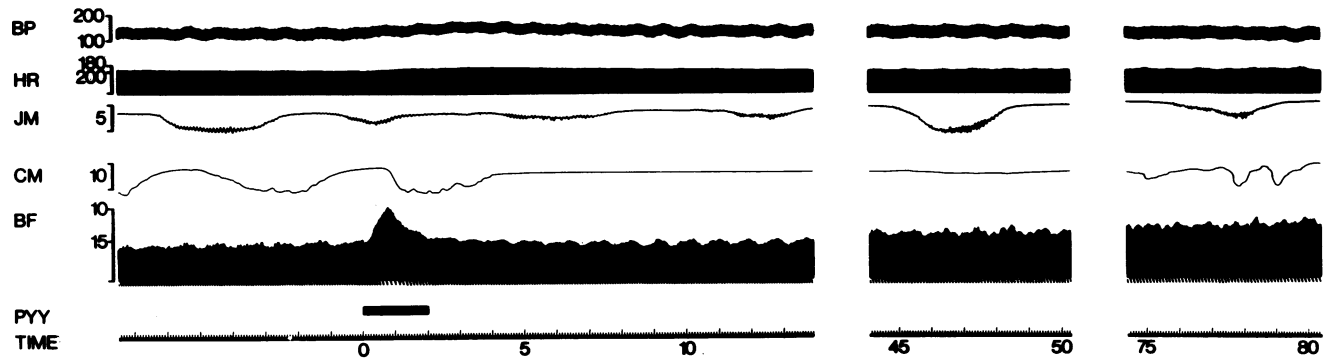

FIG. 5. Effects of local intraarterial infusion of PYY into the superior mesenteric artery (bar) at a dose of 150 pmol/kg per min for 2 min on systemic arterial blood pressure (BP, mm Hg; 1 mm Hg = 133 Pa), heart rate (HR, beats per min), jejunal motility (JM, volume change in ml), and colonic motility (CM, volume change in ml) as well as intestinal venous blood flow (BF, ml/min). Time scale is in minutes. The animal had been pretreated with guanethidine (3 mg/kg) 30 min prior to the infusion.

In conclusion, PYY is localized in endocrine cells mainly in the distal intestine and is a candidate for a gastrointestinal hormone.

We are grateful to Prof. J. Kimmel (Department of Biochemistry, McIlwain Laboratories, Kansas City, KS) and Dr. R. E. Chance (Eli Lilly, Indianapolis, IN) for generously supplying avian and bovine PP, respectively. For skillful technical assistance we thank Ms. A. Edin, Mrs. W. Hiort, Mr. C. Sundquist, Ms. I. Eriksson, and Ms. A. Peters. For expert secretarial help we thank Mrs. B. Frideen and Mrs. I. Sandberg. The present study was supported by grants from the Swedish Medical Research Council (04X-2887, 14X-6554, 13X101-1010, 04X-3766).

1. Kimmel, J. R., Pollock, H. G. & Hazelwood, R. L. (1971) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **30,** 1318 (abstr. 1550).
2. Kimmel, J. R., Hayden, L. J. & Pollock, H. G. J. (1975) *J. Biol. Chem.* **250,** 9369–9376.
3. Lin, T. M. & Chance, R. E. (1974) in *Endocrinology of the Gut*, eds. Chey, W. Y. & Brooks, F. P. (Slack, Thorofare, NJ), pp. 143–145.
4. Lorén, I., Alumets, J., Håkanson, R. & Sundler, F. (1979) *Cell Tissue Res.* **200,** 179–186.
5. Lundberg, J. M., Hökfelt, T., Änggård, A., Kimmel, J., Goldstein, M. & Markey, M. (1980) *Acta Physiol. Scand.* **110,** 107–109.
6. Tatemoto, K. & Mutt, V. (1980) *Nature (London)* **285,** 417–418.
7. Tatemoto, K. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2514–2518.
8. Hunter, W. N. (1974) *Br. Med. Bull.* **30,** 18–23.
9. Lundberg, J. M., Hökfelt, T., Änggård, A., Terenius, L., Elde, R., Markey, K., Kimmel, J. & Goldstein, M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 1303–1307.
10. Hultén, L. (1969) *Acta Physiol. Scand.* **78,** Suppl. 335, 1–116.
11. Larsson, L. I. (1980) *Clin. Gastroenterol.* **9,** 485–516.
12. Folkow, B., Lewis, D. H., Lundgren, O., Mellander, S. & Wallentin, I. (1964) *Acta Physiol. Scand.* **61,** 445–457.
13. Larsson, L. I., Goltermann, N., Magistris, L. D., Rehfeld, J. F. & Schwartz, T. W. (1979) *Science* **205,** 1393–1394.
14. Eklund, S., Jodal, M., Lundgren, O. & Sjöquist, A. (1979) *Acta Physiol. Scand.* **105,** 461–468.
15. Larsson, L.-I., Fahrenkrug, J., Schaffalitzky de Muckadell, O., Sundler, F., Håkanson, R. & Rehfeld, J. F. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3197–3200.
16. Gernandt, B. (1942) *Acta Physiol. Scand.* **3,** 270–280.
17. Burcher, E., Atkinig, J. H., Pernow, B. & Rosell, S. (1977) in *Substance P*, eds. von Euler, U. S. & Pernow, B. (Raven, New York), pp. 261–268.
18. Nilsson, G., Larsson, L. I., Håkanson, R., Brodin, E., Pernow, B. & Sundler, F. (1975) *Histochemistry* **43,** 97–99.
19. Harper, A. A., Hood, J. C., Muskens, J. & Smy, J. R. (1979) *J. Physiol. (London)* **292,** 455–467.
20. Lundberg, J. M., Änggård, A., Hökfelt, T. & Kimmel, J. (1980) *Acta Physiol. Scand.* **110,** 199–201.