Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 2514–2518, April 1982
Biochemistry

Isolation and characterization of peptide YY (PYY), a candidate gut hormone that inhibits pancreatic exocrine secretion

(chemical assay/COOH-terminal α-amide/amino acid sequence/pancreatic polypeptide family)

KAZUHIKO TATEMOTO

Department of Biochemistry II, Nobel Medical Institute, Karolinska Institute, S-104 01 Stockholm, Sweden

Communicated by U. S. von Euler, January 19, 1982

ABSTRACT **A new candidate hormone—designated peptide YY (PYY)—has been isolated from extracts of porcine upper intestinal tissue by using a novel chemical assay method. This peptide has been detected in the extracts by the presence of its unusual COOH-terminal tyrosine amide structure. The peptide consists of a linear chain of 36 amino acids and its complete amino acid sequence is: Tyr-Pro-Ala-Lys-Pro-Glu-Ala-Pro-Gly-Glu-Asp-Ala-Ser-Pro-Glu-Glu-Leu-Ser-Arg-Tyr-Tyr-Ala-Ser-Leu-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2. This peptide has a distinct sequence homology to the pancreatic polypeptide. It is therefore proposed that PYY and the pancreatic polypeptide together form a new peptide family. PYY has been found to strongly inhibit both secretin- and cholecystokinin-stimulated pancreatic secretion in the anesthetized cat.**

The COOH-terminal α-amide structure is a unique chemical characteristic of many biologically active peptides. The mechanism of the COOH-terminal amidation is at present unknown, but it has been suggested that the amide structure is formed enzymatically during the cleavage reactions from precursors to the active peptides (1, 2). The loss of the amide structure, in many cases, results in loss of biological activities of the peptides.

A novel chemical method for the assay of peptide hormones that have the COOH-terminal α-amide structure has been described (3). By using this chemical method several *hitherto* unknown peptide amides in porcine intestinal extracts have been detected—in addition to the known peptide hormones (3). Because naturally occurring peptides that have this type of structure generally are biologically active, a systematic isolation of these unknown peptide amides has been undertaken, leading to the discovery of a series of biologically active peptides. One of the peptide amides isolated from the intestine that contains isoleucine amide at its COOH terminus—designated peptide HI (PHI)—is a new member of the glucagon–secretin family and exhibits various biological activities (4, 5). From a side fraction obtained during the purification of PHI, another peptide amide that contains tyrosine amide at its COOH terminus has also been isolated (4). Based on its NH_2- and COOH-terminal amino acids (one-letter system), this peptide has been designated peptide YY (PYY)—the peptide (P) having NH_2-terminal tyrosine (Y) and COOH-terminal tyrosine (Y) amide. The peptide may possess a wide range of biological actions in the gastrointestinal tract and may be a new gut hormone.

This paper describes the isolation, complete amino acid sequence, and biological activities of this new intestinal peptide.

MATERIALS AND METHODS

The following chemicals were used: CM-cellulose; DEAE-cellulose (Whatman); Sephadex G-25 (Pharmacia); tyrosine amide (Vega Biochemicals, Tucson, AZ); dansyl-(Dns) amino acids (Calbiochem); phenylthiohydantoin amino acids (Mann Research Laboratories); DnsCl, pyridine, phenylisothiocyanate, CF_3COOH, BuOAc (sequanal grade, Pierce); Hepes (Fluka); polyamide thin-layer plates (Schleicher & Schuell); trypsin (EC 3.4.21.4; L-1-tosylamide-2-phenylethyl chloromethyl ketone-treated, Worthington); chymotrypsin (EC 3.4.21.1; Merck); thermolysin (EC 3.4.24.4; Daiwa Kasei K. K., Osaka, Japan); HCl, CH_3COOH (aristar, British Drug House, Poole, England); CH_3CN (HPLC grade, Rathburn Chemicals, Peeblesshire, Scotland). Other reagents were of analytical grade and were used without further purification. Dns tyrosine amide was prepared from tyrosine amide by the reaction with DnsCl (6). Secretin was prepared according to Jorpes *et al.* (7) and cholecystokinin (CCK), according to Mutt and Jorpes (8). Synthetic somatostatin was from Peninsula Laboratories (San Carlos, CA) and porcine pancreatic polypeptide was a gift from R. E. Chance (Lilly Research Laboratories). Glassware used for chemical assays and sequence determinations was cleaned by heating overnight (500°C). Amino acid analysis, NH_2-terminal determinations, and TLC on silica gel layer were performed as described (5).

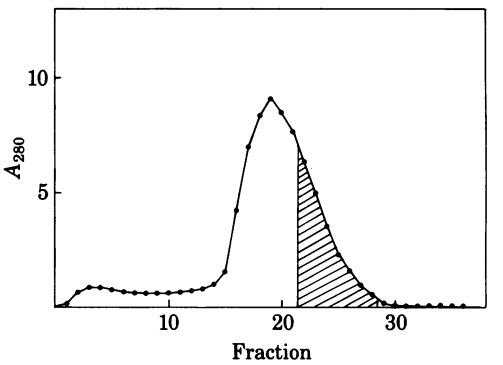

FIG. 1. Gel filtration of starting material on Sephadex G-25. The PYY-containing fraction (650 mg) obtained during purification of PHI (5) was applied to a Sephadex G-25 column (2.5 × 90 cm) and eluted with 0.2 M HOAc at a flow rate of 1.5 ml/min. The fractions (7.2 ml each) were lyophilized and an aliquot of each was subjected to the chemical assay for PYY. ▨, Fractions containing PYY.

Chemical Assay for PYY. PYY was measured by using the COOH-terminal amide determination method previously described (3, 5) after degradation of the sample with trypsin or thermolysin. The tyrosine amide released from the sample was identified in the form of fluorescent Dns derivative by TLC on polyamide layer.

HPLC. HPLC was performed by using a Waters liquid chromatographic instrument consisting of a U6K injector, two M-6000 A pumps, a 450 variable wavelength detector, and a model

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: PYY, peptide YY; PHI, peptide HI; PP, pancreatic polypeptide; CCK, cholecystokinin; Dns, dansyl.

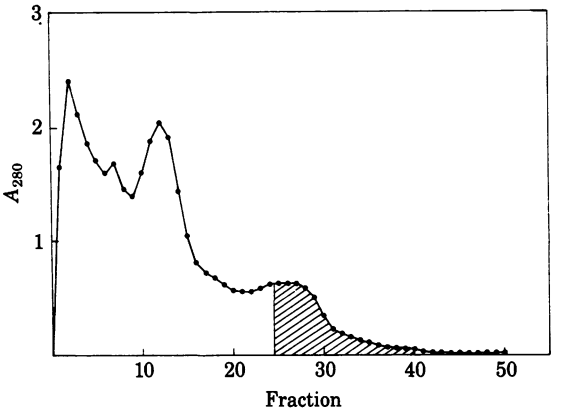

FIG. 2. Ion exchange chromatography of fractions 21–28 (see Fig. 1) on CM-cellulose. The fractions (125 mg) were applied to a CM-cellulose column (1.6 × 15 cm) equilibrated with 0.02 M NH_4HCO_3 (pH 8) and were eluted with the same buffer at a flow rate of 0.55 ml/min. Each fraction (5 ml) was lyophilized and an aliquot of each was subjected to the chemical assay. ▨, Fractions containing PYY.

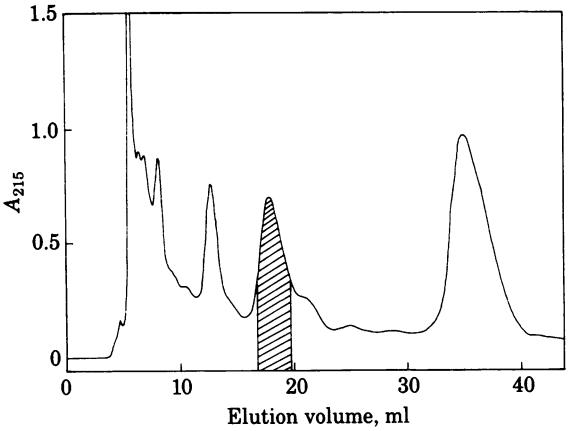

FIG. 3. HPLC separation of fractions 25–40 (see Fig. 2). Four milligrams of the combined fractions (8.8 mg) was applied to a reversed-phase HPLC column (μBondapak C_{18}, 7.8 × 300 mm) and was eluted with 34% EtOH/5 mM NH_4OAc/0.2% HOAc at a flow rate of 2 ml/min under isocratic conditions. ▨, The peak containing PYY.

660 solvent programmer. A reversed-phase HPLC column, μBondapak C_{18} (Waters) was used for peptide separation. Peptides were monitored by changes in A_{215} of the eluate.

Separation of the Tryptic and Chymotryptic Fragments by HPLC. The PYY preparation (70–100 μg) was dissolved in 50 μl of 1% NH_4HCO_3 solution and 2 μl of the trypsin or chymotrypsin solution (2 mg/ml) was added. The solution was incubated at room temperature for 4 hr (tryptic digest) or 10 min (chymotryptic digest) and then was incubated at 100°C for 6 min and lyophilized. The lyophilized material was dissolved in 50 μl of water and injected into the HPLC instrument. A μBondapak C_{18} column (3.8 × 300 mm) was used for separation of the peptide fragments. The fragments were separated by a linear gradient elution system that used 0.12% CF_3COOH/H_2O (solvent A) and 0.1% CF_3COOH/CH_3CN (solvent B, 0–50% for 40 min). The fractions that contained peptides were collected in test tubes and concentrated to approximately 1/5th of the volume under reduced pressure; they were then diluted by addition of water and lyophilized. The isolated peptide fragments thus obtained were subsequently subjected to amino acid analysis and sequence determinations.

Amino Acid Sequence Determination. The sequences of the tryptic fragments separated by the HPLC technique were determined by using a modified Dns/Edman method (9). Identification of Asp/Asn and Glu/Gln was according to Chen (10).

The Biological Model. The inhibitory effects of PYY on pancreatic exocrine secretion were studied by using an anesthetized adult (4 kg) cat. The operation was performed according to Jorpes and Mutt (11). Infusion of secretin and CCK (1.5 units/kg/hr each) in saline solution was made through the saphenous vein by using a perfusion pump at a flow rate of 10 ml/hr. Infusion or single injection of PYY was also made through the saphenous vein. Pancreatic juice from the cannulated pancreatic duct was collected in test tubes for 10-min periods by using a fraction collector. The volume of the juice and A_{280} were measured. Bicarbonate was determined by the titration method (11).

RESULTS

Isolation Procedures. Details of the extraction method and preparation of the peptide concentrate (the starting material)—obtained from 4000 kg of porcine intestine—have been described (5). The starting material (2.0 g) was applied to a CM-cellulose column (5 × 15 cm). It was first eluted with 0.02 M NH_4HCO_3 (adjusted to pH 6.5 by CO_2 gas) and then was eluted with 0.2 M NH_4HCO_3 (pH 8) in a stepwise fashion as described

Table 1. Amino acid compositions and NH_2 termini of PYY and its isolated tryptic fragments

Amino acid residues	T-1	T-2	T-3	T-4	T-5	T-3-a	T-3-b	PYY-36
Ala	3.0 (3)	1.1 (1)						4.1 (4)
Arg	1.0 (1)	1.0 (1)	0.9 (1)	1.0 (1)			0.9 (1)	4.0 (4)
Asx	1.0 (1)		1.1 (1)			1.0 (1)		2.1 (2)
Glx	3.9 (4)			1.0 (1)				5.0 (5)
Gly	1.1 (1)							1.1 (1)
His			0.9 (1)			0.8 (1)		1.0 (1)
Pro	3.8 (4)							3.7 (4)
Leu	1.1 (1)	1.0 (1)	2.0 (2)			1.1 (1)	1.1 (1)	4.0 (4)
Lys	1.0 (1)							1.1 (1)
Ser	2.0 (2)	1.0 (1)						2.8 (3)
Thr			1.0 (1)				1.1 (1)	1.0 (1)
Tyr	1.1 (1)	1.9 (2)	1.1 (1)		1.0 (1)	1.0 (1)		5.1 (5)
Val			1.0 (1)				1.0 (1)	1.0 (1)
Position	1–19	20–25	26–33	34–35	36	26–29	30–33	1–36
NH_2 terminus	Tyr	Tyr	His	Glx	Tyr	His	Leu	Tyr
Recovered, nmol	10.4	14.0	6.6	9.0	11.6	5.0	4.6	17.5

Values in parentheses represent theoretical number of residues.

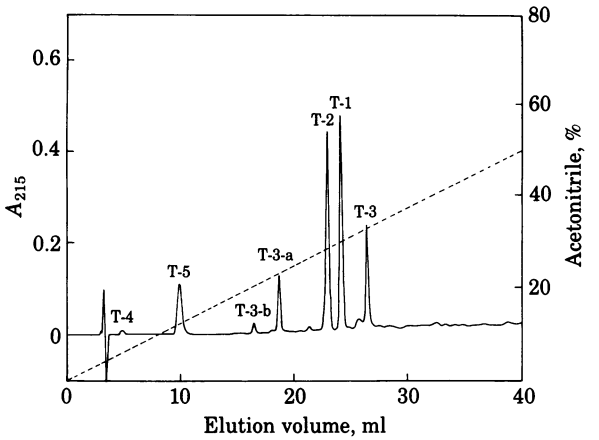

FIG. 4. HPLC separation of tryptic PYY fragments (T-1 to T-5). Trypsin-treated PYY preparation (70 μg) was applied to a μBondapak C_{18} column (3.9 × 300 mm). The tryptic peptides were separated at a flow rate of 1 ml/min by using a linear gradient system of 0.12% CF_3COOH/H_2O (solvent A) and 0.1% CF_3COOH/CH_3CN (solvent B, 0–50% for 40 min). The subfragments T-3-a and T-3-b were produced by a cleavage at the Asn-Leu bond of the T-3 tryptic fragment during the trypsin treatment.

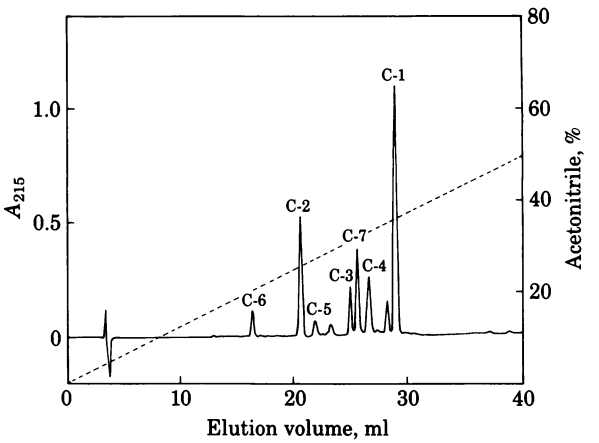

FIG. 5. HPLC separation of chymotryptic PYY fragments (C-1 to C-7). The PYY preparation (100 μg) was treated with chymotrypsin for 10 min as described. The chymotryptic peptides thus obtained were separated as described in Fig. 4.

(5). All peptide fractions that were eluted with 0.2 M NH_4HCO_3 were pooled and lyophilized. The lyophilized material (650 mg) contained PYY and was subjected to further purification by gel filtration on Sephadex G-25 (Fig. 1). Fractions 21–28 contained PYY and weighed a total of 125 mg after lyophilization. This material was purified further by rechromatography on CM-cellulose in 0.02 M NH_4HCO_3 at pH 8.0 (Fig. 2). Fractions 25–40 were pooled and lyophilized. This preparation (8.8 mg) was applied to a semipreparative HPLC column (μBondapak C_{18}, 7.8 × 300 mm) and was eluted with 34% EtOH/5 mM NH_4OAc/0.2% CH_3COOH under isocratic conditions.

Fig. 3 illustrates a typical elution profile of the HPLC separation. The eluate that contained PYY was collected and concentrated to 1/5th of the volume under reduced pressure. After addition of water to restore the original volume, it was lyophilized. This step yielded the highly purified PYY preparation (0.6 mg) that gave a single peak in the HPLC and a single spot in the TLC analyses.

Amino Acid Composition and Terminal Analysis. Amino acid analysis indicated that PYY consists of 36 amino acid residues (Table 1). A remarkably high tyrosine content (five residues per molecule) is noted. NH_2-terminal tyrosine residue of PYY was determined by the method of Gray (6) that uses DnsCl. Treatment of PYY with thermolysin or trypsin yielded COOH-terminal tyrosine amide that was identified by the method previously described (3, 5).

Separation of the Tryptic and Chymotryptic Fragments by HPLC. Treatment of PYY with trypsin yielded five major fragments and two minor ones. These were separated by the HPLC technique (Fig. 4). Amino acid analysis of the isolated tryptic fragments indicated that the two minor fragments (T-3-a and T-3-b) may be produced by a further cleavage of the tryptic T-3 fragment—due to enzyme contamination in the trypsin preparation or enzymic action of trypsin itself—because one of the minor fragments, T-3-a, contains neither arginine nor lysine and the amino acid compositions of both fragments are equal to that of T-3 (Table 1). Treatment of PYY with chymotrypsin for 10 min at room temperature yielded three major and four minor fragments that were separated by HPLC (Fig. 5). The amino acid compositions and NH_2-terminal amino acids of the chymotryptic fragments are shown in Table 2. The recovery of the tryptic and chymotryptic fragments from the HPLC column was in most cases between 50% and 80%.

Table 2. Amino acid compositions and NH_2 termini of isolated chymotryptic fragments of PYY

Amino acid residues	C-1	C-2	C-3	C-4	C-5	C-6	C-7
Ala	3.2 (3)	1.1 (1)		2.8 (3)	0.9 (1)		
Arg	0.9 (1)	0.9 (1)	1.8 (2)	1.2 (1)		1.1 (1)	1.9 (2)
Asx	1.1 (1)		1.1 (1)	1.1 (1)			1.2 (1)
Glx	4.0 (4)		1.2 (1)	3.9 (4)			1.2 (1)
Gly	1.1 (1)			1.2 (1)			
His		1.0 (1)				1.1 (1)	
Pro	3.5 (4)			3.4 (4)			
Leu	1.1 (1)	1.0 (1)	2.0 (2)	1.2 (1)	1.1 (1)		1.7 (2)
Lys	1.0 (1)			0.9 (1)			
Ser	2.0 (2)	1.0 (1)		2.1 (2)	1.1 (1)		
Thr			1.0 (1)				1.1 (1)
Tyr	3.1 (3)	1.1 (1)	1.0 (1)	1.6 (2)	0.9 (1)	0.8 (1)	1.1 (1)
Val			0.8 (1)				0.9 (1)
Position	1–21	22–27	28–36	1–20	21–24	25–27	28–36
NH_2 terminus	Tyr	Ala	Leu	Tyr	Tyr	Arg	Leu
Recovered, nmol	7.6	10.6	6.6	2.0	1.8	2.4	3.2

Values in parentheses represent theoretical number of residues.

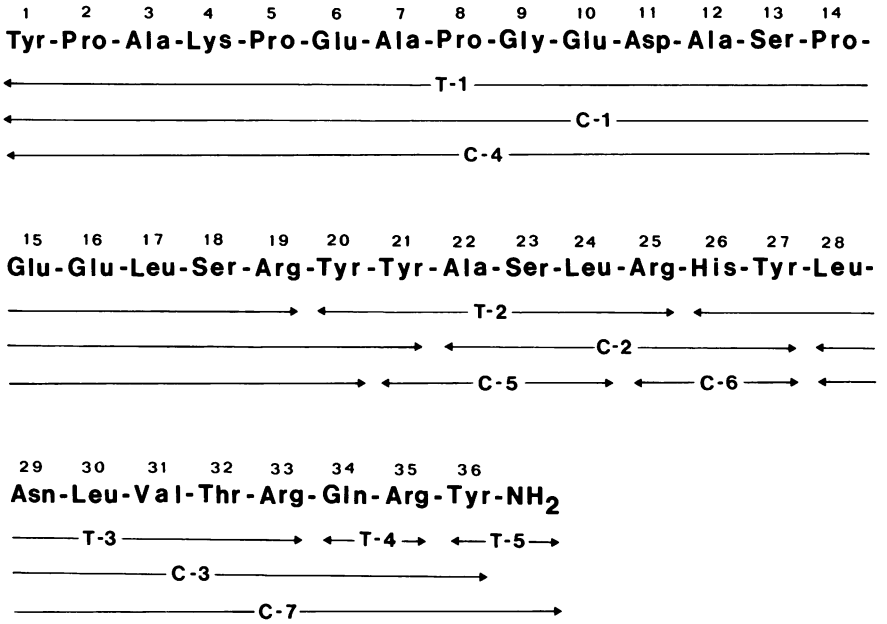

FIG. 6. The complete amino acid sequence of PYY. T-1 to T-5, tryptic fragments; C-1 to C-7, chymotryptic fragments.

Amino Acid Sequences of the Tryptic Fragments. The sequences of the tryptic fragments and amino acid compositions of the chymotryptic fragments. The amino acid sequence of PYY (PYY-36) is thus: Tyr-Pro-Ala-Lys-Pro-Glu-Ala-Pro-Gly- carried out by identifying the corresponding phenylthiohydantoin amino acids that were derived from the thiozolinone derivatives of these amino acids present in the Edman degradation products (10). The sequence of the T-1 fragment was found to be Tyr-Pro-Ala-Lys-Pro-Glu-Ala-Pro-Gly-Glu-Asp-Ala-Ser-Pro-Glu-Glu-Leu-Ser-Arg. Amino acid analysis of the T-1 fragment indicated the content of ammonia to be <0.6 mol/mol of peptide, which confirmed the sequence result that none of the COOH side chains in the T-1 fragment was amidated. The sequence of the T-2 fragment was Tyr-Tyr-Ala-Ser-Leu-Arg and that of the T-3 fragment was His-Tyr-Leu-Asn-Leu-Val-Thr-Arg. The dipeptide T-4 sequence was Gln-Arg and the COOH-terminal fragment (T-5) sequence was Tyr-NH_2, which was identified by the chemical method described (3, 5).

Complete Amino Acid Sequence of PYY. The NH_2-terminal sequence of the PYY molecule was determined by the Dns/Edman method that yielded the sequence: Tyr-Pro-Ala-Lys-Pro-Glu-Ala-Pro-Gly-, indicating that the T-1 fragment was the NH_2-terminal tryptic fragment. The complete amino acid sequence of PYY was deduced from the results of the sequences of the tryptic fragments and amino acid compositions of the chymotryptic fragments. The amino acid sequence of PYY (PYY-36) is thus: Tyr-Pro-Ala-Lys-Pro-Glu-Ala-Pro-Gly-Glu-Asp-Ala-Ser-Pro-Glu-Glu-Leu-Ser-Arg-Tyr-Tyr-Ala-Ser-Leu-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (Fig. 6).

Effect of PYY on the Pancreatic Secretion. When the pure PYY preparation was injected intravenously into the anesthetized cat, it was found that PYY was capable of strongly inhibiting secretin-stimulated pancreatic secretion. A very low dose of PYY (10–20 pmol/kg) significantly decreased the pancreatic secretion of bicarbonate as well as fluid that was stimulated by a single dose of secretin (0.1 unit/kg) (Fig. 7). A dose of PYY of 100–200 pmol/kg caused a 70–80% reduction of the pancreatic bicarbonate and fluid secretion and larger doses of PYY appeared to cause no further reduction. PYY inhibited not only

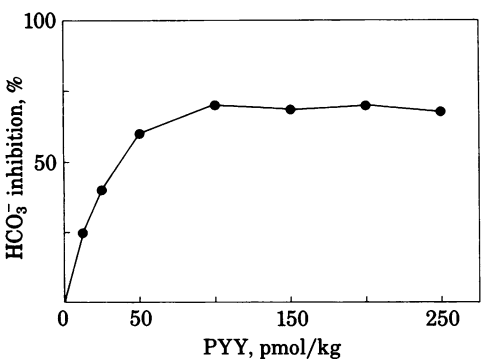

FIG. 7. A dose–response curve for inhibition by PYY of secretin-stimulated pancreatic bicarbonate secretion in the anesthetized cat. One minute after injection of PYY (10–250 pmol/kg), secretin (0.1 unit/kg) was injected to stimulate pancreatic secretion. The pancreatic juice was collected from the cannulated pancreatic duct for 20 min and the amount of bicarbonate in the juice was determined by titration with 0.1 M HCl.

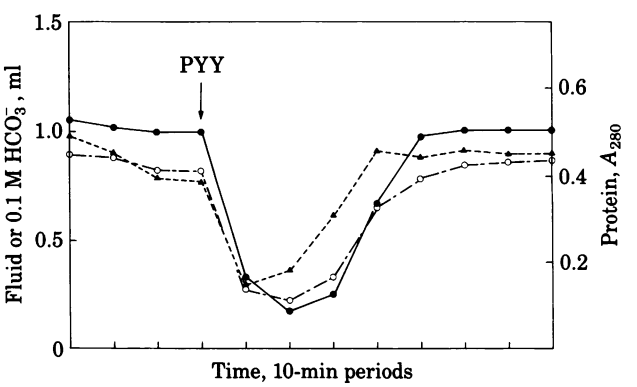

FIG. 8. Effect of PYY on pancreatic secretion of protein, fluid, and bicarbonate stimulated by a steady infusion of secretin and CCK. PYY (200 pmol/kg) was injected (arrow) into the anesthetized cat during the submaximal secretion that was stimulated by a steady infusion of secretin and CCK (1.5 units/kg each). ▲, Protein (A_{280}); ○, fluid (ml); ●, bicarbonate (expressed as milliliter of 0.1 M HCO_3^- solution).

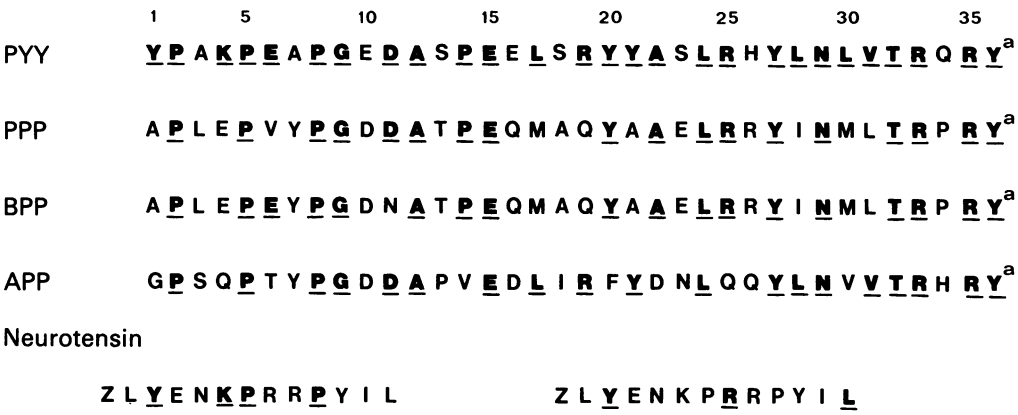

FIG. 9. Comparison of the amino acid sequence of porcine PYY with those of the pancreatic polypeptides of porcine (PPP), bovine (BPP), and avian (APP) origins and neurotensin. Identities are underlined. a, Amidated COOH terminus.

the pancreatic secretion of fluid and bicarbonate but also inhibited protein secretion that was stimulated by secretin alone or together with CCK. Fig. 8 shows the inhibitory effect of PYY during the submaximal pancreatic secretion that was stimulated by a steady infusion of secretin and CCK (1.5 units/kg each). A single injection of PYY (200 pmol/kg) caused a marked and rapid reduction (70–80%) of the fluid, bicarbonate, and protein secretion, which returned to the preinjection level after a period of 30–40 min (the protein secretion level usually returned to the preinjection level in a shorter time period). A steady infusion of PYY (200 pmol/kg/hr) decreased the pancreatic secretion by 50% during the infusion period under the submaximal conditions. A single injection of somatostatin or the pancreatic polypeptide in a dose range of 1 to 5 μg/kg did not change the level of secretion in the anesthetized cat.

DISCUSSION

PYY has been found to possess a distinct sequence homology to the pancreatic polypeptide (PP) (Fig. 9). The sequence similarities to PP are observed in the entire region of the PYY molecule; there are 18 identical positions to porcine (12) and bovine PP and 19 identical positions to avian PP in the 36 amino acid residues. The four proline residues of PYY are located in identical positions (positions 2, 5, 8, and 14) to those in porcine and bovine PP, forming a unique tertiary structure at the NH_2-terminal region. All five COOH side chains are located in the NH_2-terminal and middle regions, giving a characteristic acidic segment, whereas the COOH-terminal region is highly basic. In addition, PYY possesses some sequence similarities to neurotensin (Fig. 9). The similarities are seen in two portions of the PYY molecule. PYY also seems to have some degree of sequence homology to members of the glucagon–secretin family (unpublished data). Avian PP has some sequence homology to glucagon (13), but porcine and bovine PP have no significant homology to it. On the other hand, sequence homology between PYY and PP is unquestionably distinct. I therefore propose that the two peptides together form a new peptide family.

PYY and PP may also have similarities in biological activities. Like PP, PYY inhibits exocrine pancreatic secretion stimulated by infusion of secretin and CCK. In a dose range of 10 to 100 pmol/kg, PYY rapidly decreased the pancreatic secretion of fluid, bicarbonate, and protein in the anesthetized cat. It is of interest that PP and somatostatin—which are known to inhibit the pancreatic secretion in the dog (14, 15)—had no such effect in the cat. Therefore, PYY may be the most potent inhibitor of the pancreatic secretion known in this species.

The inhibitory effect of PYY on pancreatic secretion resembles the action of "pancreatone"—named after observation of the biological actions of the alcohol extract obtained from the ileal and colonic mucosa of cat (16). It is possible that PYY or its related peptide might be responsible for the pancreatic inhibitory action of pancreatone. However, PYY does not inhibit contraction of the gallbladder caused by injection of CCK in the anesthetized guinea pig, whereas pancreatone has been reported to inhibit the CCK effect (16). Therefore, this activity might be due to another peptide in the extract. If so, the pancreatone actions might be caused by at least two peptides.

PYY has a vasoconstrictory action and inhibits jejunal and colonic motility (unpublished data). PYY is localized in gut endocrine cells of several mammalian species including man (unpublished data). These results—together with the results of the chemical structure and biological actions reported here—indicate that PYY may be a new gut hormone.

Another new biologically active peptide, PHI, has been isolated from the intestine by using the same chemical assay method (4, 5). Discovery of PYY again demonstrates the usefulness of such chemical approach for isolation of *hitherto* unknown peptides of biological interest and suggests that there may be many more peptides to be isolated.

The author thanks Prof. V. Mutt for his support and useful discussions. Excellent technical assistance by Mrs. L. Melin is gratefully acknowledged. This study was supported by a grant from the Swedish Medical Research Council (Project 13X-1010).

1. Smyth, D. G. (1975) *Nature (London)* **257**, 89–90.
2. Suchanek, G. & Kreil, G. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 975–978.
3. Tatemoto, K. & Mutt, V. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4115–4119.
4. Tatemoto, K. & Mutt, V. (1980) *Nature (London)* **285**, 417–418.
5. Tatemoto, K. & Mutt, V. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6603–6607.
6. Gray, W. R. (1972) *Methods Enzymol.* **25**, 121–138.
7. Jorpes, J. E., Mutt, V., Magnusson, S. & Steele, B. B. (1962) *Biochem. Biophys. Res. Commun.* **9**, 275–279.
8. Mutt, V. & Jorpes, J. E. (1968) *Eur. J. Biochem.* **6**, 156–162.
9. Hartly, B. S. (1970) *Biochem. J.* **119**, 805–822.
10. Chen, R. (1976) *Hoppe-Seyler's Z. Physiol. Chem.* **357**, 873–886.
11. Jorpes, J. E. & Mutt, V. (1966) *Acta Physiol. Scand.* **66**, 316–325.
12. Chance, R. E., Johnson, M. G., Hoffman, J. A. & Lin, T.-M. (1979) in *Proinsulin, Insulin, C-peptide*, eds. Baba, S., Kaneko, T. & Yanaihara, N. (Excerpta Medica, Amsterdam), pp. 419–425.
13. Kimmel, J. R., Hayden, J. & Pollock, H. G. (1975) *J. Biol. Chem.* **250**, 9369–9376.
14. Lin, T.-M., Evans, D. C., Chance, R. E. & Spray, G. F. (1977) *Am. J. Physiol.* **232**, E311–E315.
15. Taylor, I. L., Solomon, T. E., Walsh, J. H. & Grossman, M. I. (1979) *Gastroenterology* **76**, 524–528.
16. Harper, A. A., Hood, J. C., Mushens, J. & Smy, J. R. (1979) *J. Physiol. (London)* **292**, 455–467.