IMMUNOCYTOCHEMICAL EVIDENCE FOR THE OCCURRENCE OF PYY, A NEWLY ISOLATED GUT POLYPEPTIDE, IN ENDOCRINE CELLS IN THE GUT OF AMPHIBIANS AND REPTILES¹

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ABSTRACT

Various parts of the alimentary tract of an anuran amphibian, the toad (*Bufo regularis*) and of a squamate reptile, the grass lizard (*Mabuya quinquetaeniata*) were investigated immunocytochemically for the occurrence of PYY (the polypeptide having N-terminal tyrosine and C-terminal tyrosine). PYY-immunoreactive cells were observed in the duodenum and ileum of both the toad and the lizard. These cells were of the open-type. The frequency and distribution of PYY-immunoreactive cells were different from those of BPP- and neurotensin-immunoreactive cells.

The present observations show that PYY, a newly postulated gut hormone, has a wide distribution in vertebrates. Moreover, the present study indicates that PYY, PP and neurotensin family of peptides occur in independent endocrine cell types in the gut at the evolutionary level of amphibians and reptiles.

PYY (the polypeptide which has an N-terminal tyrosine and a C-terminal tyrosine) has recently been isolated from the porcine duodenum and has been found to have structural similarities to both pancreatic polypeptide (PP) and neurotensin (8). PYY-immunoreactive cells have been observed in the small and large intestine of several mammalian species including man (J. M. Lundberg, K. Tatemoto, L. Terenius, P. M. Hellerström, V. Mutt, T. Hökfelt and B. Hemberger, Localization of the polypeptide YY (PYY) in gastro-intestinal endocrine cells and effects on intestinal blood flow and motility; in preparation). Experimental studies have shown

that PYY has a vasoconstrictor action and an inhibitory effect on the jejunal and colonic motility (Lundberg *et al.*, in preparation: *vide supra*).

The molecular structural similarity between PYY, PP and neurotensin suggests that they form a family of hormonal peptides. Phylogenetic studies of this newly added family of hormonal peptides have been expected to throw light on the possible overlapping and divergence of these polypeptides during the course of the evolution. Thus, in the present study the occurrence of PYY-immunoreactive cells in the gut of an anuran amphibian, the toad (*Bufo regularis*) and of a squamate reptile, the grass lizard (*Mabuya quinquetaeniata*) was investigated. The choice of these animals was due to the prominent mid-position *Amphibia* and *Reptilia* occupy in the vertebrate evolution (1).

¹ Preliminary results were presented in abstract form in the 7th International Conference Defined Immunofluorescence, Immunoenzyme Studies and Related Labeling Techniques. Held in Niagara Falls, New York, from 8 to 11 June, 1982.

MATERIALS AND METHODS

Adult specimens of both sexes of the toad (*Bufo regularis*) and of the grass lizard (*Mabuya quinquetaeniata*) were captured in July from the cultivated area around Cairo, Egypt. The animals were lightly anaesthetized with ether and decapitated. The oesophagus, the upper half of the stomach, the lower half of the stomach, duodenum, ileum and rectum were dissected out and fixed in Bouin's fluid for 24 h. After paraffin embedding, 5 μ m thick sections were cut and immunostained for the occurrence of PYY, BPP or neurotensin by the peroxidase-antiperoxidase (PAP) method of Sternberger (7). The antisera used were rabbit antiserum against porcine PYY No. 69D, rabbit anti-bovine PP (a gift from R.E. Chance, Lilly Res. Lab., Indianapolis, IN, U.S.A.) and rabbit antiserum against synthetic bovine neurotensin No. HC 8 (a gift from S.E. Leeman, Lab. Hum. Reprod. Harvard Med. Sch., Boston, U.S.A.). They were used at the dilution of 1:2,500, 1:2,000 and 1:1,500, respectively. The controls were as follows: 1) the first layer antiserum was replaced by normal rabbit serum; 2) The antisera were pre-incubated with 75–100 μ g/ml diluted antisera with the corresponding peptide for 24 h at 4°C and 3) the anti-PYY was pre-incubated with 75–120 μ g/ml diluted antiserum for 24 h at 4°C with the following hormonal peptides: PYY, bovine pancreatic polypeptide (BPP) (a gift from R.E. Chance, Lilly Res. Lab., Indianapolis, IN, U.S.A.), avian pancreatic polypeptide (APP) (a gift from J. Kimmel, Department of Biochemistry, McIlwain Lab., Kansas City, U.S.A.), porcine pancreatic polypeptide (PPP) (Novo Research Institute, Copenhagen, Denmark, Lot No. PPP 79062), porcine glucagon (Novo Industri A/S, Copenhagen, Denmark, Lot No. G 427144), and bovine neurotensin (Bachem, Bubendorf, Switzerland, Lot No. 9322).

PYY Antiserum (No. 69D)

The PYY antiserum was produced in rabbits as described in detail elsewhere (Lundberg *et al.*, in preparation: *vide supra*).

Immunochemical characterization of the PYY antiserum was performed by radioimmunoassays (RIA) (Lundberg *et al.*, in preparation: *vide supra*). In RIA system this antiserum did not cross-react with any of the following polypeptides: APP, neurotensin, glucagon GIP, VIP, somatostatin, cholecystokinin (CCK) and en-

kephalin. A weak cross-reaction (<0.1%) with BPP was, however, observed.

Immunocytochemical characterization of PYY antiserum was done on formalin- and Bouin-fixed human small intestine and colon using the peroxidase-antiperoxidase (PAP) method of Sternberger (7). The sections were incubated overnight with a serial dilution of the anti-PYY from 1:50 to 1:6,000. PYY-immunoreactive cells were observed up to 1:3,500 diluted antiserum with optimal immunostaining at the dilution 1:2,500. Pre-incubation of the antiserum with 100 μ g PYY/ml of 1:2,500 diluted antiserum at 4°C for 24 h abolished completely the immunostaining obtained. On the other hand, pre-incubation of the antiserum with 100 μ g/ml of 1:2,500 diluted antiserum with the following hormonal peptides had no effect on the immunostaining: APP, BPP, PPP, bovine neurotensin, porcine insulin, porcine glucagon, synthetic somatostatin, synthetic human gastrin-I, porcine CCK, synthetic pentagastrin, bovine secretin, synthetic bombesin, β -endorphin and enkephalin.

RESULTS

In both the toad and the grass lizard PYY-, PP- and neurotensin-immunoreactive cells were seen. The frequency and distribution of these three cell types in various parts of the alimentary canal of the toad and the grass lizard are given in Table 1. The description of PP and neurotensin-immunoreactive cells was similar to that given previously (2, 3). PYY-immunoreactive cells were observed in the duodenum and ileum of both the toad and the grass lizard. The oesophagus, stomach and rectum were negative. In the duodenum and ileum of both the toad and the grass lizard PYY-immunoreactive cells were located among the epithelial cells mostly in the lower portion of the villi and seldom in the deep bits between the villi. They were tall thin cells which seem to reach the lumen *i.e.* they were of the open type (Figs. 1 and 2).

Specificity Controls

No immunostaining was observed when the first layer antiserum was replaced by normal rabbit serum. The antisera were completely neutralized after the pre-incubation with the corresponding peptide. Pre-incubation of anti-PYY with APP, BPP, PPP, glucagon and neurotensin had no effect on the immunostaining.

Table 1 The Distribution and Frequency of PYY-Immunoreactive Cells as Compared with BPP- and Neurotensin-Immunoreactive Cells in Various Parts of the Gut of the Toad, *Bufo regularis* and the Grass Lizard, *Mabuya quinquetaeniata*

	<i>Bufo regularis</i>			<i>Mabuya quinquetaeniata</i>		
	PYY	BPP	Neurotensin	PYY	BPP	Neurotensin
Oesophagus	—	—	—	—	—	—
Stomach:						
The upper half	—	—	—	—	—	—
The lower half	—	+++	—	—	++++	+++
Duodenum	++	++	—	++	+	—
Ileum	++	+++	+	++	+	+
Rectum	—	—	—	—	—	—

A semi-quantitative subjective assessment was made, using the following five-grade scale: +++++, abundantly occurring cells; ++++, numerous cells; ++, moderate number of cells; +, few cells; —, absent

Fig. 1 PYY-immunoreactive cell in the duodenum of the toad. (*Bufo regularis*). They are thin cells among the mucosal epithelial cells of the lower part of the villi. $\times 650$

Fig. 2 Open-type PYY-immunoreactive cell in the ileum of the grass lizard (*Mabuya quinquetaeniata*). $\times 650$

DISCUSSION

PYY and PP molecules have the same C-terminus (Fig. 3). This similarity recalls another two well known gut hormonal polypeptides which share also the C-terminus namely, gastrin

and cholecystokinin (CCK). Gastrin and CCK have been assumed to originate from an ancestral molecule which occurs in the gut of lower vertebrates up to amphibians and starts to diversify in the gut of reptiles into several molecular forms containing different parts of the

Fig. 3 Comparison between the partially elucidated molecular structure of PYY (8) and corresponding parts in the molecule of APP (4) and BPP (5). As porcine PP, canine PP, ovine PP and human PP molecular structures differ only with one or two amino acids residues from BPP (6), BPP molecular structure can be taken as an example for mammalian PP. It is clear that PYY and PP have the same C-terminus. The structural similarities are framed.

ancestral molecule until in birds and mammals separate gastrin and CCK forms occur (2, 3). However, PYY and PP immunoreactivities seem to occur in two independent cell types, as judged from their distribution and frequency, in the gut of amphibian and reptiles. The present observation of PYY-immunoreactive cells in the gut of an amphibian, the toad (*Bufo regularis*) and of a reptile, the grass lizard (*Mabuya quinquataeniata*) indicates that PYY is a widely occurring polypeptide in the vertebrate gastrointestinal tract and that it is not limited to the mammalian gut. Moreover, it suggests that PYY is an evolutionary old polypeptide. However, further speculation on the evolutionary age of this polypeptide must await the results of studies of the nervous system as well as the gastro-entero-pancreatic endocrine systems of lower vertebrates and invertebrates, which are now in progress in our laboratories. The distribution of PYY-immunoreactive cells in the gut of the toad and the grass lizard differs from that in the mammalian gut. Whereas PYY-immunoreactive cells in mammals are rare in the stomach and duodenum and abundant in the distal part of the intestine, colon and rectum (Lundberg *et al.*, in preparation: *vide supra*), PYY-immunoreactive cells in the toad and the grass lizard occur in the duodenum and ileum.

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REFERENCES

1. EL-SALHY M. (1981) On the phylogeny of the gastro-entero-pancreatic (GEP) neuroendocrine system. *Acta Univ. Upsal.* **385**, 1-39
2. EL-SALHY M. and GRIMELIUS L. (1981) The endocrine cells of the gastrointestinal mucosa of a squamate reptile, the grass lizard (*Mabuya quinquataeniata*). A histological and immunohistochemical study. *Biomedical Res.* **2**, 639-658
3. EL-SALHY M., GRIMELIUS L., WILANDER E., ABUSINNA G. and LUNDQVIST G. (1981) Histological and immunohistochemical studies of the endocrine cells of the gastrointestinal mucosa of the toad (*Bufo regularis*). *Histochemistry* **71**, 53-65
4. KIMMEL J. R., HAYDEN L. J. and POLLOCK H. G. (1975) Isolation and characterization of a new pancreatic polypeptide hormone. *J. Biol. Chem.* **250**, 9369-9376
5. LIN T.-M. and CHANCE R. E. (1974) Candidate hormones of the gut: Bovine pancreatic polypeptide (BPP) and avian pancreatic polypeptide (APP). *Gastroenterology* **67**, 737-738
6. LIN T.-M. and CHANCE R. E. (1978) Spectrum of gastrointestinal actions of bovine PP. In *Gut Hormones* (ed. BLOOM S. R.) Churchill Livingstone, Edinburgh, pp. 242-246
7. STERNBERGER L. A. (1979) *Immunocytochemistry* Second Ed., John Wiley and Sons, New York
8. TATEMOTO K. and MUTT V. (1980) Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptides. *Nature* **285**, 417-418