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Distribution and Endocrine Morphology of Polypeptide YY (PYY) Containing Cells in the Human Gut

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ABSTRACT

Using human materials, the distribution of PYY containing cells was determined by immunocytochemical methods and discussion was made on their morphological endocrinology. PYY cells were fairly numerous in the lower gastrointestinal tract of man, particularly in the colon and rectum. The cells were also present in the pancreas and duodenum but quite rarely. PYY cells were not observed at all in the lower part of the esophagus, stomach and gall bladder. Their peculiar and characteristic shapes as well as distribution suggest that PYY may have some action (probably specific) on the function of the distal gastrointestinal tract.

A polypeptide which has N-terminal and C-terminal tyrosin (PYY) was recently isolated from the porcine duodenum[9,10]. The PYY molecule contains 36 amino acids, similar to those of PP and NPY, and PP as well as NPY molecules, furthermore, have in common the same amino acid residues[9,10] and they are characterized in particular by a C-terminal tyrosine amide. PYY immunoreactive cells have been found in the gastrointestinal mucosa of mammals, birds, reptiles and amphibians[5,6]. The precise localization of PYY-immunoreactive cells and endocrine morphology, however, remains to be elucidated in the gastrointestinal tract, gall bladder and pancreas in man and other mammals.

In the present study, the authors have attempted to elucidate the localization and morphology of PYY-like immunoreactive cells in the gastrointestinal tract, gall bladder and pancreas of man.

MATERIALS AND METHOD

Tissue material

Specimens of the lower esophagus, gastric body, antrum, pylorus, duodenum, jejunum, ileum, proximal colon, distal colon, rectum, gall bladder, and pancreas were obtained at surgery or by biopsy of patients suffering from various gastrointestinal, pancreas or gall bladder diseases (n=11). Specimens (about 0.5 × 0.5) of one group were fixed by immersion in Bouin's solution at 4°C for 18 hr. The tissue specimens were then embedded in paraffin wax through routine procedure and cut 4 microns in thickness. A routine histopathological examination of tissue sections stained by hematoxylin and eosin was made to ascertain whether the mucosal morphology was normal in each area to be examined for distribution and endocrine morphology of PYY-like immunoreactive cells.

Immunocytochemistry

(1) Indirect immunofluoresent method[2]

The de-waxed sections 4μ in thickness were left to react with the primary antiserum of PYY for 18 hr at 4°C. The labeled second layer (FITC) was applied for 1 hr at room tempera-

ture. FITC comjugated goat antirabbit globulin (MBL) was used at a solution of 1:100. The sections were examined under a fluorescent microscope (UV, Nikon) and photographed.

(2) Unlabeled antibody enzyme method[8]

Prior to immunostaining, paraffin-wax sections were deparaffinized by immersion in xylene and petroleum ether and then dried. The sections were first treated with hydrogen peroxide to exhaust the endogenous peroxidase activity and then were incubated with normal goat serum to block non-specific background staining. The specific antisera were applied for 12-18 hr at 4°C, while the conjugated antiglobulins were applied at room temperature. The following peroxidase anti-peroxidase complexes were then applied: Horseradish peroxidase rabbit anti-horse radish peroxidase (Dakopatt A/S) at 1:300 at room temperature. The peroxidase activity was revealed by incubation for 2-3 min in a freshly prepared solution of 0.05% 3, 3-diaminobenzidine tetrahydrochloride in phosphate buffered saline pH 7.2, containing 0.01% hydrogen peroxide. The sections which reacted were observed under a light microscope (Leitz) and then photographed.

Antiserum

The antiserum used was rabbit antiserum 1101 against porcine PYY (a kind gift from J.M. Polak, Hammersmith Hospital, London UK). The anti-PYY was used at a dilution of 1:200 for immunofluorescence and 1:2000 for PAP.

Specificity for immunocytochemistry

In order to demonstrate that the immunocytochemical reactions were specific, the following tests were performed.

(1) Prior to immunostaining, the diluted antiserum was absorbed with an excess amount of synthetic porcine PYY (a kind gift from V. Mutt and K. Tatemoto, Karolinska Institute, Stockholm, Sweden)[4].

(2) Normal rabbit serum was used instead of the primary antiserum as the 1st layer.

(3) PAP complex was applied alone and developed by the unlabeled antibody technique.

RESULTS

PYY-like immunoreactive cells were very rare or absent in the mucosa of the lower part of the esophagus, gastric body and antropylorus. One PYY-like immunoreactive cell was observed in the deep crypts of the proximal duodenum, while a small number of PYY-like immunoreactive cells were located in the deep crypts of Lieberkühn of ileal mucosa (Fig. 1). Contrary to these observations, a good number of cells were found

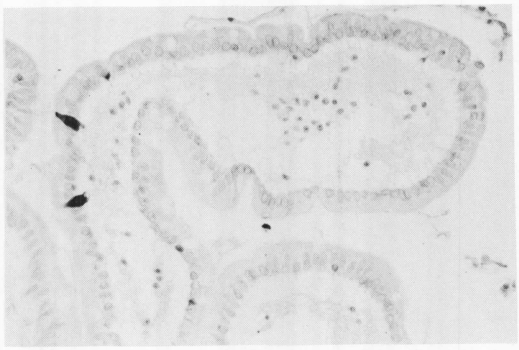


Fig. 1. Some PYY-like immunoreactive cells are present in the ileal mucosa (PAP, original magnification 100).

Fig. 2. Numerous PYY-like immunoreactive cells, which are various in shape, are observed in the rectal mucosa (PAP, original magnification 100).

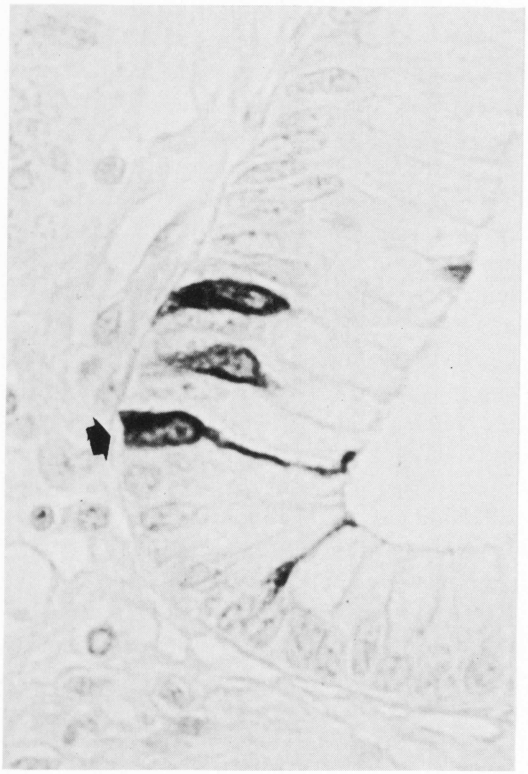


Fig. 3. Almost all the cells are open-typed (PAP, original magnification 100).

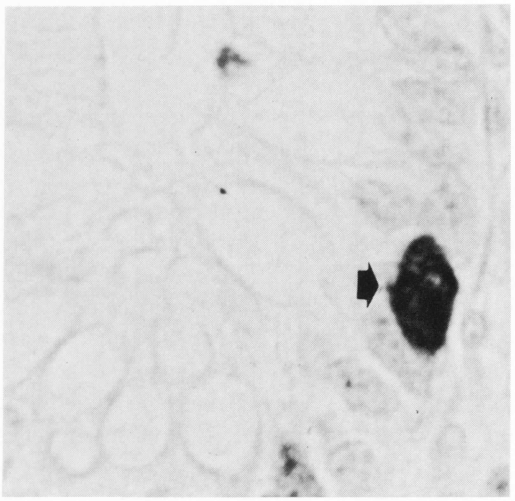


Fig. 4. A very rare cell showed PYY-like immunoreactivity is closed-typed (PAP, original magnification 100).

in the crypts of the mucosa of the distal colon and rectum (Fig. 2). They were generally open-typed endocrine cells (Fig. 3) but occasionally closed type (Fig. 4). The distribution of PYY-like immunoreactive cells in the human gut is summarized in Table 1. PYY-like immunoreactivity was thus localized in the population of endocrine cells mainly in the intestinal mucosa, particulary in the sigmoid colon and rectum.

Table 1

	RYY Distribution
Lower esophagus	—
Stomach Body	—
Antrum	—
Duodenum	+ (?)
Jejunum	not tested
Ileum	+
Proximal colon	+
Distal colon	«
Rectum	»

— not detected
+ positive
« many
» quite a lot

PYY-like immunoreactive cells had various shapes such as pyramidal, goblet-like, gourd-shaped and flask-shaped, as shown in Fig. 5. They all had long apical portions reaching the lumen and some showed long cytoplasmic processes from the base to the neighbouring cells (Fig. 6). Quite a few PYY-like immunoreactive cells were seen in the parenchyma of the pancreas, while not a cell with PYY-like immunoreactivities was observed in the gallbladder wall (Fig. 7).

Using serial sections, the specificities of PYY-like immunoreactivities in the cells were examined and all the cells showed negative PYY-

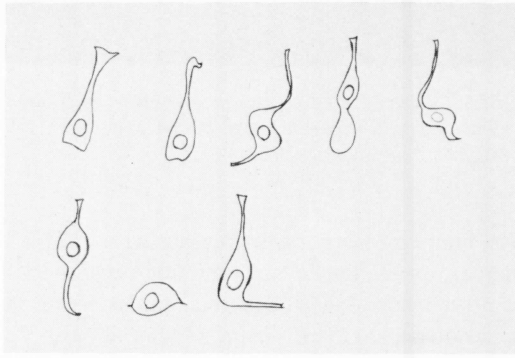


Fig. 5. PYY-like immunoreactive cells appear in various and peculiar shapes.

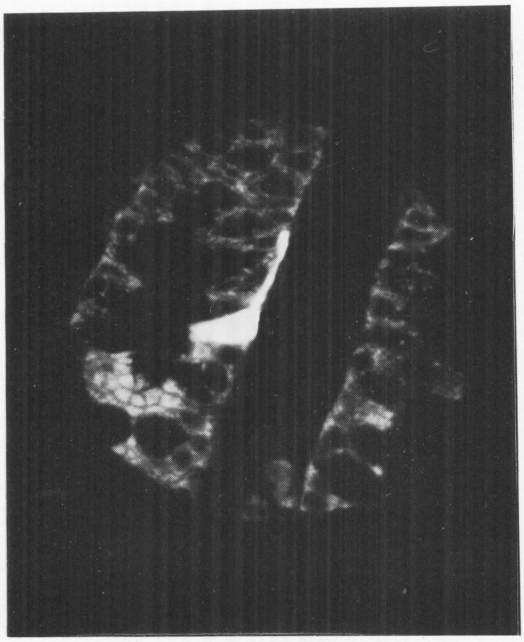


Fig. 6. Some of PYY-like immunoreactive cells possesses a long process from the basement to a neighbouring cell (IF, original magnification 100).

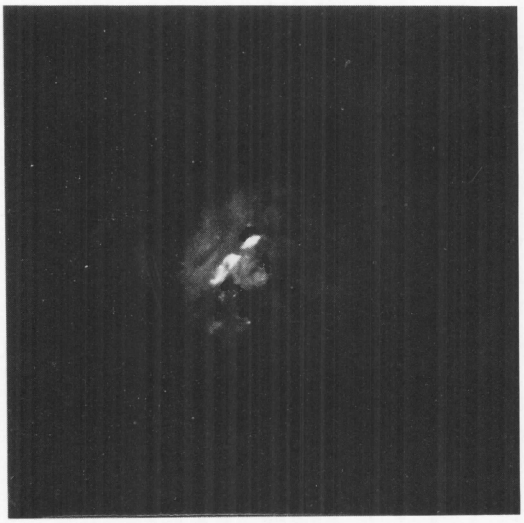


Fig. 7. Two PYY-like immunoreactive cells are identified in the parenchyma of the pancreas (IF, original magnification 100).

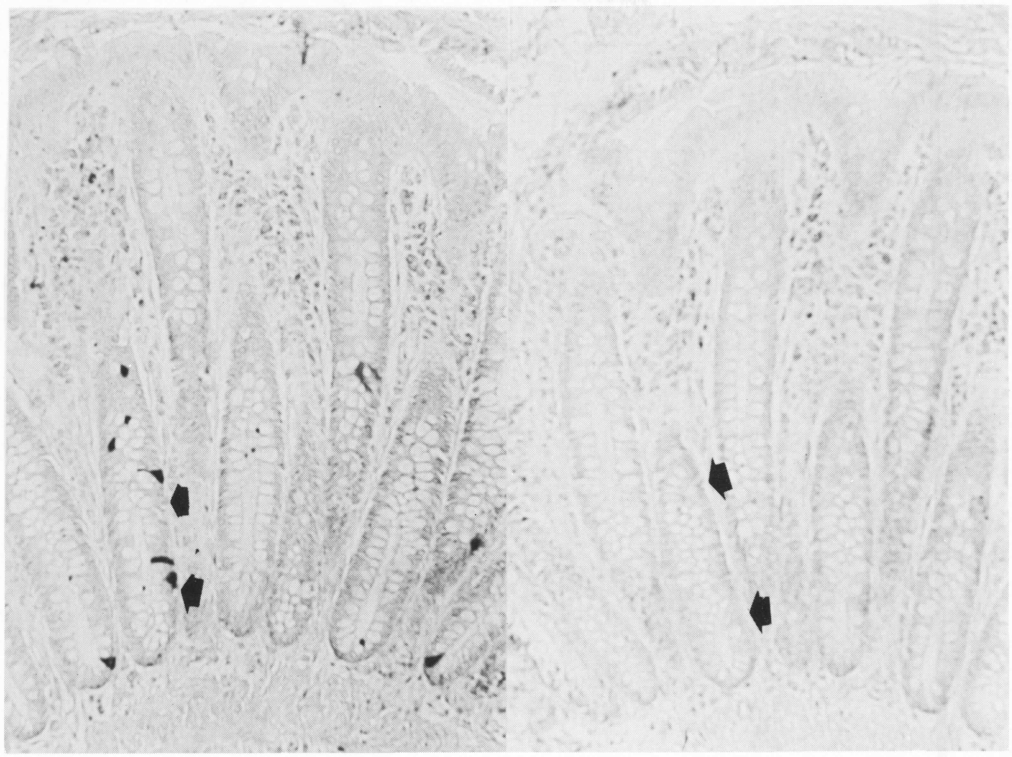
like immunoreactivities after absorption of adding an excess amount of synthetic porcine PYY or after incubating with normal rabbit serum instead of primary antiserum as well as with applying PAP complex alone (Fig. 8).

DISCUSSION

The present data showed that PYY (exactly PYY-like immunoreactivities), a recently isolated 36 amino acid peptide of the PP family[9,10], was present in a large population of endocrine cells in the lower gastrointestinal tract, particularly in the distal colon and rectum and further in a small population of the duodenum and parenchyma of the pancreas. PYY-like immunoreactivities observed in these organs were authentic PYY, since the immunoreactivities abolished with PAP after preincubation of an excess amount of synthetic PYY (kind gift from Drs. Tatemoto and Mutt). These data are similar to those in the gut of the guinea pig, rat, cat and pig by Lundberg et al[6], who found that there were numerous PYY cells in the distal small intestine, colon and rectum, while rarely in the stomach and duodenum. Their data and ours suggest that PYY cells may have in a similar localization in mammals. By radioimmunoassay, however, the same authors have found a considerable amount of PYY-like immunoreactivities in the ileum and pancreas of rats. Our morphological data showed quite a few PYY cells in the ileum and pancreas of man. The distribution of PYY may be different in concentration between human and rats although there were some differences between the immunocytochemistry data and immunochemistry data.

The lower part of the esophagus, gastric body and antrum, as well as the gall bladder did not reveal any immunoreactivities with PYY. Although these data supported the previous data[5,6], it is the view of the authors that further consideration should be made before deciding that PYY cells are not present in the upper gastrointestinal tract. Accumulation of more cases (tissue samples) of the upper gastrointestinal organs, particulary the esophagus and proximal small intestine including the jejunum should be made before this.

The number of PP-immunoreactive cells present in the human colon and rectum differs by authors[3,7]. El-Salhy et al[5] have concluded that PYY immunoreactive cells are independent cell type from PP- or enteroglucagon-immunoreactive cells, which are also present in the human colon, and that PYY immunoreactive cells are H cells[1] based on electron microscop-



(A) (B)

Fig. 8. Absence of PYY-like immunoreactive cells after absorption (B). Compare with the immunoreactive cells without absorption (A). (PAP, Serial sections, original magnification 100).

ic data (shape and type of secretory granules). As the antiserum used here cross-reacted with BPP very slightly, PYY-immunoreactive cells observed in the colon and rectum are not BPP.

PYY-immunoreactive cells are tall and various in shape with its apical portions reaching the lumen and occasionally with processes at the basal position to the neighbouring cells. These data suggest that the cells are open typed and endocrine as well as paracrine. However, closed typed cells are present but very rarely. El-Salhy et al[5] have suggested that PYY-cells may exert a paracrine action on the mucus-secreting goblet cells in view of the parallel distribution and frequency of goblet cells and PYY cells, and their cytoplasmic processes to the neighbouring goblet cells. Their hypothesis could not be verified in the present study. The reasons for the peculiar distribution of PYY immunoreactive cells and their action, however, should be elucidated before too long.

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REFERENCES

1. **Buffa, R., Capella, C., Fontana, P., Usellini, L. and Solcia, E.** 1978. Types of endocrine cells in the human colon and rectum. Cell Tissue Res. **192**: 227-240.
2. **Coons, A.H., Leduc, E.H. and Connolly, J.M.** 1955. Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application of a study

of the hyperimmune rabbit. J. Exptl. Med. **102**: 49-60.

3. **Cristina, M.L., Lehy, T., Zeiloun, P. and Dufougeray, F.** 1978. Fine structural classification and comparative distribution of endocrine cells in normal human large intestine. Gastroent. **75**: 20-28.
4. **De Mey, J.** 1983. Raising and testing antibodies for immunocytochemistry, p. 43. *In* J.M. Polak and S. Van Noorden (ed.), in Immunocytochemistry Practical Application in Pathology and Biology. Wright, S.G.
5. **El-Salhy, M., Grimelius, L., Wilander, E., Rhyberg, B., Terenius, L., Lundberg, J.M. and Tatemoto, K.** 1983. Immunocytochemical identification of polypeptide YY (PYY) cells in the human gastrointestinal tract. Histochemistry **77**: 15-23.
6. **Lundberg, J.M., Tatemoto, K., Terenius, L., Hellerström, P.M., Mutt, V., Hökfelt, T. and Hamberger, B.** 1982. Localization of the polypep tide YY (PYY) in gastrointestinal endocrine cells and effects on intestinal blood flow and motility. Proc. Natl. Acad. Sci. U.S.A. **79**: 4471-4475.
7. **Sjölund, K.** 1982. Gut neurohormonal peptides in health and disease. Ph. D. Thesis, University of Lund Sweden. Quoted from Ref. 5
8. **Sternberger, L.A. and Cuclis, J.J.** 1967. Method for enzymatic intensifications of the immunocytochemical reaction without use of labeled antibodies. J. Histochem. Cytochem. **17**: 190-196.
9. **Tatemoto, K., Carlquist, M. and Mutt, V.** 1982. Discovery and isolation of neuropeptide Y (NPY), a brain peptide that has structural similarities to intestinal PYY and the pancreatic polypeptide. Nature **296**: 654-660.
10. **Tatemoto, K. and Mutt, V.** 1980. Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptide. Nature **285**: 417-418.