Virchows Arch [Pathol Anat] (1983) 401:67–72

Virchows Archiv A

Polypeptide YY (PYY) and pancreatic polypeptide (PP) in rectal carcinoids

An immunocytochemical study*

E. Wilander[1], M. El-Salhy[1], M. Lundqvist[1], L. Grimelius[2], L. Terenius[1], J.M. Lundberg[3], K. Tatemoto[3], and T.W. Schwartz[3]

[1] Departments of Pathology and Pharmacology, University of Uppsala, University Hospital, S-751 85 Uppsala, Sweden
[2] Department of Pathology, University of Lund, General Hospital, Malmö, and
[3] Departments of Pharmacology and Biochemistry, Karolinska Institute, Stockholm, Sweden, and Department of Clinical Chemistry, Rigshospital, Copenhagen, Denmark

Summary. The frequency and distribution of polypeptide YY (PYY) and pancreatic polypeptide (PP) immunoreactive tumour cells of 14 small intestinal and of 27 rectal carcinoids were studied. All small intestinal and 14 rectal tumours were unreactive to both hormones. However, 13 rectal carcinoids contained a variable number of PP-immunoreactive cells. In four of these cases both PYY- and PP-immunoreactive cells were seen. The PP-immunoreactive cells greatly exceeded the number of PYY-immunoreactive cells. Two rectal carcinoids with PYY and PP immunoreactivities, but not the rest of the tumours, reacted also with an antiserum specific to the C-terminus of PP. This indicates that most PP immunoreactive rectal carcinoids lack the C-terminus sequence of the PP molecule.

Key words: Human rectum – Carcinoids – PP – PYY – Immunocytochemistry

The number of endocrine cell types identified and their peptide hormone production in the diffuse endocrine cell system of the gastrointestinal tract has increased greatly recently (Håkanson and Sundler 1979). This has resulted in a growing interest in endocrine cell types that are constituents of the carcinoid tumours at various locations in the stomach and intestine.

A newly isolated gut peptide, the polypeptide YY (PYY) (Tatemoto and Mutt 1980; Tatemoto 1982) has been found to be stored in endocrine cells of the intestinal mucosa and these cells appear to be most abundant

Offprint requests to: E. Wilander at the above address

* Preliminary results were presented in XIV Acta Endocrinol Congress held in Stockholm, Sweden, June 27–30, 1983

in the lower part of the ileum, colon and rectum (Lundberg et al. 1982; El-Salhy et al. 1983). Since the endocrine cell types constituting the carcinoids often reflect the endocrine cells normally present in the area in which the tumour arises, it was considered of interest to study the presence of PYY in small intestinal and rectal carcinoids. The PYY molecule has the same C-terminus and many amino acids in common with the pancreatic polypeptide (PP) molecule (Tatemoto 1982). For that reason the occurrence of both peptides in the tumours was investigated.

Material and methods

A collection of 14 small intestinal and 27 rectal argyrophilic carcinoids which were formalin-fixed and paraffin-embedded were received from different departments of pathology in Sweden with the aid of records obtained by the courtesy of the Cancer Registry of the National Board of Health and Welfare in Sweden. Approximately 4 μm thick sections were cut, stained with haematoxylin-eosin and immunostained with the peroxidase-antiperoxidase (PAP) method of Sternberger (1979) for PYY and PP. The antisera used were rabbit antiserum against porcine PYY No. 690 rabbit antiserum against bovine pancreatic polypeptide (BPP) No. 615-R-100-146-6, a gift from R.E. Chance, Eli-Lilly Research Laboratory, Indianapolis, USA, rabbit antiserum against BPP No. R-782308-2 (Milab, Malmö, Sweden) and rabbit antiserum against the sequence 24–36 of BPP No. 734-3. These antisera were used at the dilution of 1:2,500, 1:2,000, 1:650 and 1:500, respectively. The characterization of the antisera has been described in detail elsewhere (Lundberg et al. 1982; El-Salhy et al. 1982a and b). The controls used were as follows: a) the first layer antiserum was replaced by normal rabbit serum, b) the antiserum was preincubated with 100 μg of the corresponding peptide per ml diluted antiserum at 4° C for 24 h c) the anti-PYY was preincubated with 100 μg of BPP (a gift from R.E. Chance) or of porcine PP (Lot No. PPP 79062, Novo Research Institute, Copenhagen, Denmark) per ml diluted antiserum at 4° C for 24 h, and d) the anti-BPP was preincubated with 100 μg of porcine PYY per ml of diluted antiserum at 4° C for 24 h.

Results

In routine histology all tumours exhibited the morphology of carcinoids with regular tumour cells growing in different characteristic patterns.

The result of the study of the presence of PYY and PP in the tumour cells is demonstrated in Table 1. All small intestinal and 14 of the rectal carcinoids were without identified immunocytochemical staining. In 9 rectal tumours PP-immunoreactive cells were found. They varied in frequency in the different tumours but in no case did they constitute the majority cell population (Fig. 1). Two tumours contained both PYY and PP immunoreactive cells. In another 2 cases PP-, PYY- and PP C-terminus immunoreactive cells were detected (Fig. 2). The PYY cells were scanty in all cases and the PP cells far exceeded the number of PYY reactive cells. In one of these tumours the PP cells were so numerous that they probably constituted the majority of the tumour cell population.

The absorption tests showed that the PYY antiserum was neutralized only by the PYY antigen and not by BPP or PPP and the anti BPP with BPP and not by PYY.

Table 1. PYY- and PP-immunoreactivity in 27 rectal carcinoids

No. of cases	Immunoreactivity		
	PYY	PP	PP C-terminus
14	–	–	–
9	–	+	–
2	+	+	–
2	+	+	+

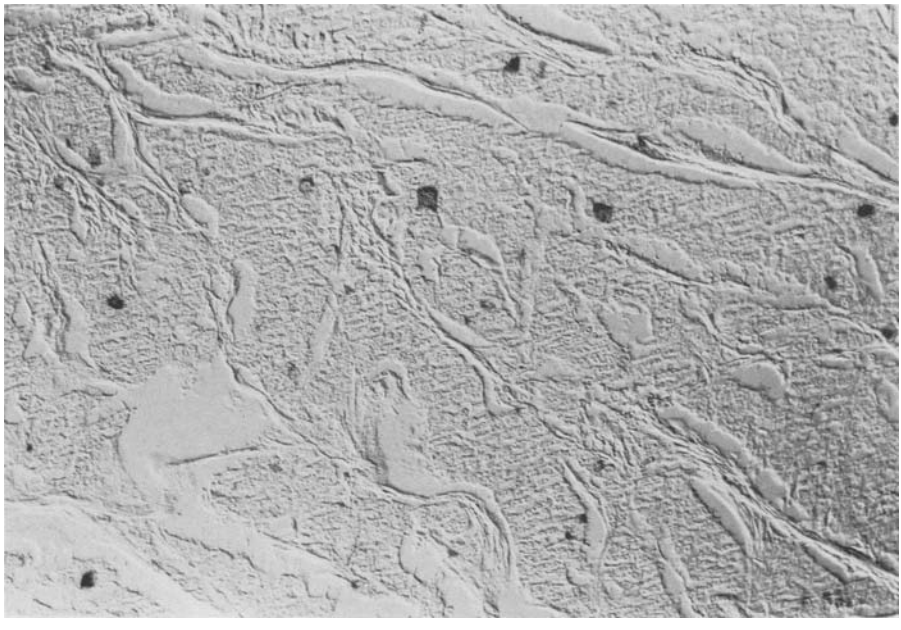
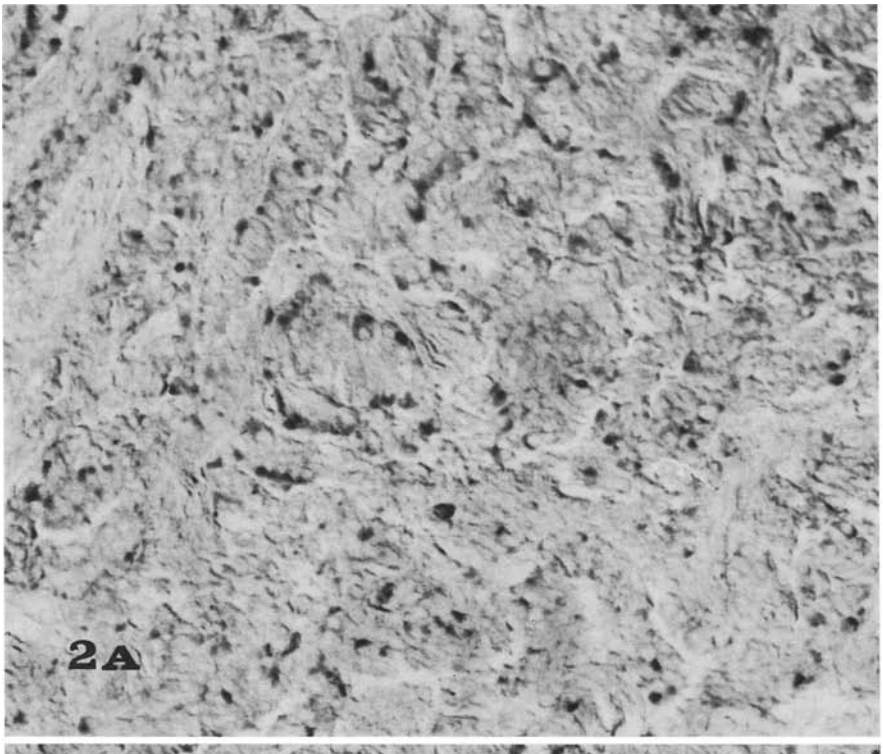
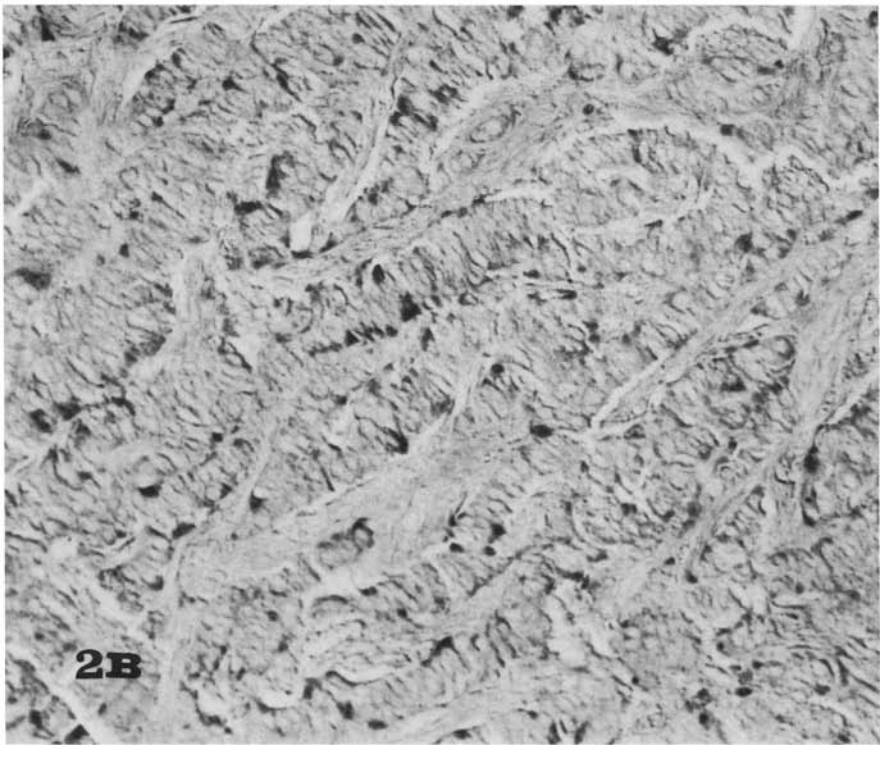


Fig. 1. PP-immunoreactive cells in a rectal carcinoid tumour which did not immunostain after incubation with anti-PYY or anti-PP C-terminus. ×120

Discussion

The rectal carcinoids are endocrine tumours. Evidence for this is given by their silver staining properties (Hosoda et al. 1975; Wilander et al. 1977), the presence of neurosecretory granules in the tumour cell by electron microscopic (Orloff 1971) and above all by the immunocytochemical demonstration of neurohormonal peptides in the tumour cells (Wilander et al. 1977; Fiocca et al. 1980; Taxy et al. 1980; Alumets et al. 1980 and 1981; O'Briain et al. 1982). Several different peptide hormones have been identified in the rectal carcinoids but PP, somatostatin and glucagon appear to be the most frequent. These three hormones have also been identified in endocrine cells of the rectal mucosa in addition to neurotensin immunoreactive cells and the argentaffin serotonin storing enterochromaffin cells (Solcia et al. 1979;


2A


2B

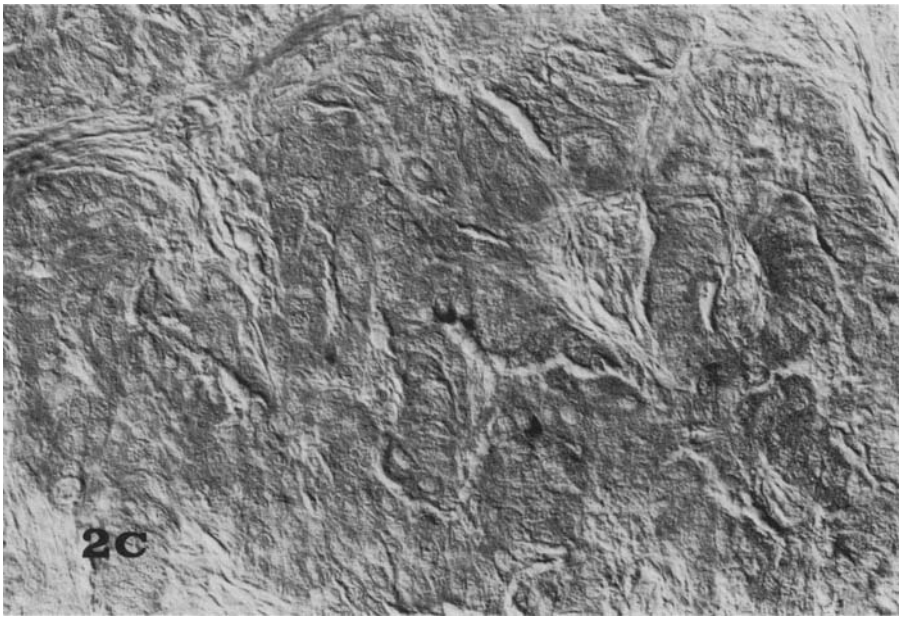


Fig. 2A–C. One of the two rectal carcinoid tumours that contains PP C-terminus, PP, and PYY-immunoreactive cells. **A** shows the PP C-terminus immunoreactive cells and **B** shows PP-immunoreactive cells and **C** the PYY-immunoreactive cells. ×210 (**A**; **B**), ×280 (**C**)

Fiocca et al. 1980; O'Briain et al. 1982). However, neurotensin has not been demonstrated in rectal carcinoids and serotonin only rarely (Taxy et al. 1980).

The present observation of PP C-terminus immunoreactivity in two rectal carcinoids only out of 13 that exhibited PP-immunoreactivity shows that in 11 cases a PP molecule that lack the C-terminus is secreted. Thus, these tumours seem to secrete a PP-like molecule rather than a genuine PP.

PYY cells appear to be one of the dominant endocrine cell types of the rectal mucosa (5) and it could be presumed that this polypeptide would be a frequent constituent of rectal carcinoids. However, this was not the case and consequently PYY seems to represent a hormone for which the statement that the hormones of endocrine tumours reflect the frequency and presence of hormones in the organ from which the tumours derive is only partly valid. Another possibility is that malignant transformation of endocrine cells has resulted in the production of abnormal hormonal peptides with altered antigenicity. The PYY hormone is not the only molecule with an unexpected distribution in endocrine tumours. It also occurs in other tumours and gastrinomas of the pancreas is one of the most conspicious representatives of the different endocrine tumours in the gastro-entero-pancreatic endocrine system (Larsson 1978).

Acknowledgement. This work was supported by grants from the Swedish Medical Research Council (Project No. 12X-102 and 04X-3766).

References

Alumets J, Falkmer S, Grimelius L, Håkanson R, Ljungberg O, Sundler F, Wilander E (1980) Immunocytochemical demonstration of enkephalin and β-endorphin in endocrine tumours of the rectum. A survey of 27 colorectal carcinoids. Acta Pathol Microbiol Scand Sect A 88:103–109

Alumets J, Alm P, Falkmer S, Håkanson R, Ljungberg O, Mårtensson H, Sundler F, Tibblin S (1981) Immunocytochemical evidence of peptide hormones in endocrine tumours of the rectum. Cancer 48:2409–2415

El-Salhy M, Grimelius L, Lundberg JM, Tatemoto K, Terenius L (1982a) Immunocytochemical evidence for the occurrence of PYY, a newly isolated gut polypeptide, in endocrine cells in the gut of amphibians and reptiles. Biomed Res 3:303–306

El-Salhy M, Wilander E, Grimelius L, Terenius L, Lundberg JM, Tatemoto K (1982b) The distribution of polypeptide YY (PYY)- and pancreatic polypeptide (PP)-immunoreactive cells in the domestic fowl. Histochemistry 75:25–30

El-Salhy M, Grimelius L, Wilander E, Ryberg B, Terenius L, Lundberg JM, Tatemoto K (1983) Immunocytochemical identification of polypeptide YY (PYY) cells in the human gastrointestinal tract. Histochemistry 77:15–23

Fiocca R, Capella C, Buffa R, Fontana R, Solcia E, Hage E, Chance RE, Moody AJ (1980) Glucagon, glicentin and pancreatic-polypeptide-like immunoreactivities in rectal carcinoids and related colorectal cells. Am J Pathol 100:81–92

Hosoda S, Kito H, Nogaki M, Masumori S, Sosuke I (1975) Is rectal carcinoid argyrophilic. Application of Grimelius silver nitrate stain in four cases. Dis Colon, Rectum 18:386–390

Håkanson R, Sundler F (1979) Peptidergic system of the gastrointestinal tract. Verh Dtsch Ges Inn Med 85:1525–1534

Larsson L-I (1978) Endocrine pancreatic tumours. Human Pathol 9:401–416

Lundberg JM, Tatemoto K, Terenius L, Hellerström PM, Mutt V, Hökefelt T, Hamberger B (1982) Localization of the peptide YY (PYY) in gastrointestinal endocrine cells and effects on intestinal blood flow and motility. Proc Natl Acad Sci USA 79:4471

O'Briain DS, Dayal Y, DeLellis RA, Tischler AS, Bendon R, Wolfe HJ (1982) Rectal carcinoids as tumours of the hind-gut endocrine cells. A morphological and immunohistochemical analysis. Am J Surg Pathol 6:131–142

Orloff MJ (1971) Carcinoid tumours of the rectum. Cancer 28:175–180 Solcia E, Capella C, Buffa R, Usellini L, Frigerio B, Fontana P (1979) Endocrine cells of the gastrointestinal tract and related tumours. Pathol Ann 9:163–204

Sternberger LA (1979) Immunocytochemistry. 2nd ed. John Wiley and Sons, New York

Tatemoto K (1982) Isolation and characterization of peptide YY (PYY), a candidate gut hormone that inhibits pancreatic exocrine secretion. Proc Natl Acad Sci 79:2514–2518

Tatemoto K, Mutt V (1980) Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptides. Nature 285:417–418

Taxy JB, Mendelsohn G, Gupta PR (1980) Carcinoid tumours of the rectum. Silver reactions, fluorescence and serotonin content of the cytoplasmic granules. Am J Clin Pathol 74:791–795

Wilander E, Portela-Gomes G, Grimelius L, Westermark P (1977) Argentaffin and argyrophil reaction of human gastrointestinal carcinoids. Gastroenterology 73:733–736

Wilander E, Portela-Gomes G, Grimelius L, Lundqvist G, Skoog V (1977) Enteroglucagon and substance P-like immunoreactivity in argentaffin and argyrophil rectal carcinoids. Virchows Arch [Cell Pathol] 25:117–124

Accepted April 22, 1983