Peptide YY-like Immunoreactivity in Normal Colon Mucosa, Muscle Layer and Adenocarcinoma

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Peptide YY (PYY)-like immunoreactivity was detected in the mucosa and muscle layer of normal human colon and rectum and in well to moderately differentiated adenocarcinoma derived from the mucosa of the colon and rectum, using a sensitive and specific radioimmunoassay for PYY. The content of PYY-like immunoreactivity in the mucosa was markedly higher than those in the muscle layer and adenocarcinomatous tissue of any part of the colon and rectum. A high concentrations of PYY-like immunoreactivity was demonstrated throughout the colon mucosa (ascending colon 94.14 ± 15.34 pmol/g, transverse colon 137.19 ± 13.44 pmol/g, descending colon 168.89 ± 15.63 pmol/g, and sigmoid colon 223.69 ± 35.31 pmol/g), the highest being observed in the rectum (313.15 ± 45.90 pmol/g). The major molecular form of PYY-like immunoreactivity both in the mucosa and muscle layer of normal human colon and rectum and in adenocarcinomatous tissue was judged by gel exclusion chromatography to be identical to pure porcine PYY. This study revealed the presence of PYY-like immunoreactivity not only in normal tissue of the colon and rectum but also in adenocarcinomas with the same elution pattern, and the mucosal concentrations of PYY-like immunoreactivity were found to be increasing distally throughout the colon and rectum.

Key Words: PYY, Human colon, Colon adenocarcinoma

Peptide YY (PYY), a newly discovered regulatory peptide, has been isolated from porcine duodenum using a novel chemical assay. In the immunohistochemical study using antiserum raised against porcine PYY, PYY-immunoreactive cells were abundantly observed in the lower part of the ileum, the colon and rectum. The physiological role of PYY is yet uncertain, but its biological actions are potent and include vasoconstriction, gastric acid secretion and reduction of gastrointestinal motility. Although the distribution of PYY has been studied by radioimmunoassay of normal tissue extract in the rat, dog, and human, it has not yet been determined in pathological tissues of the human rectum. In the present study we demonstrated the distribution of PYY in normal human colon and rectum, using a sensitive and specific assay for PYY.

MATERIALS AND METHODS

Subjects and materials
Specimens of normal mucosa, muscle layer, and mucosal side of adenocarcinomas of the colon from a total of 23 patients (10 women and 13 men, mean age 59 years, range 28–82 years) were obtained from segments of the bowel within 30 minutes after resection for the diagnosis of adenocarcinoma. All of these adenocarcinomas had extended to the subserosal layers and were histologically proved to be well or moderately differentiated adenocarcinoma and not to contain any non-cancerous tissue. The maximum diameter of the lesions was 2.5–9.0 (5.1 ± 3.3) cm. The locations

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of adenocarcinomas of the resected colon and normal tissues were as follows: 5 cases in the ascending colon, 3 cases in the transverse colon, 3 cases in the descending colon, 8 cases in the sigmoid colon, and 4 cases in the rectum. For each specimen, two full thickness samples were prepared, one being sent for histological examination and the other being immediately frozen with solid carbon dioxide after washing with saline and stored at $-70^\circ$C till extraction. Normal tissues were taken from a macroscopically normal area 10 cm distance from the tumor margin. Histological examination was made in order to exclude neoplastic infiltration, inflammation, or other abnormality in the vicinity of the processed tissue.

**Extraction of PYY-like immunoreactivity**

At the time of extraction, 200–300 mg of tissue was excised from each sample and minced while frozen, homogenized in 4.0 ml of a mixture of boiling acetic acid (1N) and 4,000 kallikrein inhibitor units (KIU) of Trasylol (Bayer, Leverkusen, West Germany), boiled for 20 minutes in a hot bath, and then centrifuged at 10,000xg at 4°C for 20 minutes. Heparin-Mn$^{++}$ solution was added to the supernatant in order to exclude lipoprotein, adjusted pH 7, lyophilized and reconstituted in phosphate buffered saline (1/15 M, pH 7.4) containing 500 KIU Trasylol. This was subjected to radioimmunoassay for PYY and also to gel exclusion chromatography.

**Radioimmunoassay for PYY**

Radioimmunoassay for PYY was performed as previously described[1]. Standards containing 0.05–10 ng porcine PYY dissolved in 0.1% bovine serum albumin (fraction V, Sigma Chemical Co., St. Louis, Mo.) in phosphate buffered saline (1/15 M, pH 7.4) or tissue extracts, antiserum (final dilution 1:10,000) and $^{125}$I-PYY (approximately 5,000 cpm) in 0.5% bovine serum albumin gammaglobulin, containing Trasylol 500 KIU/ml in phosphate buffered saline were mixed. After incubation at 4°C for 12–16 hrs, 1 ml of 20% polyethylene glycol (M.W. 6,000) in distilled water was added to each tube. Tubes were vortexed vigorously and centrifuged at 4°C for 15 min at 3,000xg. The supernatant was removed and the pellet counted in a gammacintillation counter. The sensitivity of the assay defined as the minimal detectable amount was 0.05 ng and the useful range of the standard curve was established between 0.05 ng and 1 ng. The intraassay coefficient of variation was 7.4%, while the interassay coefficient of variation was 13.4%. This radioimmunoassay was highly specific for PYY and showed essentially no cross-reactivity with related peptides such as human PP, bovine PP, and NPY. Samples were assayed in duplicate at three dilutions. Analysis of this radioimmunoassay data was made using the generalized, weighted, interactive, least squares method for logistic curve fitting[12].

**Recoveries**

Recovery of porcine PYY added to the human colon mucosa and muscle layer at the time of boiling in 1N acetic acid extraction was 104.6 ± 0.1% (mean ± SEM). Adapting these samples to the gel exclusion chromatography, no conversion of PYY into short fragments during acetic acid extraction procedure was observed.

**Gel exclusion chromatography**

One ml aliquots of reconstituted solution in the buffer were applied to a Sephadex G-50 superfine column (1.0 x 90 cm), equilibrated and eluted with a phosphate buffered saline containing 0.01% bovine serum albumin, 0.2% NaN$_3$, and 0.1% Trasylol at 4°C. The fraction volume was 1.2 ml. The column was calibrated with blue dextran, porcine $^{125}$I-PPY and Na$^{125}$I. Recoveries of PYY from the column were more than 90%.

**Statistics**

Results were expressed as nanogram per gram wet weight tissue and presented as mean ± SEM. Statistical analysis was performed by Wilcoxon’s test for comparison of median.

**RESULTS**

**PYY-like immunoreactivity in the tissues**

Extracts of the mucosa and muscle layer of normal colon and colon adenocarcinomas at different dilutions produced curves which were parallel to the PYY standard curve (Fig. 1). The concentrations of PYY-like immunoreactivity in the mucosa of normal ascending colon, transverse colon, descending colon, sigmoid colon and rectum were 94.14 ± 15.34, 137.19 ± 13.44, 168.89 ± 15.63, 223.69 ± 35.31 and 313.15 ± 45.90 pmol/g wet wt, respectively. The concentrations of PYY-like
immunoreactivity in the normal muscle layer of the ascending colon, transverse colon, descending colon, sigmoid colon and rectum were 9.21 ± 2.73, 10.54 ± 0.86, 17.97 ± 3.02, 10.37 ± 2.48 and 8.48 ± 5.19 pmol/g wet wt, respectively. In well or moderately differentiated adenocarcinoma, the concentrations of PYY-like immunoreactivity of the ascending colon, transverse colon, descending colon, sigmoid colon and rectum were 6.54 ± 1.33, 14.53 ± 4.94, 11.43 ± 3.27, 10.12 ± 2.59 and 7.12 ± 2.24 pmol/g wet wt, respectively (Fig. 2).

**DISCUSSION**

It has been demonstrated by radioimmunoassay that, in the gastrointestinal tract of the rat and dog, the levels of PYY immunoreactivities were about 10-fold to 130-fold higher in the ileum and colon than in other regions of the gastrointestinal tract. Adrian et al. has also reported that concentrations of PYY-like immunoreactivities in the human ileum and rectum are more than 10-fold and 100-fold higher, respectively, than those of the upper small intestine.

This study confirmed the presence of PYY-like immunoreactivity in the normal mucosa and mus-
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Fig. 3. Elution profile of PYY-like immunoreactivity in mucosal layer of human colon extracted in 1N acetic acid obtained by a Sephadex G-50 superfine column. Vo: void volume, Vt: total volume.

Fig. 4. Elution profile of PYY-like immunoreactivity in muscle layer of normal colon extracted in 1N acetic acid obtained by a Sephadex G-50 superfine column. Vo: void volume, Vt: total volume.

Fig. 5. Elution profile of PYY-like immunoreactivity in colon adenocarcinoma extracted in 1N acetic acid obtained by a Sephadex G-50 superfine column. Vo: void volume, Vt: total volume.

In this study, PYY-like immunoreactivity in colon adenocarcinoma was decreased and accompanied with replacement of normal intestinal endocrine cells and goblet cells by adenocarcinomatous tissue. In the gut of the mammals studied, PYY-immunoreactive cells have been reported to be the open type and to extend from the basal lamina to the gut lumen. These cells emit long cytoplasmic processes from the base to the neighboring goblet cells. This finding, together with the parallel distribution of frequency of PYY-immunoreactive cells and goblet cells, has suggested that PYY-immunoreactive cells may exert a paracrine action on mucus secreting goblet cells and be related to the process of making fecal mass. The relationship between PYY and mucus secreting goblet cells may be a contributory factor to decreased PYY-like immunoreactivity in adenocarcinomatous tissue.

Of these 23 patients six complained of constipation and two of diarrhea. Patients with abnormal bowel habits such as constipation or diarrhea did not show any significant difference in the
levels of PYY-like immunoreactivity in both normal tissues and adenocarcinomas from the patients without bowel habit disturbances (anatomical positions were matched). It is interesting to note that two patients complained of diarrhea in spite of the existence of advanced cancer in the colon and that decreased PYY-like immunoreactivity in adenocarcinomatous tissue may be related to the altered bowel habit such as diarrhea in some cases with colon cancer in view of reports suggesting that the circulating PYY is mainly derived from the colon and rectum\textsuperscript{13} and its biological action is reduction of gastrointestinal motility\textsuperscript{8}.

Taylor reported in column chromatographic studies that smaller peaks of immunoreactivity were observed eluting immediately adjacent to the major form which corresponded to the position of authentic porcine PYY in canine ileal and colonic tissue extracts\textsuperscript{9}. Chromatographic profiles of PYY immunoreactivity from the rat colon and porcine PYY on a SP-Sephadex ion exchanger also provided similar results\textsuperscript{9}. Our data demonstrated that the gel filtration patterns on Sephadex G-50 superfine column of normal mucosal layer, normal muscle layer and adenocarcinomatous tissue of the human colon showed a pattern similar to those of the whole layers of the canine ileum and colon tissue extracts\textsuperscript{9}. No difference was observed in the processing of PYY between normal mucosal tissues and well or moderately differentiated adenocarcinomatous tissues.

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