Peptide YY Abnormalities in Patients with Ulcerative Colitis

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Peptide YY concentrations in both rectal mucosa and fasting plasma, which were produced by endocrine cells in the lower gastrointestinal tract, were measured in normal subjects and patients with ulcerative colitis by specific radioimmunoassay and were examined in the relation to histopathological changes and extent of lesions of ulcerative colitis. Peptide YY levels of rectal mucosa (11.0 ± 1.4 pmol/mg protein) and fasting plasma (53.3 ± 7.9 fmol/ml) of patients with ulcerative colitis were significantly lower ($p < 0.001$) than those of normal subjects (31.5 ± 3.4 pmol/mg protein and 160.7 ± 19.7 fmol/ml). It appears that the decreased PYY level in rectal mucosa and fasting plasma is related to crypt destruction and reduction of mucosal cell. These changes of PYY levels in rectal mucosa and fasting plasma suggest a presence of relationship between concentrations of peptide YY and severity of lesions in patients with ulcerative colitis.

Key Words: Peptide YY, Ulcerative colitis, Rectal mucosa, Plasma.

Peptide YY (PYY), a recently isolated peptide from the porcine duodenum, has been found in endocrine cells of the human ileal and colonic mucosa in high concentrations, being maximum in the rectum by both radioimmunoassay and immunohistochemical procedures¹⁻⁴). PYY has been shown to have several biologic actions in animals, including vasoconstriction, reduction of pancreatic secretion, and inhibition of gastrointestinal motility^{2,5-8}). In addition, Adrian has reported that PYY circulates in plasma and concentrations rise in response to feeding, suggesting that the circulating PYY is mainly derived from the ileum and colon from its gut distribution⁴). In view of its localization and actions, changes in plasma concentrations and mucosal contents of this peptide in patients with colonic disease having mucosal inflammation are of great interest. Such digestive disorders are associated with changes in mucus secretion and motility that may be influenced by PYY. Thus, the aim of this study was to investigate the effects of colonic mucosal inflam-

mation on mucosal contents and plasma concentrations of PYY in patients with ulcerative colitis.

MATERIALS AND METHODS

Subjects

Fasting blood samples were drawn into tubes containing Trasylol (Bayer, Leverkusen, West Germany) and 1M EDTA to give a concentration of 600 kallikrein inhibitor units (KIU) and 20 μ l/ml blood, respectively. The group was composed of 10 healthy subjects (2 females and 8 males with a mean age of 54.3 years and age range of 27-78 years) having normal weight and height and 7 patients with ulcerative colitis (2 females and 5 males with a mean age of 29 years and age range of 17-43 years) (Table 1).

A group of 28 patients, in whom colon endoscopy was required for diagnostic or follow-up process were studied. In the endoscopic examination of the patients, 5 biopsied specimens were obtained endoscopically by Olympus colono-

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scopes from the rectum at a position about 10 cm distal from the anal ring. Three out of 5 biopsied specimens were frozen immediately in liquid nitrogen and stored at -70°C till extraction, and the other 2 specimens were processed for histological examination. This group was composed of 19 patients (12 females and 7 males with a mean age of 49.9 years and age range of 20-83 years), who visited our gastroenterology clinic for investigation of non-specific symptoms but in whom no alimentary tract pathology was subsequently found and 9 patients with ulcerative colitis (7 females and 2 males with a mean age of 36.6

years and age range of 21 ~ 47 years)

The diagnosis of ulcerative colitis was made based on clinical history and signs with confirmation being made by barium radiology, colonic endoscopy, and histology. The extent of disease and histology findings based on barium enema, colonoscopic appearances, and histological examination are shown in Tables 1 and 2.

Extraction of Blood Samples

Blood samples were collected in ice-chilled tubes containing 20 μl of 1 M EDTA-2Na and 600 KIU of Trasylol per ml of blood and left on

Table 1. Clinical data 1

Case No.	Age	Sex	Extent of disease	Current activity	Histological findings*		PYY in plasma (fmol/ml)
					Crypt destruction	Reduction of goblet cells	
1	29	M	Rectum	inactive	severe	severe	86.8
2	28	M	Pancolitis	inactive	mild	mild	47.5
3	31	F	Distal sigmoid and rectum	inactive	moderate	mild	39.8
4	36	F	Distal descending, sigmoid, and rectum	inactive	mild	mild	65.6
5	17	M	Descending, sigmoid, and rectum	inactive	severe	severe	27.9
6	19	M	Distal sigmoid and rectum	inactive	moderate	severe	31.6
7	43	M	Sigmoid and rectum	inactive	mild	mild	74.1

* Dysplasia was not found in any of the biopsy specimens.

Table 2. Clinical data 2

Case No.	Age	Sex	Extent of disease	Current activity	Histological findings*		PYY in tissue (pmol/mg protein)
					Crypt destruction	Reduction of goblet cells	
1	40	M	Distal 15 cm	inactive	mild	mild	14.4
2	26	F	Pancolitis	inactive	severe	severe	11.2
3	51	F	Pancolitis	inactive	mild	mild	14.8
4	47	F	Pancolitis	inactive	mild	mild	11.1
5	23	F	Distal 20 cm	inactive	severe	severe	3.2
6	31	F	Distal sigmoid and rectum	inactive	mild	mild	15.1
7	47	F	Pancolitis	inactive	moderate	moderate	10.4
8	43	F	Left side colon	inactive	moderate	moderate	3.8
9	21	M	Rectum	inactive	severe	severe	7.8

* Dysplasia was not detected in any of the biopsy specimens.

Histological findings at a level below the peritoneal reflection are shown.

ice until centrifugation at 4°C. Plasma was stored at -20°C until extraction.

For extraction, ice-cold acetone (7.5 ml) and 2M acetic acid (1.5 ml) were added to 2 ml of thawed plasma in a glass tube and vortexed vigorously. After centrifugation at 4°C (600 x g for 30 minutes), the supernatant was transferred to another glass tube and ice-cold petroleum ether (4 ml) was added to the aliquots. After vigorous vortex, petroleum ether phase was removed with a pasteur capillary pipet and acetone phase was removed using Concentrator EC 10 (TOMY SEIKO Co., Ltd., Tokyo, Japan). Materials remaining in aqueous phase were lyophilized and reconstituted in phosphate buffered saline (0.067 M, pH 7.4) (IATRON Lab., Tokyo, Japan) containing 600 KIU Trasylol, and then subjected to radioimmunoassay for PYY and also to gel exclusion chromatography.

Extraction of Rectal Biopsied Specimens

At the time of extraction, 3 biopsied specimens were homogenized by Polytron homogenizer (Kinematica GmbH, Lucern, Switzerland) in 4.5 ml of a mixture of ice-cold acetic acid (1M) and 4,000 KIU Trasylol, boiled for 20 minutes in a hot bath. An aliquot was retained for protein

assay⁹⁾ and the remaining homogenate was centrifuged at 18,000 x g at 4°C for 20 minutes.

The supernatant was lyophilized and reconstituted in phosphate buffered saline (0.067 M, pH 7.4) containing 2,000 KIU Trasylol, and then subjected to radioimmunoassay for PYY and also to gel exclusion chromatography.

Table 3. Cross-reactivity of various peptides in human peptide YY radioimmunoassay

Compound	% cross-reactivity (molar basis)
Peptide YY	100
Neuropeptide Y	0
Human pancreatic polypeptide	0
Bovine pancreatic polypeptide	0
Human β -LPH	0
Human β -EP	0
β -EP [β -EP-(1-16)]	0
Methionine enkephalin	0
α -Melanocyte stimulating hormone	0
Adrenocorticotropin (1-39)	0
Substance P	0
Vasoactive intestinal polypeptide	0
Neurotensin	0
Somatostatin	0
Human gastrin	0

β -EP, β -endorphin; β -LPH, β -lipotropin. Various amounts of each compound in the range of 1-1,000 ng were added to assay tubes. The amount of inhibition of binding of ¹²⁵I-peptide YY to the antiserum was determined. Cross-reactivities were calculated by comparison of the 50% B/Bo point (i.e., half-maximal binding point) between a given peptide and peptide YY.

Fig. 1. Dilution curves of human rectal mucosa and plasma. (Rectal mucosa, —●—; plasma, —○—.)

Table 4. Concentrations of peptide YY in rectal mucosa and plasma of human

	PYY in the rectal mucosa (pmol/mg protein)	n	Plasma PYY (fmol/ml)	n
Normal Subjects	31.5±3.4	19	160.7±19.7	10
Patient with ulcerative colitis	11.0±1.4*	9	53.3± 7.9*	7

PYY, peptide YY. Values given are the mean ± SEM.

* Significantly different from normal subjects ($p < 0.001$).

Radioimmunoassay for PYY

Radioimmunoassay for PYY was performed as previously described, using synthetic PYY (PEN-INSULA Lab. Inc., Belmont, CA) as a reference standard and for labeling¹⁰. The final dilution of the antiserum was 1:10,000, and the minimal detectable quantity of PYY was 0.05 ng/tube. The intraassay and interassay coefficient of variation were 7.4% and 13.4%, respectively. This antiserum showed essentially no cross-reactivity with related peptides (Table 3).

Gel Exclusion Chromatography

Aliquots (1.0 ml) of reconstituted solution in the buffer were applied to a Sephadex G-50 superfine (Pharmacia Fine Chemicals, Uppsala, Sweden) column (1.0 x 40 cm), equilibrated and eluted at 4°C with phosphate buffered saline, with contained 0.01% bovine serum albumin (Fraction V, Sigma Chemical Co., St. Louis, Mo.), 0.2% NaN₃, and 0.1% Trasylol. The fraction volume was 0.9 ml. The column was calibrated with blue dextran (Pharmacia Fine Chemicals, Uppsala, Sweden) for void volume and ¹²⁵I-PYY. Recoveries of PYY from the column were >90%.

Recoveries

Recoveries of synthetic PYY added to the human rectal biopsied specimens at the time of homogenizing in 1M acetic acid and the human plasma samples just before the first step of plasma extraction procedure were 90.4±0.3% and 70.0±0.6%, respectively.

RESULTS

As shown in Fig. 1, a good parallelism exists between the PYY standard curve and the dilution

curves of the extracts from rectal biopsied specimens and plasma.

The PYY contents in biopsied specimens of normal subjects and patients with ulcerative colitis were 16.3-76.3 and 3.2-15.1 pmol/mg protein, respectively (Tables 2 and 4 and Fig. 2). The PYY concentrations in plasma samples of normal subjects and patients with ulcerative colitis were 73.5-289.6 and 27.9-86.8 fmol/ml, respectively (Tables 1 and 4 and Fig. 3).

The elution profiles of PYY-like immuno-reactivity on Sephadex G-50 superfine column in

Fig. 2. Peptide YY contents in rectal mucosa. The shaded area indicates mean ± SEM.

Fig. 3. Peptide YY concentrations in plasma samples. The shaded area indicates mean $\pm$ SEM.

biopsied specimens of both normal subjects and patients with ulcerative colitis showed the same elution pattern, that is, a major peak which corresponded to synthetic PYY with two minor peaks (Fig. 4). Those in plasma extracts of both normal subjects and patients with ulcerative colitis showed the same pattern, that is, two major peaks corresponding to the position of synthetic PYY and larger in form than that of a broad minor peak eluting earlier than these two major peaks (Fig. 5).

DISCUSSION

The PYY levels of biopsied specimens and plasma from patients with ulcerative colitis was significantly lower than those of normal subjects (Table 4). Especially in biopsied specimens from patients with ulcerative colitis, all the levels of

Fig. 4. Elution profile of peptide YY like immunoreactivity in human rectal mucosa extracted in 1M acetic acid obtained by a Sephadex G-50 superfine column. Vo, void volume; Vt, total volume.

PYY were lower than the range of those of normal subjects.

In human colon and rectum, open type PYY-immunoreactive cells were observed in the deep part of the crypts and to emit cytoplasmic processes to the neighboring goblet cells, suggesting that PYY cells may exert a paracrine action on mucus-secreting goblet cells, as previously reported³.

The circulating PYY was suggested to be mainly derived from the ileum and colon according to the distribution of PYY-like immunoreactivity throughout the lower part of the human ileum, colon and rectum, increasingly distally, and the peak response manner of plasma PYY to various constituents of meal⁴.

Fig. 5. Elution profile of peptide YY like immunoreactivity in human plasma extracted in acetone and petroleum ether obtained by a Sephadex G-50 superfine column. Vo, void volume; Vt, total volume.

In our findings, the remarkably decreased levels of PYY in rectal biopsied specimens and plasma of patients with ulcerative colitis seem to be attributable to the reduction of PYY-immunoreactive cell number in the inflamed rectal mucosa according to these previous reports^{3,4}.

In contrast, Adrian et al, reported that fasting plasma PYY concentrations were significantly elevated in patients with ulcerative colitis compared with healthy controls and suggested a relationship to motility or secretory changes associated with diarrhea¹¹). The cause of this discrepancy is unknown, but the difference in phase of the disease may be a contributory factor. The current activities of ulcerative colitis cases were all inactive and showed chronic epithelial regeneration and in Adrian's report 16 of the 24

patients with ulcerative colitis had active disease¹¹).

The effect of colonic function has not yet been adequately studied, but in view of the effects of PYY on intestinal motility and mucus secretion from goblet cells, it is possible that the decrease of both circulating PYY and PYY contents of rectal mucosa may be responsible, in part, for altered bowel habit and discharge of bloody mucus that occurs in patients with ulcerative colitis.

Gel filtration patterns of fasting plasma PYY-like immunoreactivity composed of a major peak eluting immediately in front of the major form corresponding to synthetic PYY were different from those of rectal mucosa. This probably represents incomplete processing of PYY precursor and may therefore present prohormonal forms. Those of both plasma and rectal biopsied specimens showed no differences between normal subjects and patients with ulcerative colitis.

Taylor¹²) reported chromatographic profiles of canine PYY-like immunoreactivity in postprandial blood and colonic tissue extracts, while Adrian et al.¹³) reported those of basal and postglucose PYY-like immunoreactivity in plasma from patients with dumping syndrome. Their chromatographic findings were not different from ours.

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