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Changes of the Peptide YY Levels in the Intestinal Tissue of Rats with Experimental Colitis following Oral Administration of Mesalazine and Prednisolone

Yoshihiko HIROTANI,*[,a,b] Kyoko MIKAJIRI,[b,c] Kenji IKEDA,[a,b] Michiaki MYOTOKU,[a] and Nobuo KUROKAWA[b]

[a]*Laboratory of Clinical Pharmaceutics, Faculty of Pharmacy, Osaka Ohtani University, 3–11–1 Nishikiorikita, Tondabayashi City 584–8540, Japan,* [b]*Department of Pharmacy, Osaka University Hospital, 2–15 Yamadaoka, Suita City 565–0871, Japan, and* [c]*Department of Hospital Pharmacy, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160–8582, Japan*

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Few studies have reported the changes in the peptide YY (PYY) levels in the intestinal tissue of rats with ulcerative colitis (UC) following oral administration of mesalazine and prednisolone. We investigated the effects of these drugs on the intestinal mucosal PYY levels in a rat model of UC. We confirmed that the PYY levels in the rat intestinal mucosal tissue were high in the lower intestinal tract. The leukocyte count and hemoglobin levels approached the normal values after administering mesalazine or prednisolone to rats treated with 3% dextran sulfate sodium (DSS). The PYY levels in the caecum and colon decreased significantly after administering DSS but increased when mesalazine was administered in a tissue-specific manner. Unlike mesalazine, the PYY levels increased in the ileum in addition to the colon and rectum after administering prednisolone. However, neither of the drugs induced any changes in the plasma PYY levels. These findings indicate that changes in the intestinal tissue PYY levels may be partially involved in the improvement of DSS-induced UC in rats following the administration of these drugs.

Key words——peptide YY; experimental colitis; mesalazine; prednisolone; intestinal mucosa

INTRODUCTION

Ulcerative colitis (UC) is a nonspecific inflammatory bowel disease and a chronic refractory disease with unknown causes which involves the formation of erosions or ulcers and repeated a remission and relapse in the colorectal mucosa. The main therapeutic drugs for UC are adrenocorticotropic hormones, mesalazine (5-aminosalicylic acid), salazosulfapyridine, and immunosuppressive agents (*e.g.*, 6-mercaptopurine and cyclosporine); recently, infliximab has also been used. Mesalazine and prednisolone, which are the main drugs in clinical use, have been specified as therapeutic drugs in the treatment guidelines for UC.[1] Mesalazine (in tablet form) is used for treating mild and moderate symptoms, while prednisolone (in tablet or injection form) is used for patients who are unresponsive to mesalazine and for those with severe and fulminant colitis. In addition, mesalazine is used for treating acute inflammatory bowel disease (IBD) and for maintenance treatment during remission in UC and Crohn's disease.

Various methods have been reported for inducing UC in animal models.[2–4] In an experimental model of UC, we used dextran sulfate sodium (DSS), which is known to induce an enteritis that closely resembles UC within a relatively short time period.[2,3] In animal models of UC, DSS, when ingested along with drinking water, can induce pathologic changes similar to those occurring in human UC in the caecum and large intestine of the animals.

The gut hormone peptide YY (PYY) comprises a 36-amino acid residue, similar to neuropeptide Y (NPY), and is secreted by the intestinal L-type endocrine cells.[5,6] PYY-secreting cells are primarily located in the mucosal layer of the lumen of the ileum, colon, and rectum, and at a higher density in the colon and rectum.[7] The main gastrointestinal functions of PYY are to reduce intestinal motility,[8,9] regulate antisecretory effects in the gastrointestinal tract,[10] decrease the gastrointesinal blood flow volume,[11] extend the gastric emptying time,[12] and promote the net absorption of water and electrolytes in the colon;[13,14] further, it has trophic action.[15,16]

*e-mail: hirotay@osaka-ohtani.ac.jp

PYY is an important regulator of postprandial fluid and electrolyte transport in the intestine. Since fluid loss is a common feature of IBD including UC, it may be speculated that the production and secretion of PYY, which has proabsorptive effects, could be affected as a part of the regulatory mechanism. It has been found that PYY levels in patients and model animals with IBD including UC are altered.[17–19] However, there are no reports regarding the tissue content of PYY associated with therapeutic agents in IBD patients and UC model rats. Therefore tissue levels of PYY were studied in DSS-induced UC rats. In this study, we investigated the effects of PYY on the distal intestine under the influence of mesalazine and prednisolone. We analyzed the effects of administering DSS and these therapeutic agents on PYY levels in the intestinal tissue as an index of internal changes in the intestinal tract.

MATERIALS AND METHODS

Materials We used 3% DSS solution (molecular weight, 5000) (Wako Pure Chemical Industries Ltd., Osaka, Japan) for the induction of UC in a rat model. Mesalazine (Nisshin Kyorin Pharmaceutical Co. Ltd., Tokyo, Japan) was suspended in 0.5% carboxymethyl cellulose (CMC) solution, while prednisolone (Wako Pure Chemical Industries Ltd.) was dissolved in distilled water for administration.

Animals Male Wistar rats (Clea Japan Inc., Tokyo, Japan) weighing 180–190 g were used. The rats were maintained on an animal chow (MF diet, Oriental Yeast, Tokyo, Japan) for 1 week. They had free access to rat chow and water and were housed in a room maintained at 23±2℃ on a 12-h/12-h light/dark cycle. All experimental procedures were conducted in accordance with the Osaka University Medical School Guidelines for the Care and Use of Laboratory Animals.

DSS Administration The rats were divided into 3 groups and provided distilled drinking water supplemented with 3% (w/v) synthetic DSS *ad libitum* for 14 days. The normal group received drinking water during all the experiments.

Mesalazine and Prednisolone Administration Mesalazine (100 mg/kg) and prednisolone (1 mg/kg) were administered orally once a day with 1% DSS solution after 3% DSS treatment for 14 days. We administered 0.5% CMC solution or distilled water to the animals in the control group.

Preparation of Rat Plasma and Intestinal Tissue Samples The animals were anesthetized with pentobarbital sodium administered intraperitoneally (30 mg/kg body weight). Blood samples were collected in tubes containing heparin and aprotinin (500 KIU/ml, Bayer, Dussendorf, Germany) for estimating PYY; they were then immediately centrifuged on ice. The plasma was separated and stored at −30℃ until assay. Next, *via* laparotomy, sections of the duodenum; jejunum; proximal, middle, and distal ileum; caecum; upper and lower colon; and rectum were quickly excised and flushed with ice-cold saline solution. The excess liquid was removed by blotting the tissue segments with filter paper, and each segment was immediately frozen at −80℃ until assay of PYY levels.

Tissue Extraction Each segment of the rat intestine was thawed, slit open longitudinally, gently patted dry, and weighed. Then, using a homogenizer (Model BM-1, Nihon Seiki Seisakusho, Tokyo, Japan), tissue samples from each segment were homogenized on ice in 10-fold the weight of ice-cold acetic acid 0.1 M. These samples were then boiled for 15 min. After cooling the samples, glacial acetic acid was added (final concentration: acetic acid 1 M solution), followed by centrifugation. The supernatant was lyophilized and frozen at −30℃ until assay.

Measurement of Leukocyte Count We added 0.1 ml of whole blood to 0.9 ml of Turk's diluting fluid, mixed the solution, and counted the leukocytes contained under a microscope.

Measurement of Hemoglobin We used the Nescauto Hemo Kit for measurement of hemoglobin. We added 8 μl of whole blood to 2 ml of coloration reagents, mixed the solution well, and let it stand for 5 min at room temperature. Using a spectrophotometer, we measured the absorbance of the coloration reagent or purified water (ES) to contrast, and of the sample (EA) at 541 nm, and we calculated the hemoglobin concentration as follows: Hemoglobin concentration (g/dl) $=EA/ES\times 16.0$

Myeloperoxidase Activity Large intestine tissue samples taken for measurement of myeloperoxidase (MPO) activity were then frozen on dry ice. The samples were stored at −20℃ until assay. MPO activity was measured using the technique of Bradley *et al.*[20]

PYY Measurement For the PYY assay, phosphate buffer 0.1 M (pH 7.4) containing NaCl 0.14 M, EDTA 25 mM, 0.5% BSA, and 0.02% sodium azide was used as the assay buffer. To each assay tube, 0.4

ml of assay buffer, an unknown sample, or 0.1 ml of standard antigen solution (synthetic rat PYY, Yanaihara Institute Inc., Shizuoka, Japan), 0.1 ml of anti-rat PYY serum RY32 (diluted to 1 : 56000, Yanaihara Institute Inc.) antibody, and 0.1 ml of ^{125}I (about 5000 cpm)-labeled synthetic rat PYY antigen were added and mixed, and the mixture was incubated for 22 h at 4℃. Next, we added 50 μl of goat anti-rabbit γ-globulin serum (1 : 10, Daiichi Radioisotope Laboratories Ltd., Tokyo, Japan), 50 μl of normal rabbit serum (1 : 50, Daiichi Radioisotope Laboratories Ltd.), and 0.5 ml of 5% (w/v) polyethylene glycol 6000 (Wako Pure Chemical Industries Ltd.) solution in phosphate buffer 10 mM (pH 7.4), and the solution was incubated for 4 h at 4℃. Following centrifugation, we removed the supernatant and measured the radioactivity of the precipitate using a gamma counter.

Data Analysis Experimental data are expressed as mean±S.D. Statistical analysis was performed using Student's unpaired *t*-test with a significance level of $p<0.05$.

RESULTS

Changes in Leukocyte Count, Hemoglobin Level, and Colon Length Following the Administration of Mesalazine and Prednisolone in the DSS-induced Rat Model of UC Changes in the leukocyte count in rats with DSS-induced UC are shown in Table 1 and Fig. 1. By the 14th day of DSS administration, the leukocyte count had increased to more than ~8-fold the original count. The hemoglobin levels decreased significantly by approximately 50%, accompanied by bleeding in the large intestine (Table 1, Fig. 1). Bloody stools were observed from days 8 to 14 of DSS administration. Following the appearance of bloody stools, no increases were observed in body weight (data not shown). In the rats with UC, shortening of the large intestines was observed on day 7 of DSS administration, and the animals did not recover up to day 14. On the other hand, in the rats with 5% DSS-induced UC, intense bloody stools were observed

Table 1. Effects of Leukocyte Count, Hemoglobin Levels and Colon Lengths in Rats after 3% DSS and/or Drug Treatment

Treatment	Leukocyte count ($\times 10^3/mm^3$)		Hemoglobin level (g/dL)		Colon length (cm)	
	mesalazine	prednisolone	mesalazine	prednisolone	mesalazine	prednisolone
Normal	3.09±1.22	2.88±0.88	14.60±1.39	15.47±0.50	22.30±1.83	22.70±1.40
Control (3% DSS)	25.52±5.63**	27.55±2.19**	7.35±2.62**	8.90±2.47**	15.90±1.61**	16.30±1.87**
3% DSS+drug	10.90±3.74**,††	10.48±4.33**,††	9.74±1.96**	9.09±4.95*	17.10±2.20*	17.53±0.92**

Values are mean±S.D. of 6–8 rats. * $p<0.05$, ** $p<0.01$ compared normal rat. †† $p<0.01$ compared with control rat.

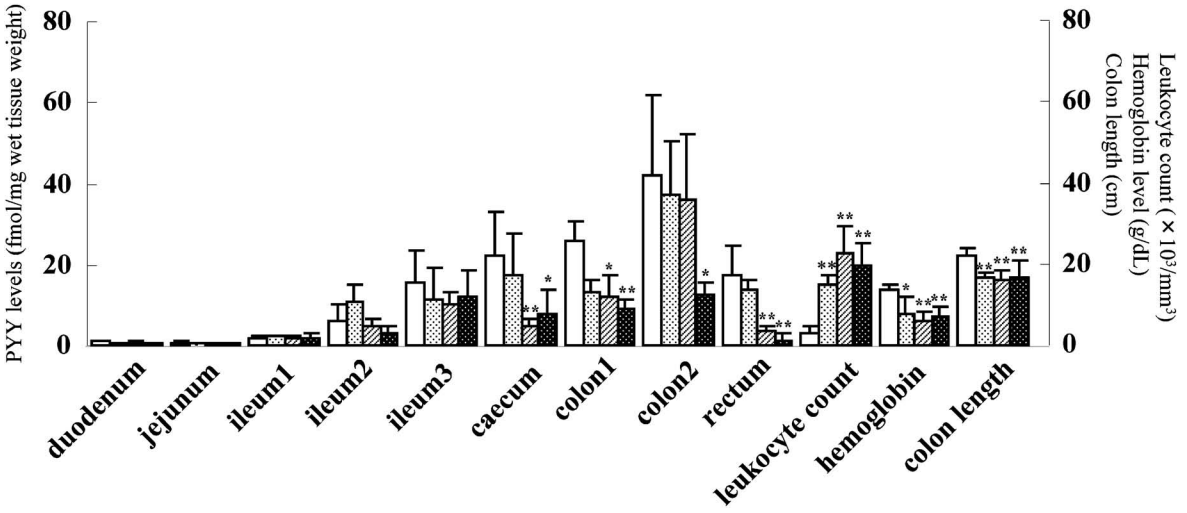

Fig. 1. Changes of Intestinal Mucosal PYY Levels and Inflammatory Makers in DSS Administrating Rat

Data are presented as the mean±S.D. of 6–8 rats. * $p<0.05$, ** $p<0.01$ compared with control rat. □; normal rat, ▩; 3% DSS-treated rat for 1 week, ▨; 3% DSS-treated rat for 2 weeks, ■; 5% DSS-treated rat for 1 week.

from day 7, with remarkable weight loss and mortality in some cases (data not shown). Therefore, we induced UC with 3% DSS in this study.

In the rat model of 3% DSS-induced UC, the leukocyte count decreased significantly after administering mesalazine or prednisolone ($p<0.01$) (Table 1, Fig. 2). The hemoglobin level and colon length tended to increase with either of the drugs (Table 1, Fig. 2). Based on these results, we confirmed the therapeutic effects of these drugs.

Changes in PYY Levels in Intestinal Mucosal Tissue The PYY levels in the intestinal tissues of normal rats were high, particularly in the lower part of the ileum, colon, and rectum (Fig. 1). The changes in the PYY levels in the intestinal tissue samples obtained from DSS-treated rats are shown in Fig. 1. The PYY levels 14 days after 3% DSS administration decreased significantly in the caecum ($p<0.01$), colon 1 ($p<0.05$), and rectum ($p<0.01$), where the pathologic changes of DSS-induced colitis are characteristically observed.[3] The PYY levels 7 days after 5% DSS administration decreased significantly throughout the large intestine (Fig. 1). However, no changes were observed in the PYY levels in 7 days after 3% DSS administration (Fig. 1). Leukocyte counts, hemoglobin levels, and intestinal length changed significantly after 3% and 5% DSS administration.

Effects of Mesalazine and Prednisolone Administration on the PYY Levels in Intestinal Mucosal Tissue and Plasma of Rats with UC The PYY levels after administering mesalazine to rats with DSS-induced

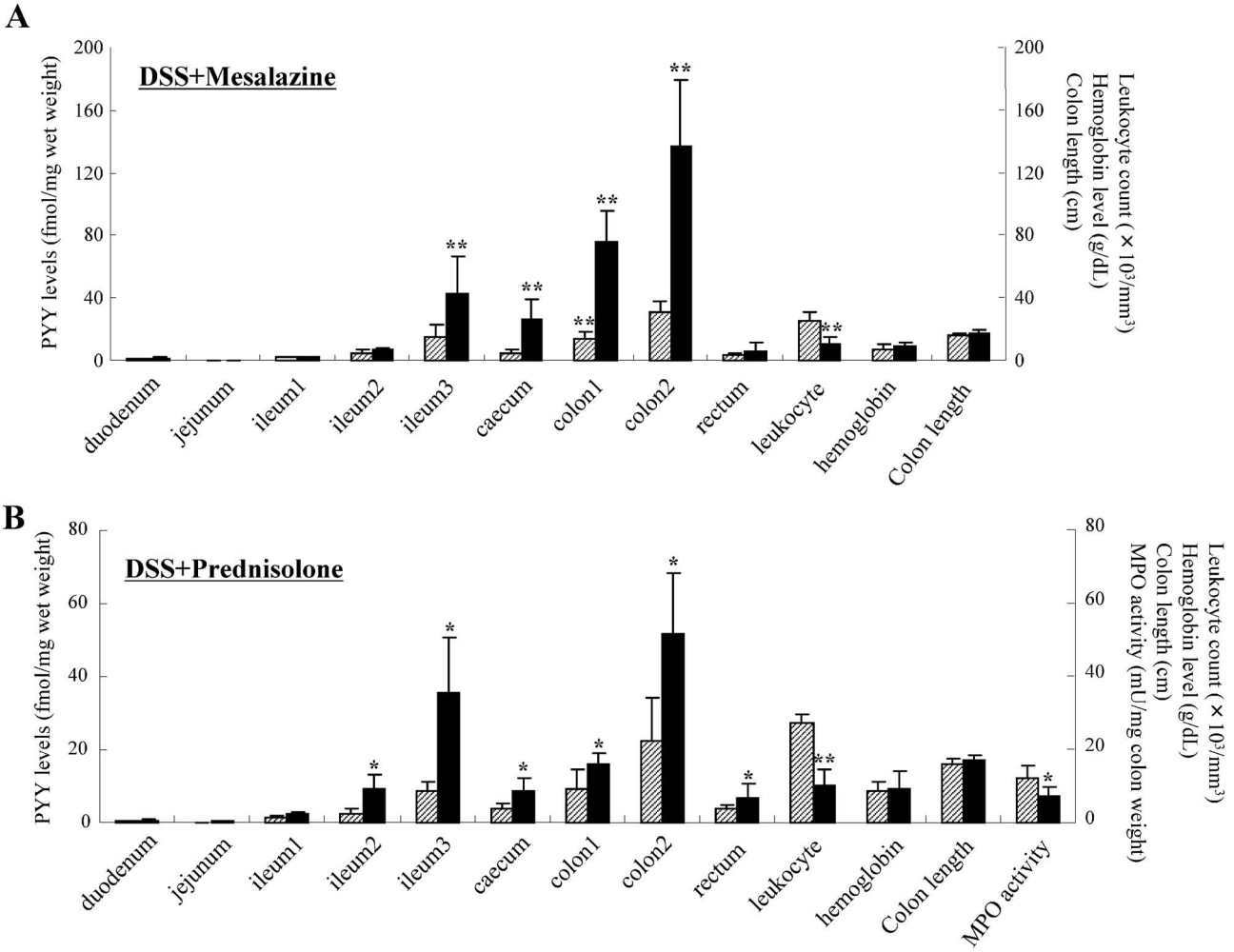

Fig. 2. Effects of Mesalazine and Prednisolone on Intestinal Mucosal PYY Levels and Inflammatory Makers in Rats after 3% DSS Administration

(A) PYY levels of rat received daily treatment with mesalazine (100 mg/kg/day) for 14 consecutive days after 3% DSS administration. (B) PYY levels of rat received daily treatment with prednisolone (1 mg/kg/day) for 14 consecutive days after 3% DSS administration. Data are expressed as mean±S.D. of 6–8 rats. * $p<0.05$, ** $p<0.01$ compared with control rat. ▨; vehicle administrating rats after 3% DSS-treated rat for 2 weeks, ■; drug administrating rats after 3% DSS-treated rat for 2 weeks.

UC are shown in Fig. 2A. The PYY levels in the caecum decreased markedly after administering 3% DSS but increased significantly after administering mesalazine (26.72±11.87 *vs.* 4.62±2.32 fmol/mg wet tissue, $p<0.01$). Moreover, we observed similar significant increases in the levels in the colon segments (colon 1: 75.82±19.14 *vs.* 13.78±4.72 fmol/mg wet tissue, $p<0.01$; colon 2: 136.75±42.11 *vs.* 30.73±7.54 fmol/mg wet tissue, $p<0.01$, ileum 3: 42.86±23.33 *vs.* 15.10±8.00 fmol/mg wet tissue, $p<0.01$). However, the plasma PYY levels did not change (data not shown). The PYY levels in prednisolone-treated rats are also shown in Fig. 2B. The PYY levels increased throughout the tract, and the increase was particularly significant in the caecum (8.93±3.11 *vs.* 4.09±1.19 fmol/mg wet tissue, $p<0.05$), colon 1 (16.27±2.80 *vs.* 9.17±5.42 fmol/mg wet tissue, $p<0.05$), and colon 2 (51.76±16.74 *vs.* 22.63±11.66 fmol/mg wet tissue, $p<0.05$); moreover, the levels in the ileum segment increased markedly (ileum 1: 2.26±0.58 *vs.* 1.29±0.51 fmol/mg wet tissue, $p<0.05$; ileum 2: 9.20±3.92 *vs.* 2.41±1.61 fmol/mg wet tissue, $p<0.05$; ileum 3: 35.53±15.43 *vs.* 8.90±2.20 fmol/mg wet tissue, $p<0.05$). However, no changes were observed in the plasma PYY levels (data not shown). The leukocyte count decreased significantly after administering mesalazine or prednisolone ($p<0.05$ or $p<0.01$, respectively) (Fig. 2A, B). MPO activity decreased significantly after administering prednisolone ($p<0.05$) (Fig. 2B). However, no significant changes were observed in the hemoglobin level and colon length after the administration of the two drugs (Fig. 2A, B).

DISCUSSION

Although the involvement of immunologic, genetic, or environmental factors in the progression of UC has been reported, the precise pathogenic mechanism involved remains unclear. Currently, it is reported that UC symptoms arise due to a specific autoimmune reaction in the large intestine.[21] However, despite many reports on UC research, the determining factor for UC progression has not been clarified. Therefore various studies have been performed using UC animal models in attempts to elucidate the cause of UC and establish a treatment for it. In a similar UC model, it was previously reported that repeated DSS administration induced epithelial atrophy, extensive loss of mucus production, widespread hyperplasia over large areas of the colonal mucosa, complete loss of crypt epithelium in the mucosa of the large intestinal tract, crypt abscesses, and severe ulcers.[22] Further, from the clinical viewpoint, morphologic changes occurring after chronic DSS administration are reportedly accompanied by clinical symptoms that closely resemble those of severe human UC, including diarrhea and bloody stools.[22] The standard drugs (mesalazine and prednisolone) currently used in UC therapy have been effective in this model of repeated DSS administration.

In this study, we used a rat model of enteritis resembling UC induced by DSS (molecular weight, 5000). Bloody stools and weight loss were observed from day 8 of treatment with 3% DSS solution. We measured the leukocyte counts, hemoglobin levels, and colon lengths as indices for assessing the degree of injury induced in the intestinal tract due to 3% DSS administration and the effects of the drugs.[23,24] Due to the progression of inflammation with DSS administration, leukocyte counts increased markedly, accompanied by large intestinal bleeding, and hemoglobin levels decreased significantly. In these UC model rats, shortening of the colon was also observed. These observations were similar to the pathogenic changes detected in human UC. On the other hand, when administering 5% DSS, intense bloody stools were observed from day 7, with marked weight loss and mortality in some cases. The pathologic changes induced by 1% DSS administration are insufficient in the first 2 weeks. Therefore, in this study, we used 3% DSS in the acute-UC rat model. After administering mesalazine or prednisolone to the rats with 3% DSS-induced UC, leukocyte counts and MPO activity (in the prednisolone group) significantly approached values obtained for the controls; thus we confirmed the effectiveness of both drugs in treating DSS-induced UC. However, neither drug significantly affected hemoglobin levels and colon lengths.

When we measured the PYY levels in the intestinal mucosa as an endocrinologic index of the effects of mesalazine and prednisolone on rats with UC, the levels decreased markedly in the caecum, colon, and rectum in rats treated with 3% DSS for 2 weeks and with 5% DSS for 1 week. No significant changes were observed in the plasma PYY levels. Although more detailed studies are required to confirm these results, our observations suggest that the rapid release of

PYY occurs *via* a paracrine route, accompanied by acute injury to the intestinal mucosa, following the administration of DSS. In other words, local release of PYY from endocrine cells within the mucosal layer of the lower ileum, colon, and rectum may affect only the surrounding mucosal epithelia in a paracrine manner. The PYY-secreting cells are of the open type, *i.e.*, they extend from the basal lamina to the gut lumen.[25] Furthermore, El-Salhy *et al.* reported that PYY-secreting cells emit cytoplasmic processes extending to the neighboring goblet cells.[25] In the present study, the decreased levels of PYY in these intestine sections appear to be attributable to the reduction of PYY-secreting cell (L cell) numbers in the inflamed intestinal mucosa.[26] The mucosal epithelium, which functions as a barrier, is maximally affected by the series of inflammatory reactions involving the large intestinal mucosa which occur in UC; in addition, it is affected by intestinal bacteria and food intake. Accordingly, the marked changes in PYY levels that were observed in this rat model may have occurred because PYY is produced and secreted in the injured mucosal epithelium. On other hand, the PYY levels in the caecum and colon decreased significantly after administering DSS but increased after administering mesalazine in a tissue-specific manner. Unlike mesalazine, the PYY levels increased in the caecum and colon with prednisolone administration; moreover, this increase was greater than that in the rat ileum. The pharmacologic mechanisms of mesalazine and prednisolone are most probably related to their antiinflammatory effects. The PYY levels in the lower intestinal tract increased markedly after administering mesalazine and prednisolone to the DSS-treated rats. Our results do not indicate whether these observations were due to the direct or indirect effects of mesalazine or prednisolone.

In conclusion, these changes in PYY levels in the intestinal mucosa suggest a relationship among concentrations of PYY, the progression of UC, and improvement of symptoms with mesalazine and prednisolone treatment. PYY may be a useful pharmacologic agent in the treatment of UC, in which potentiation of intestinal function may be beneficial.

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