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Peptide YY enhances NaCl and water absorption in the rat colon in vivo

M. Okuno, T. Nakanishi, Y. Shinomura, T. Kiyohara, H. Ishikawa and S. Tarui

2nd Department of Internal Medicine, Osaka University Medical School, 1-1-50, Fukushima, Fukushima-ku, Osaka 553 (Japan)
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Abstract. Peptide YY (PYY) is thought to possess paracrine and endocrine functions. The highest concentrations of this peptide are in the colonic mucosa. The effect of PYY on electrolyte and water transport in the rat colon was studied in vivo. Under urethane anesthesia, rat colonic loops were perfused at a constant rate with physiological buffer solution containing phenol red as a nonabsorbable volume marker, and net movements of water, sodium, chloride and potassium in the perfused colon were determined every 10 min. Intravenous administration of PYY produced a dose-dependent increase in the net absorption of sodium chloride and water, as well as a decrease in the net secretion of potassium. PYY inhibited the reduction in net absorption of sodium chloride and water evoked by vasoactive intestinal peptide (VIP), but did not affect the VIP-evoked increase in net potassium secretion.
These findings suggest that PYY acts as an enhancer of sodium chloride and water absorption and as an antagonist to VIP-induced secretion in the colon.
Key words. Peptide YY; vasoactive intestinal peptide; colon; electrolyte and water transport; rat; in vivo.

Peptide YY (PYY), a 36 amino acid-polypeptide, is localized in the endocrine-like cells in the gastrointestinal mucosa, which are numerous in the distal intestine, particularly in the colon [1–3]. Immunohistochemical studies have revealed that PYY cells sometimes have long cytoplasmic processes extending from the basal portion to the neighboring epithelial cells [1, 3], which suggests that PYY cells may have dual endocrine and paracrine functions.

Although hormonal effects of PYY have been suggested, such as inhibition of gastric or pancreatic secretion [4, 5], inhibition of gastrointestinal motility and constriction of intestinal vasculature [1, 6], little is known about the paracrine roles of PYY. Vasoactive intestinal peptide (VIP), which is a neuropeptide widely distributed in the central and peripheral nervous systems, has been shown to stimulate intestinal secretion [7]. Recently we reported [8]

that PYY, in vitro, inhibited the increase in the short-circuit current (Isc) of rat colonic mucosa induced by VIP. The present study was therefore undertaken to investigate in vivo the effect of exogenous PYY administration on net electrolyte and water transport, and the influence of PYY on the VIP-induced secretion in the colon, using a simple anesthetized rat model.

Materials and methods

Colon perfusion. Male Sprague-Dawley rats weighing 250–350 g were used. After 18 h of fasting, the animals were anesthetized with 25% urethane (intraperitoneal, 1.5 g/kg). After tracheostomy, a catheter was inserted into the left jugular vein, and a midline incision was made in the abdomen. A colonic loop beginning 1 cm distal to the cecal-colonic junction and ending 1 cm proximal to the pelvic brim (total length: about 10 cm) was cannulated with polyethylene tubings (inner diameter: 4 mm), rinsed with warm saline until the effluent became clear, and returned to the abdominal cavity. The proximal cannula was attached to the tubing of the infusion pump, and the distal cannula was used for collection of the perfusate. After the incision had been sutured, a volume of saline equal to 1% body weight was infused intravenously to replace surgical fluid loss. The colonic loop was perfused with a physiological solution at a constant temperature (37 °C) and rate (0.5 ml/min) with a peristaltic pump (model P-1, Pharmacia Fine Chemicals, Sweden). The solution consisted of Na^+ 140, K^+ 5, Cl^- 120, HCO_3^- 25 mEq/l, with phenol red (0.02 g/l) as a nonabsorbable marker for water movement, as described by Edmonds et al.[9].

Colon perfusion was carried out for three hours. For each animal, a 90-min basal period was followed by a 90-min test period[10]. Body temperature was maintained at 37 °C to 37.5 °C with a heating board or 60 W lamp. On completion of the experiment the animal was killed, and the perfused colon was removed, dried at 110 °C overnight, and weighed.

Peptide infusion. Constant intravenous infusion of saline at a rate of 2 ml/h by an infusion pump (Atom 235, Japan) was begun as soon as colon perfusion was started. After 90 min of saline infusion, test peptides dissolved in saline containing 0.1% bovine serum albumin (BSA; Sigma Chemical Co, St. Louis, MO, USA) were infused intravenously in the experimental animals for the following 90 min at a rate of 2 ml/h. In control animals, saline was infused for 90 min and vehicle (saline containing 0.1% BSA) for the following 90 min. Porcine PYY and VIP were purchased from Peninsula Laboratories (Belmont, CA, USA).

Measurement of water and electrolyte transport. Colon perfusate was collected, using a new tube for every 10-min period. The tubes were centrifuged at 3000 rpm for 10 min, and a portion (0.5 ml) of the supernatant was mixed with 1.5 ml of 0.01 N NaOH for phenol red assay. The phenol red concentration in the mixture was calculated using a colorimetric assay (absorbance at 545 nm) as described by Nakaki et al.[11]. The residual supernatant was used for determining the concentrations of sodium and potassium by flame photometry, and chloride by coulometric assay (Hitachi autoanalyzer 710, Japan). Water and electrolyte transport for each 10-min period were calculated as described previously[12], and expressed as microliters or microequivalents per minute per gram dry weight. Positive values indicate net absorption and negative values indicate net secretion. For each animal, basal values of mean net movement of water and electrolytes were calculated by averaging the results of the three 10-min collection periods immediately before peptide infusion. Values for the test period were calculated by averaging the results of three consecutive 10-min periods during which a maximal effect of the infused peptide was observed. All results are expressed as mean ± SEM. Significance of differences was assessed by using paired or unpaired Student's t-test.

Results

In control animals, the net absorption of sodium chloride and water or net secretion of potassium in the colon remained stable over three hours of colon perfusion.

Intravenous PYY infusion (0.5, 2, 8 nM/kg · h) resulted in a progressive increase in net water absorption in a dose-dependent manner (fig. 1). The maximal effect occurred between 50 and 80 min after the beginning of the intravenous PYY infusion. In figure 2, the values for mean net movement of water and electrolytes, during three consecutive 10-min periods taken 50–80 min after the beginning of the PYY infusion (8 nM/kg · h), are compared with those found in the basal period preceding the PYY infusion. Infusion of PYY (8 nM/kg · h) increased mean net water absorption from 103.7 ± 10.3 to 165.5 ± 8.3 µl/min · g dry weight. Likewise, mean net absorption of sodium and chloride increased from 24.1 ± 1.5 and 19.4 ± 1.3 to 34.7 ± 1.5 and 25.9 ± 2.5 µEq/min · g dry weight, respectively, whereas mean net secretion of potassium decreased from 2.3 ± 0.1 to 1.3 ± 0.2 µEq/min · g dry weight.

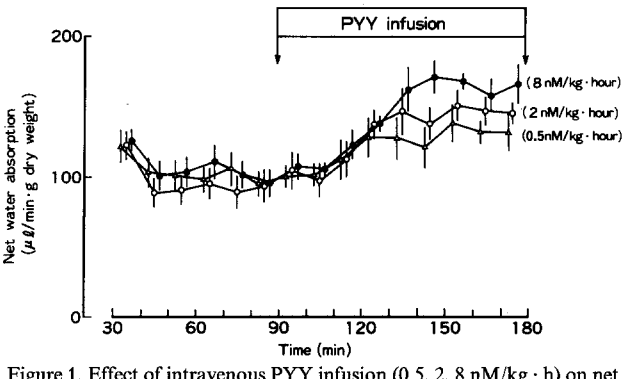

Figure 1. Effect of intravenous PYY infusion (0.5, 2, 8 nM/kg · h) on net water absorption in the perfused rat colon. Means ± SEM are shown (5 animals in each group).

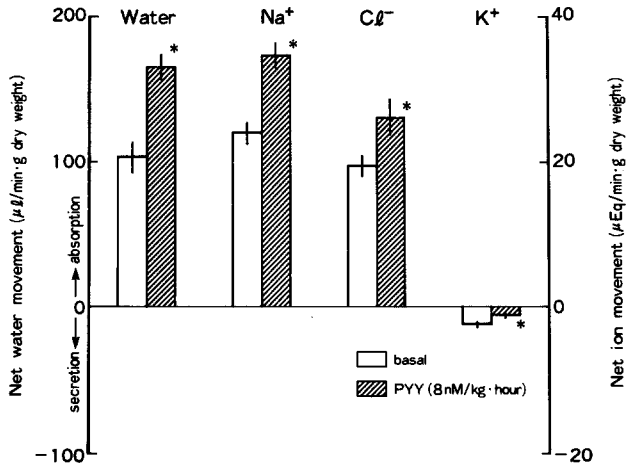

Figure 2. Comparison of the net movements of water and electrolytes in the perfused rat colon during: a) three 10-min periods taken 50–80 min after the beginning of the PYY infusion (8 nM/kg · h); b) the basal period. Means ± SEM are shown (n = 5). *p < 0.01 (test vs basal period).

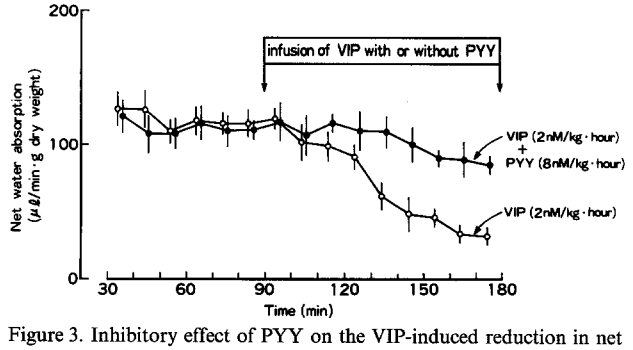

Figure 3. Inhibitory effect of PYY on the VIP-induced reduction in net water absorption in the perfused rat colon. Open circles indicate net water absorption when VIP (2 nM/kg · h) alone was infused intravenously. Closed circles indicate the same absorption when VIP (2 nM/kg · h) and PYY (8 nM/kg · h) were infused simultaneously. Means ± SEM are shown (6 animals in each group).

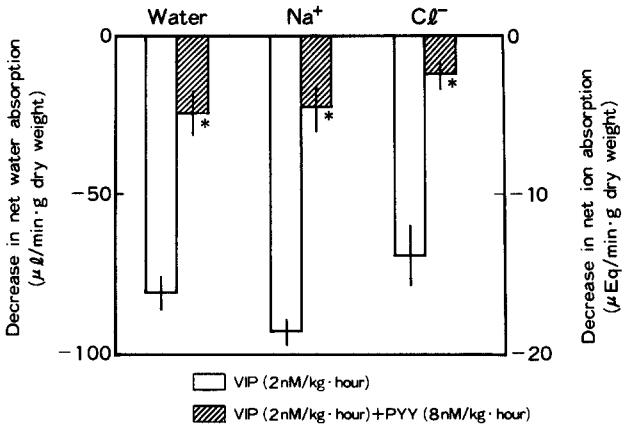

Figure 4. Inhibitory effect of PYY on the VIP-evoked reduction in net absorption of sodium chloride and water. Open columns indicate the reduction, with respect to basal values, in net absorption of sodium chloride and water observed 60–90 min after the beginning of intravenous infusion of VIP (2 nM/kg · h). Hatched columns indicate the corresponding values when VIP (2 nM/kg · h) and PYY (8 nM/kg · h) were infused simultaneously. Means ± SEM are shown (6 animals in each group). *p < 0.05 (VIP plus PYY vs VIP).

In contrast, intravenous VIP infusion (2 nM/kg·h) induced a progressive decrease in net absorption of sodium chloride and water (figs 3 and 4) as well as an increase in net potassium secretion. The phenol red recovery in the effluent determined during saline and VIP infusion were 98.7 ± 0.7 and 99.3 ± 0.8% (n = 4 animals), respectively. These results exclude the possibility that the observed reduction in net water absorption by VIP was secondary to the increase in the colonic epithelial permeability of phenol red. The maximal effect occurred between 60 and 90 min after the start of the VIP infusion. When PYY (8 nM/kg·h) and VIP (2 nM/kg·h) were infused simultaneously, the VIP-induced effect on net absorption of sodium chloride and water was significantly reduced (figs 3 and 4). Mean increase in net potassium secretion caused by the simultaneous infusion of VIP and PYY (ΔK^+ secretion = 1.19 ± 0.41 μEq/min·g dry weight) was not significantly different from that caused by the infusion of VIP alone (ΔK^+ secretion = 0.93 ± 0.48 μEq/min·g dry weight).

Discussion

The present study demonstrates that intravenous administration of PYY in the rat caused the following effects on electrolyte and water transport in the colin: 1) increase of sodium chloride and water absorption, 2) decrease of potassium secretion, and 3) inhibition of the reduction in sodium chloride and water absorption evoked by VIP. In short, PYY enhanced absorption of sodium chloride and water, and counteracted the diarrheogenic effect of VIP. This effect of PYY, observed in vivo, seems to be related to the effect observed by us in vitro [8], namely that PYY inhibited the increase in Isc induced by VIP. PYY shares amino acid sequence homologies with neuropeptide Y (NPY) and pancreatic polypeptide [13]. PYY and NPY have been shown to stimulate sodium and chloride absorption in studies with rabbit ileal [14] or rat jejunal [15] mucosa mounted in Ussing chambers. An in vivo experiment [16] indicated that both peptides inhibit prostaglandin E_2-induced secretion in the rat small intestine, and it has been demonstrated that PYY and NPY share a common receptor site in the rat small intestinal epithelium [17]. On the other hand, the effect of PYY on colonic ion transport has not been well studied. To the best of our knowledge, the present study was the first to demonstrate the in vivo effect of PYY on colonic electrolyte and water transport.

Plasma concentrations of PYY in man have been shown to rise in response to ingestion of food, particularly fat [3]. It is conceivable that certain intraluminal stimuli may cause PYY cells to release this peptide, which could act locally on the neighboring epithelial cells to enhance the absorption of sodium chloride together with water. The overflow of PYY could then enter the circulation and produce hormonal effects, involving reduction of gastric and pancreatic secretion [4, 5] or inhibition of gastric emp-

tying[1,6]. Elevated plasma PYY levels are observed in patients with diarrhea due to tropical sprue, pancreatic insufficiency, inflammatory bowel disease or acute infection[18]. It is probable that in such cases PYY cells may function fully to compensate for the loss of electrolytes and water and to maintain homeostasis.

VIP is not only an important neuropeptide in the enteric nervous system but is also thought to be responsible for watery diarrhea in patients with VIP-producing tumors. Since it has been reported that PYY infusion in man was well tolerated[19], and was effective for inhibiting VIP-stimulated ileal secretion[20], PYY or its analogues may possibly be useful as a drug for palliative therapy for patients with VIP-producing tumors.

In conclusion, PYY enhances absorption of sodium chloride with water and antagonizes the secretory effect of VIP in the rat colon.

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Proopiomelanocortin expression in the skin during induced hair growth in mice

A. Slominski[a], R. Paus[b] and J. Mazurkiewicz[c]

[a] *Department of Immunology and Microbiology,* [b] *Department of Anatomy, Cell Biology and Neurobiology, Albany Medical College, Albany (New York 12202, USA), and* [c] *Department of Dermatology, University Hospital R. Virchow, Freie Universität Berlin, D-1 Berlin 65 (Germany)*

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Abstract. We demonstrate for the first time a hair cycle-dependent gene and protein expression of proopiomelanocortin in mouse skin in vivo. Northern blot detected POMC mRNA with an apparent size of 0.9 kb in anagen but not telogen skin. Western blot emphasized a specific protein of 30–33 kDa recognized by anti β-endorphin in late but not early anagen or telogen skin. By immunocytochemistry, β-endorphin antigen was localized in the sebaceous gland in a hair cycle dependent manner.

Key words. Proopiomelanocortin; hair growth; skin; C57B1/6 mice; β-endorphin.

The proopiomelanocortin (POMC) gene is predominantly expressed in the pituitary gland and encodes a single large protein precursor for neuropeptides with multiple regulatory functions, including adrenocorticotropin (ACTH), endorphins (EP), melanotropins (MSH), and lipotrophins (LPH)[1–3]. The POMC gene is also expressed in the brain and many peripheral tissues such as testes, ovaries, placenta, lymphoid cells, adrenals, kidney, lung, liver and gastrointestinal tract[1–4].

Skin, the largest organ of the body, is considered to be a target organ for regulatory functions of some POMC-derived peptides such as MSH or ACTH[3,5–7]. Recent reports showing POMC gene expression and translation in murine and human epidermal keratinocytes in vitro[8,9] suggest that the skin may be among those peripheral tissues that are capable of expressing the POMC gene. This raises the possibility that locally generated POMC products such as β-endorphin, ACTH, LPH and the melanotropins play important roles in the physiology of the skin and its exquisitely hormone sensitive appendages. Utilizing the previously described C57BI/6 mouse model for hair growth and pigment biology stud-