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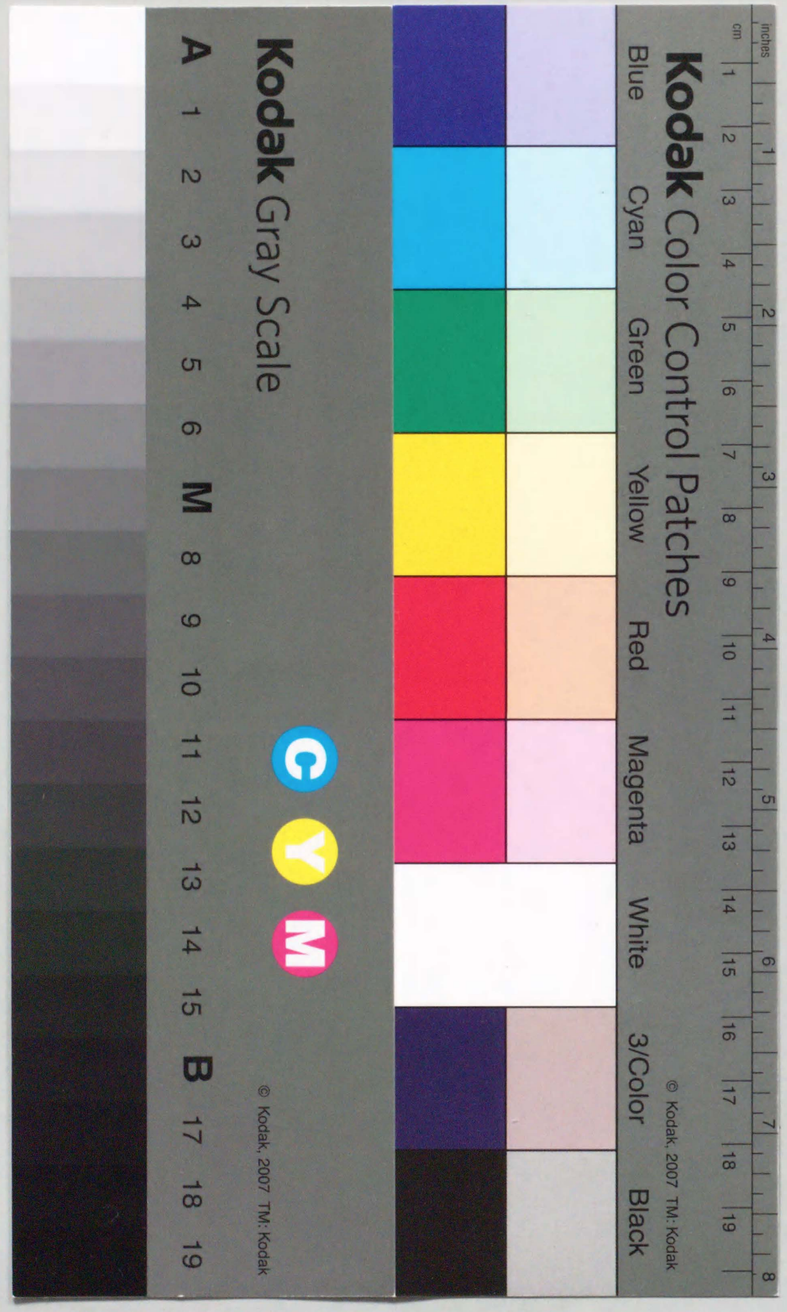

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主論文

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PEPTIDE YY INHIBITS ION SECRETION INDUCED BY VASOACTIVE INTESTINAL POLYPEPTIDE OR SEROTONIN IN THE RAT COLON *IN VITRO*

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ABSTRACT

Peptide YY (PYY) is a gut peptide localized in intestinal mucosal endocrine cells, which are especially abundant in the colon. In order to study the effect of PYY on mucosal ion transport in the colon, the transmucosal potential difference (PD) and short circuit current (Isc) of the rat colonic mucosa were measured with Ussing chambers. Addition of PYY to the serosal reservoir induced a prompt and sustained decrease in PD and Isc in a concentration-dependent manner without affecting the tissue resistance. The threshold concentration and the EC_{50} value for PYY were 10^{-9} M and 2×10^{-8} M, respectively. Moreover, PYY inhibited the increase in Isc induced by VIP, theophylline and serotonin, indicating that PYY can antagonize both cyclic AMP- and calcium-mediated secretion of ion in the colon. The results suggest that PYY acts as an antisecretory modulator in the colon.

Peptide YY (PYY) is a 36-residue peptide isolated and purified from porcine gut (21), and has considerable sequence homologies with pancreatic polypeptide (PP) and neuropeptide Y (NPY) (19). PYY is produced in many intestinal mucosal endocrine cells (4, 5, 13) which are abundant in the intestine, particularly in the colon (1, 2, 10, 22). Lundberg *et al.* (13) reported that intestinal PYY cells were sometimes found to have long processes resembling those of somatostatin-immunoreactive cells in the stomach, suggesting that PYY may have a paracrine as well as endocrine action. Since elevated plasma PYY levels have been observed in patients with diarrhea due to idiopathic inflammation or acute infection of the bowel (3, 12), it is possible that PYY has some relation with intestinal ion transport. The purpose of the present study was to examine the effect of PYY on colonic ion transport *in vitro*, especially in association with the effect of such secretagogues as vasoactive intestinal polypeptide (VIP), theophylline and serotonin.

MATERIALS AND METHODS

PYY and VIP were purchased from Peninsula Laboratories (Belmont, CA, U.S.A.), theophylline from Nakarai Chemicals (Kyoto), serotonin from Sigma Chemicals (St. Louis, MO, U.S.A.), and the other chemicals from Wako Pure Chemical Industries (Osaka).

Details of the methods adopted in the present *in vitro* study have been described elsewhere (11, 15). Briefly, nonfasting male Sprague-Dawley rats weighing 200–300 g were used, and colonic segment stripped off its serosal and muscle layers was mounted between Lucite half-chambers (exposed area= 1.13 cm^2). Both sides of the tissue samples

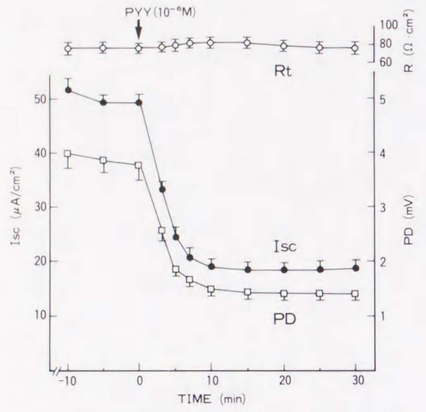

Fig. 1 Effect of PYY on short circuit current (Isc ●—●), potential difference (PD □—□) and tissue resistance (R ○—○) in rat colonic mucosa. PYY (10^{-6} M) was added to the serosal reservoir after an initial 30 min stabilization period. All values are expressed as the mean±SE of 8 experiments.

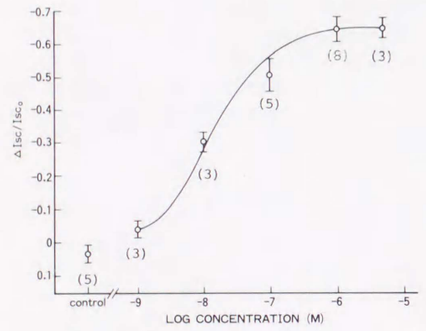

Fig. 2 Dose-response curve of the decrease in Isc by PYY. Results are expressed as the ratio of the maximal decrease in Isc (ΔIsc) to the basal Isc (Isc_0). Values represent the mean±SE, with the number of experiments in parentheses.

were bathed with 10 ml of oxygenated Ringer's solution and maintained at 37°C by means of a water-jacketed gas-lift circulating system. The Ringer's solution had the following composition (mM): 140 Na^+, 119.8 Cl^-, 5.2 K^+, 1.2 Ca^{2+}, 1.2 Mg^{2+}, 25 HCO_3^-, 2.4 HPO_4^{2-} and 0.4 $H_2PO_4^-$. Glutamine (5 mM) and 10 mM glucose were added to Ringer's solution. Two agar-KCl bridges in both mucosal and serosal sites connected the chamber to a pair of calomel electrodes for measuring the transmucosal potential difference (PD) with a high-impedance potentiometer. Direct current was passed across the tissue between two other agar bridges connected to a battery via Ag-AgCl electrodes, and the short circuit current (Isc) was measured by a micro-amperemeter with a correction for the drop in potential between PD-measuring agar electrodes by the fluid resistance. The tissue resistance (Rt) was calculated from the PD and Isc according to the Ohm's law. The tissue was incubated for 30 min to equilibrate until the first agent was added to the serosal reservoir. The second agent, if used, was added 10 min after the treatment with the first agent. The results are expressed as the mean±SE.

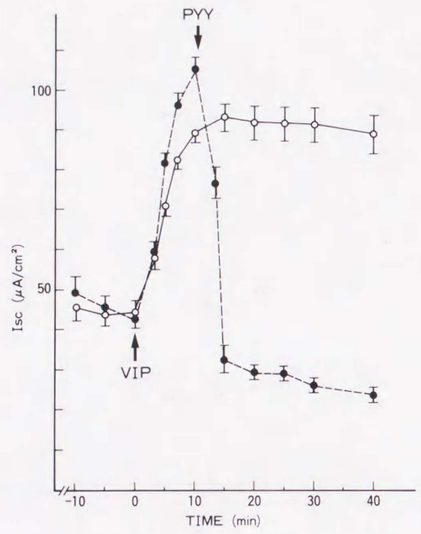

Fig. 3 Effect of 10^{-7} M VIP alone on Isc (○—○) and inhibitory effect of 10^{-6} M PYY on Isc previously increased by 10^{-7} M VIP (●--●). PYY was added 10 min after the treatment with VIP. (n=4)

RESULTS

Effects of PYY on the Basal State

The addition of 10^{-6} M PYY to the serosal reservoir after an initial 30-min stabilization period caused an immediate decrease in Isc. The maximal decrease in Isc ($-\Delta$Isc=31.3±3.7 μA/cm²) occurred 15 min after the addition of 10^{-6} M PYY. The decrease in Isc persisted over 30 min after PYY addition. The change in PD following the addition of PYY was parallel to that of Isc, indicating that the Rt was not affected by PYY in this experimental system for 40 min (Fig. 1).

Fig. 2 shows the dose-response curve of the decrease in Isc by PYY. Results are expressed as the ratio of the maximal decrease in Isc to the basal Isc just before the addition of PYY, since the decrease in Isc by PYY varies directly with each level of the initial Isc before the addition of PYY. The lowest concentration of PYY that produced a significant decrease in Isc was 10^{-9} M, and PYY at 10^{-6} M produced the maximal decrease in Isc. The EC_{50} for PYY was 2×10^{-8} M. Therefore, 10^{-6} M PYY was used in all subsequent experiments.

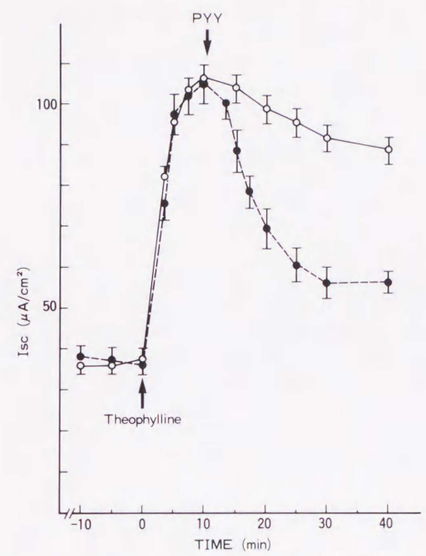

Fig. 4 Effect of 10^{-2} M theophylline alone on Isc (○—○) and inhibitory effect of 10^{-6} M PYY on Isc previously increased by 10^{-2} M theophylline (●--●). PYY was added 10 min after the treatment with theophylline. (n=5)

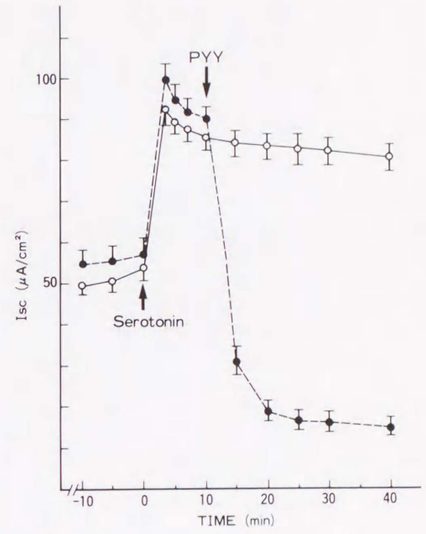

Fig. 5 Effect of 10^{-5} M serotonin alone on Isc (○—○) and inhibitory effect of 10^{-6} M PYY on Isc previously increased by 10^{-5} M serotonin (●--●). PYY was added 10 min after the treatment with serotonin. (n=7)

Effect of PYY on Secretagogue-induced Mucosal Isc Response

VIP (Fig. 3) The addition of 10^{-7} M VIP to the serosal reservoir caused a rapid and sustained increase in Isc. This concentration of VIP has been shown to produce the maximal increase in Isc (16). The addition of 10^{-6} M PYY to the 10^{-7} M VIP-treated tissue caused a rapid and marked decrease in Isc (ΔIsc=83.2±4.0 μA/cm²) that was much larger than that with PYY alone. PYY completely blocked the VIP-evoked mucosal Isc increase.

Theophylline (Fig. 4) The addition of 10^{-2} M theophylline to the serosal reservoir also resulted in an immediate and sustained increase in Isc. The addition of 10^{-6} M PYY to the 10^{-2} M theophylline-treated tissue caused a marked decrease in Isc (ΔIsc=48.1±8.5 μA/cm²), which was smaller than in the 10^{-7} M VIP-treated tissue. PYY partially inhibited the Isc-increase produced by theophylline.

Serotonin (Fig. 5) The Isc increased

markedly after the addition of 10^{-5} M serotonin to the serosal reservoir. The addition of 10^{-6} M PYY to the 10^{-5} M serotonin-treated tissue caused a rapid decrease in Isc (ΔIsc= $75.4 \pm 4.6\ \mu A/cm^2$) even below the levels of the basal Isc. PYY reversed the serotonin-evoked mucosal Isc response.

DISCUSSION

Recent experiments have suggested that PYY and NPY affect the fluid and electrolyte transport in the small intestine (9, 18). Friel *et al.* (9) reported that PYY and NPY reduced the short circuit current in the rabbit ileum *in vitro*. They showed that NPY enhanced mucosal-to-serosal Cl^- fluxes and reduced serosal-to-mucosal Cl^- fluxes across mucosa in the rabbit ileum. Seria *et al.* (18) described that NPY and, to a lesser extent, PYY reduced prostaglandin E_2-induced fluid secretion in the rat jejunum *in vivo*. While the highest NPY immunoreactivity in the gut is found in the proximal intestine (20), the highest PYY immunoreactivity is found in the colon in rats (10), dogs (10, 22) and humans (1, 2). It may well be speculated that PYY affects colonic ion transport when acting as a paracrine substance.

The present study shows that rat colonic mucosa does respond to PYY with a marked alteration in electrogenic ion transport *in vitro*. PYY produced a marked decrease in Isc and PD in the rat colonic mucosa (Fig. 1). The very low EC_{50} of PYY may support a physiological role of PYY in the regulation of ion transport (Fig. 2). The increase in net Cl^- absorption such as seen in the NPY-treated rabbit ileal mucosa (9) may be involved in the PYY-induced decrease in the short circuit current in rat colonic mucosa.

In addition, PYY was shown to block the Isc-increasing effect of VIP, theophylline and serotonin (Figs. 3, 4 and 5). VIP and theophylline are known to stimulate electrogenic Cl^- secretion in rat colonic mucosa by increasing intracellular cyclic AMP through the activation of adenylate cyclase and the inhibition of phosphodiesterase, respectively (6, 7, 16). On the other hand, serotonin is known to facilitate the intestinal fluid secretion through a calcium-dependent and cyclic AMP non-mediated process (7, 23). PYY inhibited the increase in Isc induced by all of these agents, indicating that PYY can antagonize both cyclic AMP- and calcium-mediated secretagogues in the colon. Although the mode of interaction by PYY with intracellular mediators of active electrolyte secretion remains unclear, these results suggest that PYY may interfere some intracellular steps other than the increase in the cyclic AMP and calcium concentrations.

We have previously reported that prostaglandin D_2 (PGD_2) has also very similar actions of inhibiting the Isc-increasing effect by prostaglandin E_1, theophylline and serotonin *in vitro* (11). It is thus possible that PYY affects colonic ion transport via PGD_2 synthesis. PYY, like PGD_2, appears to be an inhibitory modulator in the colonic ion transport.

In conclusion, we have shown that PYY has a strong antisecretory action in the rat colon. Further studies are expected to determine its therapeutic potential in human endocrinopathies (8, 14, 17) associated with excessive secretion of VIP and serotonin.

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