

The effect of the gastrointestinal hormones on colonic mucosal blood flow

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The effect of intravenous administration of various gastrointestinal hormones or peptides on the colonic mucosal blood flow was investigated by a reflex spectrum apparatus (TS-200, Sumitomo Denko Co). Various gastrointestinal hormones (pentagastrin, secretin, substance P, vasoactive intestinal polypeptide) were administered via the femoral vein at different doses. The hormones were administered over 30 minutes using a chronofuser at a rate of 0.1 ml/min. Serial measurements of cecal mucosal blood flow were performed. Saline was administered to the control group.

1) Pentagastrin was administered at doses of 1 $\mu\text{g}/\text{kg}/\text{hr}$, 50 $\mu\text{g}/\text{kg}/\text{hr}$, 100 $\mu\text{g}/\text{kg}/\text{hr}$, and 200 $\mu\text{g}/\text{kg}/\text{hr}$. Cecal mucosal blood flow decreased when the dose of pentagastrin was increased. The intravascular supply of oxygen also decreased in a dose dependent manner. Each dose of the gastrointestinal hormone caused a reduction in blood pressure.

2) Secretin was administered at doses of 5 $\mu\text{g}/\text{kg}/\text{hr}$, 50 $\mu\text{g}/\text{kg}/\text{hr}$, and 100 $\mu\text{g}/\text{kg}/\text{hr}$. Each tested dose of this gastrointestinal hormone acted to maintain cecal mucosal blood flow. The blood pressure remained unchanged throughout the experiment.

3) Substance P was administered at doses of 1 $\mu\text{g}/\text{kg}/\text{hr}$ and 5 $\mu\text{g}/\text{kg}/\text{hr}$. Cecal mucosal blood flow and the intravascular oxygen supply increased after administration of this gastrointestinal hormone. The blood pressure decreased transiently at the start of administration, but later gradually returned to the baseline values.

4) Vasoactive intestinal polypeptide (VIP) was administered at doses of 1 $\mu\text{g}/\text{kg}/\text{hr}$, 5 $\mu\text{g}/\text{kg}/\text{hr}$, and 10 $\mu\text{g}/\text{kg}/\text{hr}$. VIP caused the cecal mucosal blood flow to increase in a dose-dependent manner. The intravascular oxygen supply also increased significantly after administration of this gastrointestinal hormone. The blood pressure initially decreased after administration of each test dose of VIP, after which it gradually started to increase.

5) To identify the factors responsible for the increase in cecal mucosal blood flow at the start of VIP administration at a dose of 5 $\mu\text{g}/\text{kg}/\text{h}$ of VIP, the blood concentrations of VIP, cyclic AMP, phospholipase, prostaglandin E_2 , prostaglandin E, and 6 keto-prostaglandin $F_1\alpha$ were examined at regular intervals. However, no significant changes with regard to these substances were noted.

Key words : gastrointestinal hormone pentagastrin, secretin, substance P, VIP, colonic mucosal blood flow

I. Introduction

The effect of the gastrointestinal hormones on the mucosal blood flow in the upper digestive tract have been studied by many investigators. However, the effect of the gastrointestinal hormones on the mucosal blood flow in the lower digestive tract, especially that of the colonic mucosal blood flow, remains to be clarified. Gastrointestinal hormones are considered to be humoral factors that regulate complex digestive processes. Thus, it is speculated that these hormones also influence colonic function. In this study, we examined the effects of the various gastrointestinal hormones and peptides on colonic mucosal blood flow using a spectrum analyzer.

II. Materials and Methods

1) Experimental animals

Male Wistar rats weighing about 300 g were used.

2) Experimental methods

Rats were anesthetized by intraperitoneal administration of pentobarbital (30 mg/kg). They were then subjected to laparotomy while their blood pressure was monitored at the left common carotid artery. The cecum was resected at a location contralateral to its attachment to the mesentery. Feces were carefully removed from the resected cecal specimens so as not to damage the mucosa. The resected cecal specimens were then washed with physiological saline and fixed to the table where measurements were to be made. After placing an optical fiber over the cecal mucosa, the cecal blood flow was measured using a TS-200 tissue spectrum analyzer (Sumitomo Denko Co.). The following experiments were performed after the mucosal blood flow had stabilized.

a) The effect of the gastrointestinal hormones on colonic mucosal flow

Various doses of the gastrointestinal hormones (penta-

gastrin, secretin, substance P [SP], vasoactive intestinal polypeptide [VIP]) were continuously infused via the femoral vein for 30 minutes. Mucosal blood flow was serially measured to 90 minutes after the start of the administration of the gastrointestinal hormones or 60 minutes after the completion of infusion. The various test substances were dissolved in physiological saline and were infused at a rate of 0.1 ml/min using a chronofuser. O₂ saturation (SaO₂) was used as an indicator of the oxygen content in blood, and IHb was used as an indicator of blood flow. The product of SaO₂ and IHb was designated as IHbO₂, which served as an indicator of the oxygen supply in blood. The measured values were expressed as the rate of change after administration of the test substances.

b) Changes in the various factors during increases in blood flow. The cause for the significant increase in blood flow after continuous infusion of VIP (5 µg/kg/h) was by serial measurements of the concentrations of VIP, cyclic AMP, phospholipase, prostaglandin E₂, prostaglandin E, and 6 keto-prostaglandin F₁α. The measured values were expressed as rates of change after administration of VIP. The same measurements were made after infusion of physiological saline in the control group.

3) Statistical analysis

Data were expressed as means ± SE. The t-test was used for the comparison of data. Differences were considered significant when the P value was less than 0.05.

III. Results

The findings with regard to blood flow which were measured and recorded by a spectrum analyzer showed occasional markedly disruptive waves. Despite the appearance of these waves which were thought to be due to peristalsis, the results were generally satisfactory. The blood flow was represented by the means of the measured values.

A transient increase in the colonic mucosal blood flow was noted initially in the control group during continuous infusion of physiological saline. However, the colonic mucosal blood flow soon started to decrease gradually. The blood pressure showed gradual increases during this interval.

a) Administration of the gastrointestinal hormones

The colonic mucosal blood flow was lower in the group which was administered pentagastrin (ICI pharma) at a dose of 1 µg/kg over 30 minutes compared to that in the group that was administered physiological saline (Fig. 1). However, the colonic mucosal blood flow started to increase 60 minutes after administration of pentagastrin. A 6% increase in colonic mucosal blood flow was noted 90 minutes after administration of pentagastrin. Administ-

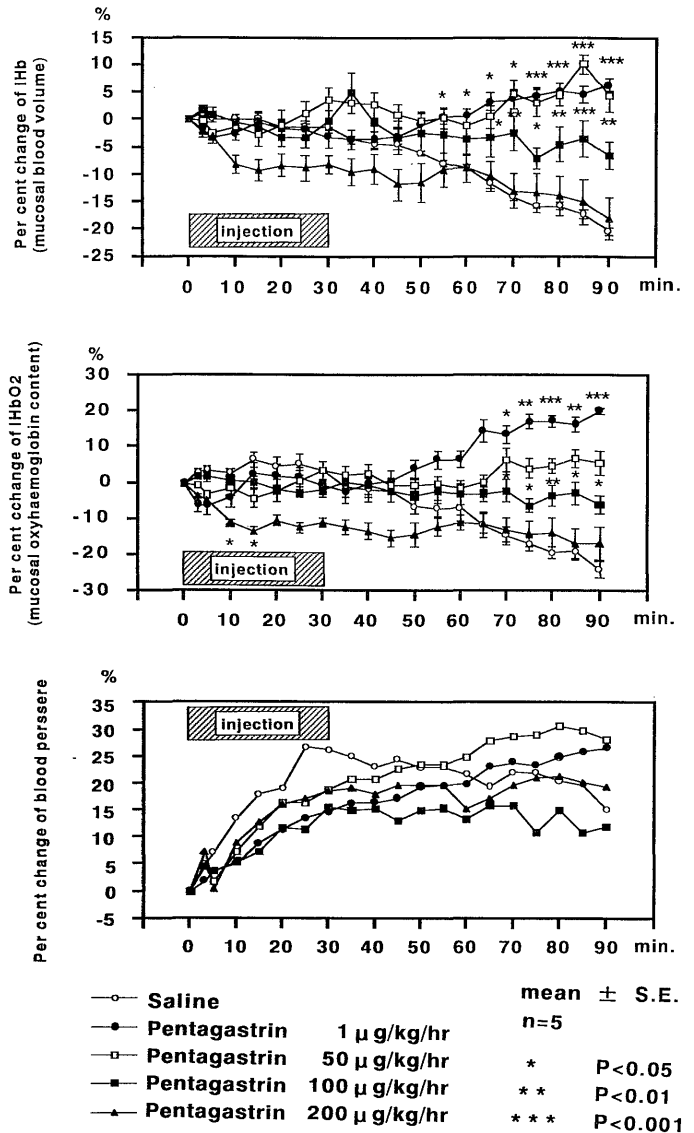


Figure 1. The effect of pentagastrin on colonic mucosal blood volume, mucosal oxyhaemoglobin content and blood pressure. Pentagastrin was continuously injected for 30 min. at a concentration of 1 µg/kg/hr (closed circles), 10 µg/kg/hr (open squares), 100 µg/kg/hr (closed squares) and 200 µg/kg/hr (closed deltas). Open circles were saline injection to control rats. Solutions were administered using the same flow rate in femoral vein of anesthetized rats. Index of colonic mucosal haemoglobin concentration (upper panel) and colonic mucosal oxyhaemoglobin content (middle panel) were measured by a tissue spectrum analyzer during monitoring of blood pressure (lower panel) during 90 min.

ration of pentagastrin at a dose of 50 µg/kg/h resulted in a 4% increase in the colonic mucosal blood flow at the end of infusion, after which it started to decrease gradually. The colonic mucosal blood flow was at its minimum 60 minutes after administration of pentagastrin at a rate and dose of 50 µg/kg/hr. However, after 60 minutes, the

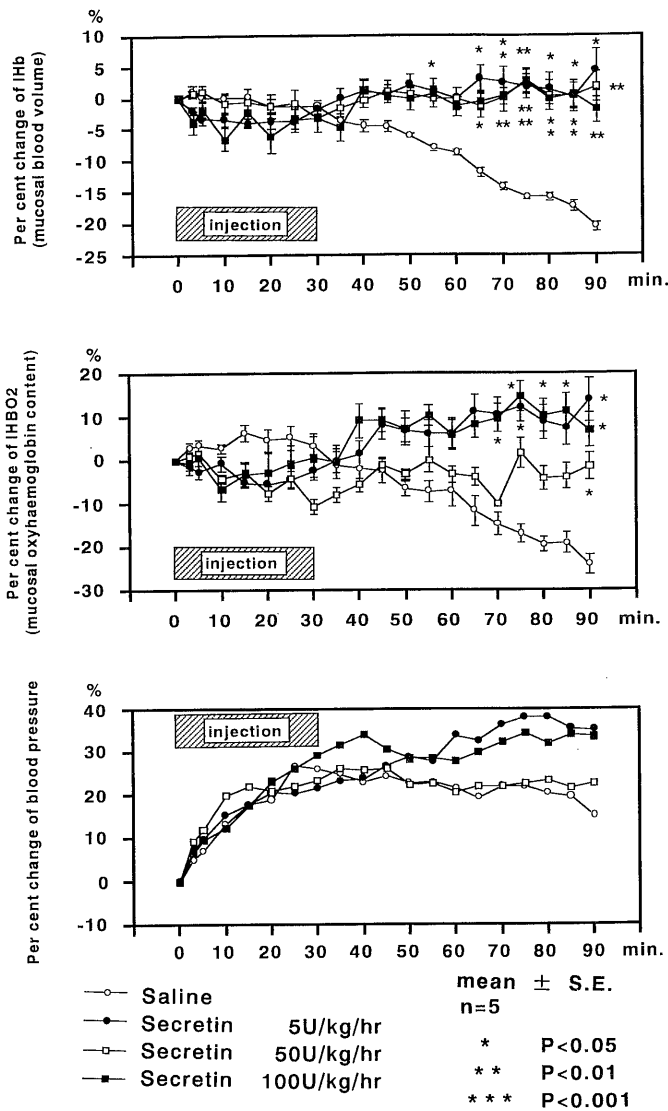


Figure 2. The effect of exogenous secretin on colonic mucosal blood volume, mucosal oxyhaemoglobin content and blood pressure. The concentration of secretin was 5 U/kg/hr (closed circles), 50 U/kg/hr (open squares) and 100 U/kg/hr (closed squares). The conditions were similar to those in Fig. 1.

colonic mucosal blood flow was at its minimum 60 minutes after administration of pentagastrin at a rate and dose of 50 µg/kg/h. However, after 60 minutes, the colonic mucosal blood flow began to increase again. A 4% was noted 90 minutes after the administration of pentagastrin at a rate and dose of 50 µg/kg/hr. Slight reductions in the blood pressure and blood flow were noted 15 minutes after the administration of the hormone at a rate and dose of 100 µg/kg/hr. The blood flow continued to decrease 60 and 90 minutes after administration. The blood pressure and blood flow were markedly reduced soon after the start of administration of pentagastrin at a dose and rate of 200 µg/kg/h. These reductions were significant compared

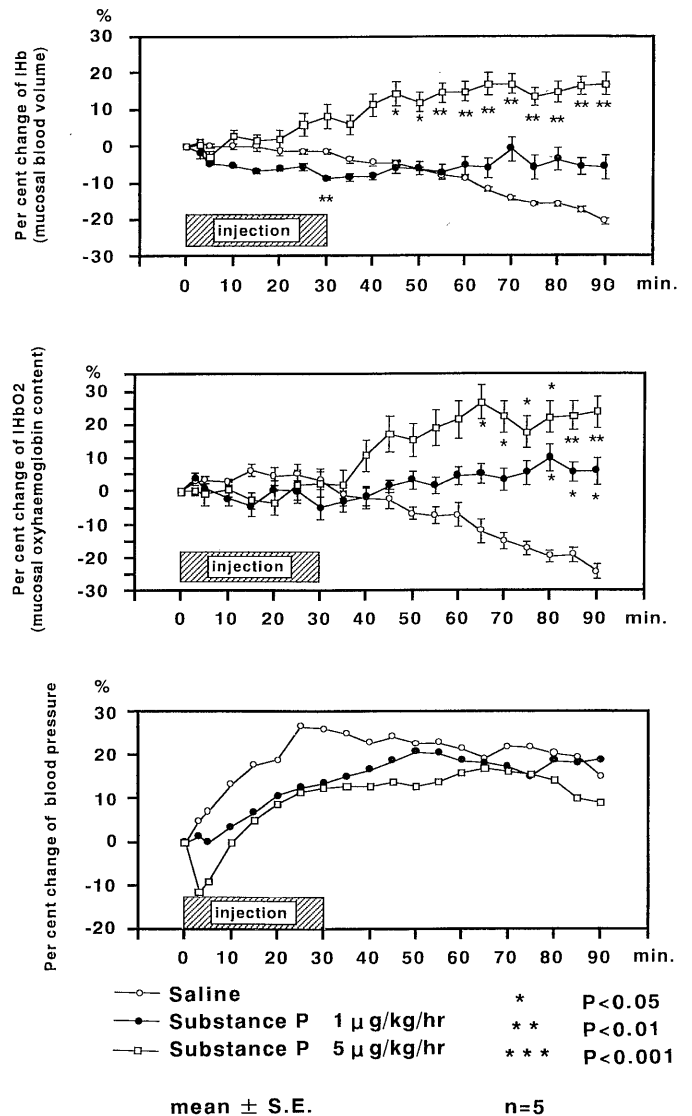


Figure 3. The effect of exogenous substance P on colonic mucosal blood volume, mucosal oxyhaemoglobin content and blood pressure. The concentration of substance P was 1 µg/kg/hr (closed circles) and 5 µg/kg/hr (open squares). The conditions were similar to those in Fig. 1.

to those seen after administration of physiological saline. However, the levels of the blood pressure and blood flow were similar in the group administered pentagastrin at a dose and rate of 200 µg/kg/hr and in the control group, 60 minutes after administration of the gastrointestinal hormone.

The effect of secretin (Sigma) at doses of 5 µg/kg/hr, 50 µg/kg/hr, and 100 µg/kg/hr on blood pressure was less compared to that of physiological saline (Fig. 2). The mucosal blood flow was unchanged before and after administration of secretin. The effect of secretin on cecal mucosal blood flow was greater than that of physiological saline.

The administration of substance P (SP) (Sigma) at doses of 1 $\mu\text{g}/\text{kg}/\text{hr}$, and 5 $\mu\text{g}/\text{kg}/\text{hr}$ produced a transient reduction in blood pressure at first. Subsequently, the blood pressure started to increase again, albeit gradually. At a dose of 1 $\mu\text{g}/\text{kg}/\text{h}$ of SP, the mucosal blood flow was reduced 15 and 30 minutes after the start of its administration. The mucosal blood flow still showed a 5% reduction 45 minutes after infusion. After this, the mucosal blood flow showed a slightly higher increase than that in the control group. The mucosal blood flow started to increase soon after administration of SP at a dose of 5 $\mu\text{g}/\text{kg}/\text{h}$. A state of increased mucosal blood flow was maintained until 90 minutes after the administration of SP. This increase in blood flow was statistically significant compared to that in the control group. The rats died soon after the administration of SP at a dose of 10 $\mu\text{g}/\text{kg}/\text{h}$, and this portion of the experiment could not be completed.

The blood pressure was slightly lower during administration of VIP (Sigma) than during the administration of physiological saline (Fig. 4). The blood flow slightly decreased during the administration of 1 $\mu\text{g}/\text{kg}/\text{h}$ of VIP. However, the blood flow gradually returned to its pre-administration level after infusion of the gastrointestinal hormone. The blood flow was reduced at first when either 5 or 10 $\mu\text{g}/\text{kg}/\text{h}$ of VIP was administered. However, the blood flow showed small increases 20 minutes after the administration of VIP at these doses. Later, the blood flow was observed to be markedly increased in this group.

b) Measurements of various factors

Factors which might have contributed to the increase in blood flow during the administration of VIP were investigated. For this purpose, the blood levels of VIP, cyclic AMP, phospholipase, prostaglandin E_2 , prostaglandin E, and 6 keto-prostaglandin $F_{1\alpha}$ were measured. The results of these measurements showed no significant changes with regard to these factors after the experiments.

IV. Discussion

Gastrointestinal hormones are thought to be humoral transmitters which regulate complex digestive functions. There have been many reports with regard to the effect of these hormones on mucosal blood flow in the upper digestive tract, especially the stomach²⁰. However, the effect of these gastrointestinal hormones on mucosal blood flow in the lower digestive tract, especially that in the colon, has not been fully investigated. In this study, we evaluated the effect of the gastrointestinal hormones (or gastrointestinal peptides) on mucosal blood flow in the colon.

Mucosal blood flow at different colonic sites have been studied for many years. Steinberg et al. measured the mucosal blood flow at different sites in the rat large intestine using the ^{86}Rb clearance technique. They reported

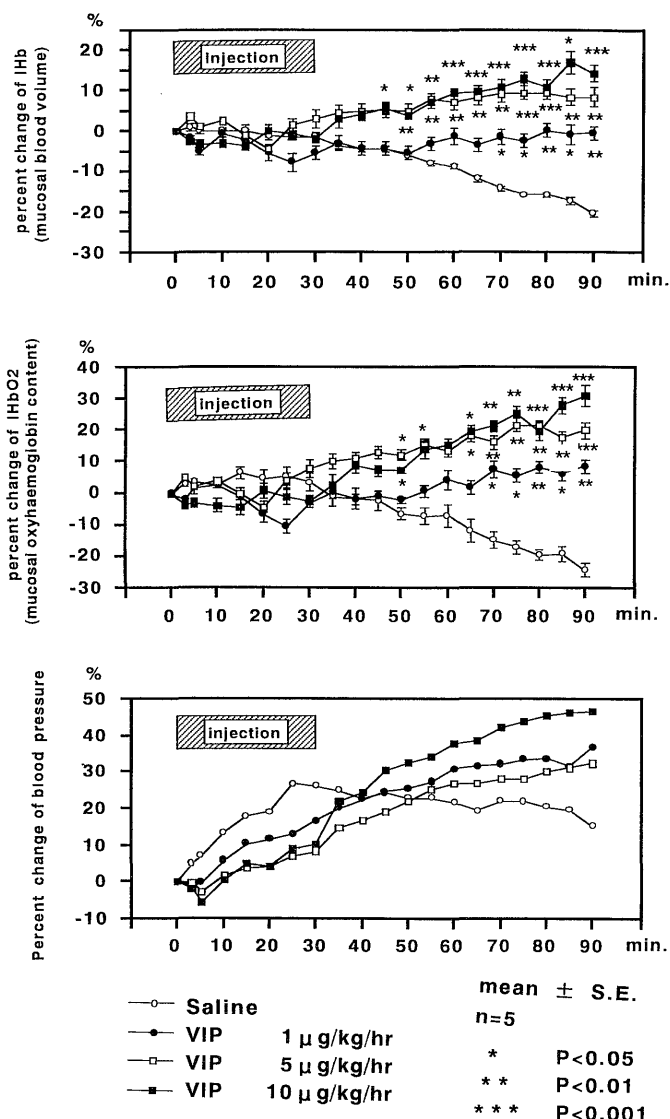


Figure 4. The effect of exogenous VIP on colonic mucosal blood volume, mucosal oxyhaemoglobin content and blood pressure. The concentration of VIP was 1 $\mu\text{g}/\text{kg}/\text{hr}$ (closed circles), 5 $\mu\text{g}/\text{kg}/\text{hr}$ (open squares) and 10 $\mu\text{g}/\text{kg}/\text{hr}$ (closed squares). The conditions were similar to those in Fig. 1.

that the mucosal blood flow in the cecum, colon, and rectum (and sigmoid colon) was 0.5 ± 0.19 ml/min/g, 0.6 ± 0.17 ml/min/g, and 0.2 ± 0.20 ml/min/g, respectively¹³. Coxon et al. showed that the mucosal blood flow at different colonic sites in pigs all similar, except at the sigmoid colon, using the ^{133}Xe clearance technique¹⁴. Wang et al¹² found no difference in the regional blood flow at different colonic sites in healthy adults, except at the rectum where the blood flow was significantly less than at other sites. Given that the mucosal blood flow is similar at all colonic sites, we chose to measure the regional blood flow at the cecum, where measurements could be most easily made.

Methods of measuring the regional blood flow in the colonic membrane consist of the isotope clearance technique and the hydrogen gas clearance technique. Absolute values of the regional blood flow may be obtained using these techniques. However, there are drawbacks with regard to these techniques which included the long time required for making measurements and the difficulty in serial measurements. In contrast, the TS-200 tissue spectrum analyzer is easy to operate and can be used for serial noninvasive measurements of mucosal blood flow. Each measurement was made over a 5 second interval and the mean value was taken to be the blood flow. Even if differences in pulse waves had been present, the blood flow could be measured by taking the average.

Low dose administration of pentagastrin also resulted in an increase in mucosal blood flow. However, the blood pressure was unchanged at this low dose. The blood flow peaked 30 minutes after the administration of pentagastrin at a dose of 50 $\mu\text{g}/\text{kg}/\text{hr}$. Discontinuation of infusion was followed by reduction in blood flow in a dose-dependent manner. However, administration of 200 $\mu\text{g}/\text{kg}/\text{h}$ of pentagastrin resulted in reductions of both the mucosal blood flow and the blood pressure. The administration of a low dose of pentagastrin produced an increase in mucosal blood flow. Higher doses resulted in reductions of blood flow as well as blood pressure. After the completion of pentagastrin infusion, its blood level decreased in a dose-dependent manner.

Many studies have confirmed that pentagastrin increases the mucosal blood flow of the upper digestive tract¹⁶. It is believed that this gastrointestinal hormone mainly acts to increase the gastric blood flow and secretion. We showed that these substances modified the arterial flow in different ways according to the affected organ system. Pentagastrin had the effect of increasing the colonic mucosal blood flow up to a certain concentration, just as it did the gastric blood flow. However, concentrations above this critical level reduced the colonic mucosal blood flow instead. We speculated 3 reasons which might explain this phenomenon. First, the 200 $\mu\text{g}/\text{kg}/\text{hr}$ dose resulted in a much higher blood concentration than is the case when a conventional dose of 5 or 6 $\mu\text{g}/\text{kg}/\text{hr}$ was used. The abnormally high blood level may have caused the reduction of the colonic mucosal blood flow. Second, the secondary metabolic products of pentagastrin may have played a role in reducing the colonic mucosal blood flow. Third, the increase in gastric blood flow due to pentagastrin could have produced an imbalance in the blood distribution in the abdominal organs, which in turn may have resulted in the reduction of the colonic mucosal blood flow.

Secretin was discovered in the porcine duodenal and jejunal mucosa by Baylis and Starling in 1902¹⁷. Its structure was determined by Mutt and Jorpes in 1966²⁰. Secretin is a normal chain peptide which is composed of 27 amino

acids. The 27 amino acids comprise 11 kinds of amino acids and has a molecular weight of 3055. This gastrointestinal hormone acts to inhibit gastrin secretion, but enhances duodenal secretion. Secretin also increases the blood flow in the celiac artery³ and superior mesenteric artery^{1,16}. The arterial blood in the right side of the colon and cecum is supplied by the superior mesenteric artery. Naturally, we expected an increase in the blood flow in these areas as a result of administration of secretin. In our experiments secretin caused an increase in blood flow, regardless of its concentration. This increase in the blood flow was enhanced by treatment with atropine. From this, one may infer that the observed increase in blood flow was a manifestation of the secondary metabolic effect of secretin on the intestinal glands. The increase in blood flow may also be mediated by the release of intermediate metabolites having vasoactive properties¹⁶. In this study, we did not investigate the secondary factors that might have been associated with the effect of secretin. Although secondary factors may play a role in causing an increase in mucosal blood flow, their effects were not clearly seen in our experiments.

Substance P (SP) was first extracted from intestinal and brain tissues by Euler and Gaddum in 1931¹⁸. Today, it is known as a potent vasoactive substance. In 1975 Pernow and Rosell intraarterially administered SP to dogs which resulted in an increase in the mucosal blood flow¹⁹. Hallberg and Pernow reported in 1975 that the intravenous administration of SP increased the blood flow in the carotid artery, hepatic artery, mesenteric artery, and portal vein without compromising the renal circulation²¹. Gronstad et al.²⁰ showed that small intestinal blood flow increased after SP administration in cats by endoluminal perfusion. This experimental observation indicates that SP acts as a vasodilator, regardless of its route of administration. SP triggers relaxation of the cerebral vasculature in the presence of vascular endothelial cells, just as acetylcholine does⁶. Furchgott and Zawadzki found that acetylcholine did not show a vasodilating effect in extracted blood vessel specimens⁷. They ascribed this phenomenon to endothelial cell injury that might have been caused by the investigator during the experiment. They hypothesized that acetylcholine manifests its vasodilating action by releasing an unknown vasodilating substance from the vascular endothelial cells. Given that the vasodilating action of SP is dependent upon the integrity of the vascular endothelial cells, it is speculated that the vasodilating effect of SP is caused by an endothelium-derived relaxing factor (EDRF) or prostaglandin (PG) I₂. PGI₂ seems to mediate the vasodilating action of SP. PGI₂ is not only produced in the vascular endothelial cells, but is also synthesized in the subendothelial tissues. Thus, removal of the vascular endothelial cells is not sufficient for complete inhibition of the vasodilating action of SP. Because of this, it is likely that EDRF is associated with

the vasodilating effect of SP. It has been shown that at least a portion of the EDRF is constituted by nitric acid. However, much still remains unknown with regard to the nature of EDRF, and need to be further investigated.

VIP, extracted and purified from porcine intestine in 1970 by Said and Mutt, is a peptide which acts as a vasodilator and enhances blood flow in porcine intestine⁴. The cardiovascular, gastrointestinal, endocrine, metabolic, and hematological effects of VIP have been identified so far. Duckles and Said et al.¹⁷ and Lee et al.⁶ found that acetylcholine did not dilate cerebral vessels of monkeys when their endothelial cells were experimentally removed. However, dilatation of these vessels by VIP and neural stimulation still occurred even after the endothelial cells were removed. This observation suggests that noncholinergic vasodilation of cerebral vessels may be mediated by VIP. VIP activates adenylate cyclase and increases the level of intracellular cyclic AMP in vascular smooth muscle. An increase in the level of intracellular cyclic AMP results in vasodilation⁶. There have been many reports that VIP increases hepatic and portal venous blood flow⁹.

VIP causes only a slight increase in the blood flow in the superior mesenteric artery^{10,11}. Initially, we expected marked increases in colonic mucosal blood flow after the administration of VIP. However, our findings showed the vasodilating effects of VIP to be weaker than the other gastrointestinal hormones that we tested in this study.

Cerebrovascular vasodilation by VIP may be prevented if the production of PG is inhibited by indomethacin⁵. This suggests that cerebrovascular vasodilation may be related to the production of PGI₂ in the vascular walls. However, no significant changes regarding the blood concentration of 6 keto-PGF₁α were noted in our experiments (6 keto-PGF₁α is produced during the synthesis of PGI₂). Thus, our findings failed to support the hypothesis that PGI₂ is somehow related to cerebrovascular vasodilation. If microdialysis had been performed in an experimental system confined to the intestinal circulation, our findings may have been very well different from what we have obtained in this study.

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