Effects of Avian Pancreatic Peptide on Smooth Muscle Relaxations by Vasoactive Intestinal Peptide and Inhibitory Nerve Stimulation

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Abstract—Experiments were carried out to investigate whether avian pancreatic peptide (APP) antagonized relaxations of vascular and gastrointestinal smooth muscles induced by vasoactive intestinal peptide (VIP) and non-adrenergic, non-cholinergic inhibitory nerve stimulation using the cat submandibular gland, cat colon and dog stomach in vivo and several isolated smooth muscles in vitro. APP caused a dose-dependent inhibition of an atropine-resistant vasodilatation induced by chorda-lingual and pelvic nerve stimulation and by VIP. APP did not reduce VIP output induced by pelvic nerve stimulation. APP shifted the dose-response curve for VIP and the frequency-response curve for pelvic nerve stimulation to the right. The vasodilatation induced by isoproterenol or bradykinin was inhibited by somewhat higher doses of APP. APP failed to inhibit the vago-vagal reflex relaxation of the dog stomach. In guinea pig and rat fundic strips and in isolated chick rectum, APP did not exert any significant effects on relaxation caused by VIP or transmural stimulation in the presence of atropine and guanethidine. The result indicates that APP inhibits VIP-mediated relaxation of the vascular smooth muscle, but not that of the gastrointestinal smooth muscle. The inhibitory effect of APP on VIP-mediated vasodilatation is suggested to be due to the physiological competition.

It is generally accepted that the gastrointestinal tract is innervated by autonomic nerves which contain not only adrenergic and cholinergic nerves but also nerves with unknown neurotransmitter(s). For example, a vago-vagal reflex has been reported to cause a relaxation of the stomach which is not blocked by cholinergic and adrenergic blocking agents (1–4). It has also been reported that stimulation of the pelvic nerve, chorda-lingual nerve and chorda tympani cause marked atropine-resistant vasodilatations in the colon (5), submandibular gland (6) and submaximally gland (7), respectively.

Many peptides with biological activity have been isolated and specific radioimmunoassays developed. In particular, vasoactive intestinal peptide (VIP) has been found to cause powerful relaxations of smooth muscles of the gastrointestinal tract and blood vessels in various species (8–10). Furthermore, VIP-like immunoreactivity has been found in the intramural nerves and the nerves around small blood vessels in the gastrointestinal tract (11–13) and is released by stimulation of the vagus nerve, pelvic nerve and chorda-lingual nerve (14–17). Therefore, VIP has been suggested to mediate the inhibitory responses described above.

Recently, avian pancreatic peptide (APP)-like immunoreactivity has been reported to be present in adrenergic neurones around blood
vessels in the cat submandibular gland (18). Furthermore, APP was found to inhibit the atropine-resistant vasodilatation of this gland induced by chorda-lingual nerve stimulation and by close arterial infusion of VIP, suggesting that APP, or a structurally similar peptide, interacts with mechanisms related to VIP in the exocrine gland (19). If so, it is possible that APP acts universally as a VIP antagonist in the tissues innervated by non-adrenergic, non-cholinergic inhibitory nerves. The purpose of the present experiments was to study this possibility using the cat colon and the dog stomach in vivo and several isolated smooth muscles of the gastrointestinal tract in vitro. A preliminary account of part of this work has been published elsewhere (20).

Materials and Methods

Animals and general operative procedure of the in vivo experiments: The experiments on the colon and the submandibular gland were performed on cats anesthetized with chloralose (50 mg/kg, i.v.) and urethane (100 mg/kg, i.v.) after induction with ether. The experiments on the stomach were carried out on dogs anesthetized with pentobarbitone (30 mg/kg, i.v.). A tracheal cannula was inserted to allow a free airway. Polyethylene cannulae were inserted into the femoral artery to record systemic blood pressure through a pressure transducer (Gould Statham, Inc., P23 ID) and into the cephalic vein for systemic administrations of drugs.

Experiments on the submandibular gland: Experiments on the cat submandibular gland were made according to the method of Lundberg et al. (17). Briefly, a polyethylene cannula was retrogradely inserted into the facial artery for close arterial infusion of drugs to the gland. All branches of the cranial part of the carotid artery were ligated except one branch of the facial artery supplying the submandibular gland. A polyethylene cannula was inserted into the salivary duct to monitor salivary flow by a drop counter. In order to monitor the venous outflow from the gland, a polyethylene cannula was introduced into the extrajugular vein. All veins, except the one from the submandibular gland, were ligated, and drops of the venous outflow from the cannula were measured by a cadmium sulphide photocconductive cell (CdS cell). The electrical signals from the CdS cell were converted to pulses by a pulse generator, and the pulse interval was successively monitored by a tachometer (San Ei 1321). The venous flow was expressed as drops/min. The blood was continuously returned to the animal via the cephalic vein by a peristaltic pump (Mitsumi, SJ1210). Heparin (1000 u/kg) was injected intravenously before the cannulation in the jugular vein. A close arterial infusion of drugs was made using an infusion pump (EYELA, MP-101) at a rate of 100 μl/min or 100 μl/30 sec. The peripheral cut end of the submandibular branch of the chorda-lingual nerve was stimulated electrically for 30 sec (8 Hz, 1 msec, 10 V).

Experiments on the colon: Experiments on the cat colon were performed according to the modified method of Hultén (5). The abdomen of the cat, fasted for 24 hr, was opened by a mid-line incision, and the small intestine was removed. The caudal mesenteric artery and its perivascular nerves were separated, and the vein running along the colonic border was ligated at the boundary between the colon and rectum. The venous flow from the colon was recorded via a wide bore polyethylene cannula inserted into the cranial mesenteric vein by means of the drop counter described above. The venous outflow pressure was set at 10 cmH2O. The blood was continuously returned to the animal via the jugular vein by a peristaltic pump. Heparin (1000 u/kg) was injected intravenously before the cannulation in the cranial mesenteric vein. In order to record colonic motility, a rubber balloon was inserted into the transverse colon. The balloon was connected to a water reservoir by means of tubing, and the system was filled with warm water. The change in the weight of the water reservoir was recorded by a displacement transducer (3). The intraballoonal pressure was usually set at 10 cmH2O. Bilateral pelvic nerves were stimulated with an electronic stimulator (5 msec, 8 V) at frequencies from 1 to 16 Hz for 30 sec. A thin polyethylene cannula was retrogradely inserted from the jejunal artery until its tip reached the bifurcation of the cranial mesenteric and commun colic
artery. Through this cannula, close arterial infusions of drugs were made using an infusion pump at a rate of 150 μl/15 sec or 100 μl/min.

Samples of venous blood were collected from the cannulated cranial mesenteric vein for 30 sec at intervals in ice cooled plastic tubes containing 50 μl of a protease inhibitor, aprotinin (500 μl) and 100 μl of EDTA (250 mM) for determination of VIP concentration and hematocrit. The output of VIP was calculated as the veno-arterial differences in plasma VIP concentrations times colonic plasma flow which was estimated from blood flow times (1-hematocrit) and expressed as fmol/30 sec. The tube was kept on ice until centrifugation, and the plasma was frozen at −70°C.

Experiments on the stomach: Experiments on the dog stomach were conducted as described previously (3). In order to record gastric motility, a rubber balloon was introduced into the stomach via the mouth, and changes in the volume of the stomach were measured as described for colonic motility. The intraballoon pressure was usually set at 15 cmH2O and was kept constant despite changes in the volume of the stomach because of the wide dimension of the reservoir. The chest was opened by removing the 7th to 13th ribs of the left side. A communicating branch of the left vagus nerve to the dorsal vagus trunk was dissected free. The central and peripheral cut ends of the branch were mounted on silver electrodes for vagal afferent and efferent nerve stimulation (20 Hz, 1 msec, 50 V), respectively. For close arterial infusions of drugs to the stomach (0.4 ml/5 sec), a polyethylene cannula was retrogradely inserted into a branch of the splenic artery, until its tip reached the bifurcation of the splenic and left gastroepiploic artery.

Experiments on the isolated smooth muscle preparations: The guinea pig, rat and chick were stunned and bled to death. Fundic strips of the stomach were prepared from the guinea pig and rat. The rectum was isolated from the chick. The fundic strip or the isolated rectum was suspended in a 5 ml bath containing Krebs solution of the following composition (mM): NaCl, 118; KCl, 4.5; CaCl2, 2.5; MgSO4, 1.2; KH2PO4, 1.2; NaHCO3, 25 and glucose, 10 (pH 7.3–7.4). The solution was bubbled with 5% CO2 in O2 and maintained at 30°C. The preparations were allowed to equilibrate for 60 min under a resting tension of 1 gram. Mechanical activity of the muscle was recorded isometrically by a force-displacement transducer (San Ei, 45196). Drugs were added to the bath in a volume of less than 50 μl. Transmural electrical stimulation of the fundic strip was applied through two parallel Ag-AgCl electrodes placed at either side along the whole length of the preparation as described by Ohga and Taneike (21). Electrical stimulation of the isolated chick rectum was applied using the coaxial electrodes described by Paton (22).

Isolation and identification of avian pancreatic peptide: APP was isolated and purified according to the method of Kimmel et al. (23). Briefly, chicken pancreas was collected at a slaughterhouse, immediately frozen with dry ice, and stored at −20°C until use. The isolation procedure involves four main steps, acid-alcohol extraction, gel filtration, DEAE-cellulose chromatography and droplet countercurrent chromatography. The fraction containing the peptide obtained by droplet countercurrent chromatography, the final step of the purification, was evaporated to dryness. The residue was dissolved in 1 M acetic acid and desalted by gel filtration with Sephadex G 25, and then the fractions were pooled and freeze-dried. The homogeneity of this peptide was evaluated by gel electrophoresis and thin layer chromatography. A single band was visible at levels up to 100 μg/tube by gel electrophoresis and thin layer chromatography showed only one yellow ninhydrin-positive spot. The amino acid composition of this isolated peptide was the same as that of APP, and the peptide map of this peptide digested by trypsin was similar to that of APP digested by this enzyme (23).

Determination of VIP concentration: Plasma VIP concentration was measured by radioimmunoassay. VIP was coupled with bovine serum albumin by carbodiimide according to the method of Fahrenkrug and Schaffalitzky de Muckadell (24). Anti-VIP
serum was generated by intradermal injection of this VIP-conjugate homogenized in Freund's complete adjuvant at several sites in three rabbits. Booster injections were made at monthly intervals by similar injection of the VIP-conjugate homogenized in Freund's incomplete adjuvant. After the 5th injection, each rabbit developed antiserum to VIP usable for radioimmunoassay. Antisera were stored at -70°C.

VIP (0.6 nmol) was iodinated by the chloramine T method using 1 mCi Na125I. The reaction mixture was applied to a column (1.5 x 13) of Sephadex G 10, and the column was eluted with 10 mM sodium phosphate buffer (pH 7.4) containing 150 mM NaCl. The first radioactive peak fraction of gel filtration was then applied to a column (1 x 10) of CM Sephadex C-25 equilibrated with 50 mM ammonium bicarbonate (pH 7.9). Iodinated VIP was eluted by a linear gradient of equal volumes (30 ml) of 50 mM and 500 mM ammonium bicarbonate, and the third peak fraction which eluted at about 250 mM ammonium bicarbonate was pooled and freeze-dried. This iodinated VIP was used for radioimmunoassay.

Radioimmunoassay procedure was performed according to the method of Pandian et al. (25). Briefly, the first incubation was carried out at 4°C for 16 to 20 hr in 0.2 ml of assay buffer (10 mM phosphate buffer (pH 7.4), 150 mM NaCl, 25 mM EDTA, 0.5% bovine serum albumin, 0.005% protamine sulphate and 0.005% bacitracin), 0.2 ml of antisera to VIP (1:80,000) diluted with 1% normal rabbit serum and 0.2 ml of sample or standard solution. After the first incubation, 0.2 ml of iodinated VIP in assay buffer (10,000 cpm/tube) was added, and the second incubation was made at 4°C for a further 40 hr. Free and bound iodinated VIP were separated by the addition of 0.1 ml of anti-rabbit IgG goat serum. One ml of polyethylene glycol (6.6%) in 50 mM sodium phosphate buffer (pH 7.4) was then added to the reaction mixture to facilitate the production of immune precipitate. Standards contain 0.78 fmol/tube VIP at the lowest level and doubling concentration up to 100 fmol at the highest concentration.

The antibody used (VK208) reacts with the sequence 7–28 and partly with 11–28, but reacts poorly with the 18–28 VIP fragment, indicating that VK208 is a mid- and COOH-terminal-specific antibody. The intra-assay (n=5) and inter-assay (n=5) coefficients of variation were ±8.2% and ±5.5% at the lower level of standard (1.6 fmol/tube) and were ±13.4% and ±14.3% at the highest level of standard (100 fmol/tube), respectively. The non-specific binding of labelled ligand was less than 5%. The 95% confidence limit in the estimation of percent bound iodinated VIP at zero hormone concentration was 6.6% bound so that the detection limit was 1.2 fmol VIP/tube.

Statistical analysis: The results were expressed as the mean±S.E.M., and statistical significance was assessed using Student's t-test (unpaired). P values less than 0.05 were considered significant.

Chemicals: The following drugs were used: vasoactive intestinal peptide, and bradykinin (Protein Ins. Inc.); bovine serum albumin, bacitracin, and aprotinin (Sigma); carbodiimide, chloramine T and atropine sulfate (Wako); protamine sulfate (Nakarai); and Na125I (Amersham).

Results

Blood flow of cat submandibular gland:

In order to re-examine the result reported by Lundberg et al. (19), the first experiments were carried out to determine whether or not APP inhibited atropine-resistant increases in the blood flow induced by chorda-lingual nerve stimulation and infusion of VIP in the cat submandibular gland.

Chorda-lingual parasympathetic nerve stimulation (8 Hz, 1 msec, 10 V) caused marked increases in submandibular venous outflow and salivary secretion. The systemic administration of atropine (0.5 mg/kg) completely abolished salivary secretion but not the increase in the venous outflow, indicating that this response was attributed to an atropine-resistant vasodilatation. APP was applied to the submandibular gland by a close arterial infusion for 2 min at a flow rate of 100 µl/min. A representative result is shown in Fig. 1. APP (0.48–12 nmol) caused a dose-dependent inhibition of the atropine-resistant vasodilatation induced by
parasympathetic nerve stimulation, APP itself caused small decreases in the basal blood flow with the doses of 12 and 2.4 nmol (Fig. 1A: b and c).

A close arterial infusion of VIP (1.1 nmol/100 μl) for 30 sec (Fig. 1B: a) caused a vasodilatation with a time course similar to that of the response to nerve stimulation (Fig. 1A: a). With this rate of infusion, saline solution also increased the blood flow, and this response had to be subtracted from the response to VIP (Fig. 1B: e). APP was applied simultaneously with VIP for 30 sec. As shown in Fig. 1B: b and c, APP (10 and 2 nmol/100 μl) inhibited the maximum vasodilatation induced by VIP. Furthermore, APP significantly shortened the duration of the vasodilatation.

**Blood flow of cat colon:** Pelvic nerve stimulation is well-known to cause an atropine-resistant colonic vasodilatation and contraction and to produce an atropine-sensitive mucous secretion (5). Therefore, it is of interest to study the effects of APP on vasodilatation induced by pelvic nerve stimulation and VIP. The resting blood flow of the cat colon measured by the drop-counter was about 100 drops/min. A close arterial infusion of APP (0.375–6 nmol/150 μl) for 15 sec caused a dose-dependent decrease in the basal blood flow without any significant effects on the colonic motility and blood pressure. The lowest effective dose of APP was 0.375 nmol, and the 50% inhibitory dose was about 3 nmol.

Pelvic nerve stimulation (8 Hz, 5 msec, 8 V) elicited a rapid and significant increase in the blood flow and a sustained contraction of the cat colon. The increase in the blood flow, but not colonic contraction, tended to decline during stimulation. These responses to nerve stimulation were resistant to the systemic administration of atropine (0.5 mg/kg) as reported by Hultén (5). The increase in the blood flow induced by nerve stimulation was partially inhibited by APP. A typical result is shown in Fig. 2. In this case, the colonic contraction in response to nerve stimulation was somewhat decreased by APP. However, the inhibitory effect of APP on the colonic motility was different from preparation to preparation.

It was reported that pelvic nerve stimulation caused a release of VIP-like immunoreactivity (VIP) in colonic venous plasma in the cat (14, 26, 27). If so, it is possible that APP affects the output of VIP. In this experiment, the pelvic nerve was stimulated 3 times at intervals of 20 to 30 min, and APP (1.5 nmol/150 μl) was applied 30 sec before the second stimulation. The resting arterial blood plasma (femoral artery) contained VIP in the concentration of 43±5 pmol/L (mean±S.E.M., n=6). VIP concentration of the colonic venous plasma was 133±10
pmol/L, indicating that there is a marked veno-arterial difference. Pelvic nerve stimulation caused an increase in VIP output in the colonic venous plasma in addition to colonic vasodilatation. These nerve-mediated responses tended to decrease on repetition. The release of VIP induced by nerve stimulation was rapid in onset and decreased near the resting level 3 min after the cessation of stimulation (Fig. 3). A close arterial infusion of APP for 15 sec did not modify resting VIP output in the plasma. However, the peak value of VIP output in the plasma induced by subsequent pelvic nerve stimulation was reduced by APP. Difference in the peak value of VIP output between the first and second stimulation was 0.05 < P < 0.1 (n=6). There was no statistically significant difference in VIP concentration in the venous plasma collected during stimulation.

In order to estimate the effect of APP on the increase in blood flow elicited by pelvic nerve stimulation and VIP quantitatively, dose- or frequency-response curves were compared in the presence and absence of APP. APP (1.5 nmol or 6 nmol/150 µl) was infused for 15 sec, and then the pelvic nerve was stimulated for 30 sec, when the APP-induced decrease in the resting blood flow had attained its lowest level. Pelvic nerve stimulation caused a frequency-dependent increase in colonic blood flow which began to increase from 1 Hz and attained a maximum at about 8 Hz. APP was found to decrease the blood flow in response to nerve stimulation in all frequencies used (Fig. 4A). A close arterial infusion of VIP (15 pmol-0.48 nmol/150 µl) for 15 sec caused a dose-dependent increase in vasodilatation which attained a maximum at about 0.24 nmol. APP (1.5 nmol or 3 nmol/150 µl) was infused for 15 sec, and VIP was applied after APP. The results obtained in this series of experiments are summarized in Fig. 4B. The preceding application of APP shifted the dose-response curve for VIP to the right and depressed a maximum vasodilatation in a dose-dependent manner. These results seem to indicate that the inhibitory effects of APP on vasodilatation in response to pelvic nerve stimulation and VIP are in a non-competitive manner.

In order to estimate the specificity of the action of APP as a VIP antagonist, inhibitory effects of APP on drug-mediated vasodilatation were compared. Close arterial infusions of VIP, bradykinin and isoproterenol for 15 sec caused dose-dependent increases in colonic blood flow in the atropinized cat. In some experiments, these vasodilators were
applied together with various doses of APP. A typical result obtained from one experiment is shown in Fig. 5. Under this condition, a lower dose of APP (<0.75 nmol) partially inhibited the increase in the blood flow induced by VIP, but not those caused by

Fig. 3. Release of VIP-like immunoreactivity in colonic venous plasma and venous flow in response to pelvic nerve stimulation in the presence or absence of APP. Filled squares (■) indicate the period (30 sec) of pelvic nerve stimulation (8 Hz, 5 msec, 8 V). Thirty sec before the second stimulation, close arterial infusion of APP (↓, 1.5 nmol/150 μl) was made for 15 sec. Symbols (mean±S.E.M.) represent the amount of venous plasma flow for 30 sec (A), the amount of VIP output in venous plasma for 30 sec (B) and plasma VIP concentration (C) (1st and 2nd stimulation, n=6; 3rd stimulation, n=4).

Fig. 4. Frequency- and dose-response curves showing peak vasodilator response in cat colon to pelvic nerve stimulation and VIP infusion in the presence or absence of APP. Symbols indicate the percentage (mean±S.E.M.) of response to 16 Hz (A, n=5–8) and 0.48 nmol VIP (B, n=5–7) in the absence (○) or presence (■, 1.5 nmol; ▲, 3 nmol; ○, 6 nmol) of APP. Abscissa scales represent frequencies (Hz) of nerve stimulation (A) and doses (pmol/150 μl) of VIP (B) on a logarithmic scale. Stars near symbols indicate statistically significant difference from the control value in the absence of APP (*P<0.05, **P<0.01, ***P<0.001).
bradykinin and isoproterenol (Fig. 5: b and c). However, when higher doses of APP (>1.5 nmol) were used, the responses to bradykinin and isoproterenol were also inhibited in a dose-dependent manner (Fig. 5: d, e and f).

In order to compare inhibitory effects of APP on vasodilatations, pelvic nerve stimulation, VIP, and bradykinin were applied when the APP-induced decrease in the resting blood flow had attained its lowest level. The peak value of response in the presence of APP was expressed as a percentage of a mean peak value of before and after control responses. The results are summarized and plotted against log doses of APP (Fig. 6). APP caused an inhibitory effect on vasodilatation induced by pelvic nerve stimulation and VIP within the range of doses examined (0.375–6 nmol/150 µl). In the presence of 1.5 nmol APP, there was a statistically significant difference between the response to bradykinin and those to pelvic nerve stimulation and VIP (P<0.05), but not between response to nerve stimulation and VIP (bradykinin, VIP, n=5; nerve stimulation, n=8). However, a higher dose of APP (3 and 6 nmol) inhibited all responses to VIP, bradykinin and pelvic nerve stimulation.

Motility of dog stomach: Stimulation of the peripheral and central cut ends of the vagal communicating branch in the thorax causes a marked contraction and relaxation of the dog stomach, respectively. The contraction is reversed to relaxation by the systemic administration of atropine. Both relaxations induced by vagus nerve stimulation are resistant to adrenergic blocking agents (3). Therefore, the study was carried out to investigate whether APP inhibited the gastric relaxation of the dog induced by vagus nerve stimulation and VIP.

The vago-vagal reflex relaxation of the stomach returned gradually to the resting level. On the other hand, peripheral vagal stimulation produced a relaxation which was recovered quickly to the resting level or was followed by a poststimulation contraction (Fig. 7). A close arterial infusion of VIP (37.5 pmol–6 nmol/0.4 ml) for 5 sec caused a dose-dependent relaxation of the dog stomach. The application of APP in a high dose (12 nmol/0.4 ml) did not produce any effects on the gastric motility. Furthermore, as shown in Fig. 7, the relaxations induced by...
the central and peripheral vagal stimulation for 30 sec and the close arterial infusion of VIP (3 nmol/0.4 ml) for 5 sec were not affected by preceding applications of APP.

Mechanical response of isolated smooth muscle in vitro: Transmural electrical stimulation of the guinea pig and rat fundic strips and isolated chick rectum elicited frequency-dependent relaxations in the presence of atropine (1 nM) and guanethidine (1 μM). VIP, added to the bathing medium, caused relaxations of all these preparations in a dose-dependent manner. The pD2 value for VIP causing the relaxation was 7.89±0.07 in the guinea pig fundic strips and 7.84±0.05 in the isolated chick rectum (n=6). APP itself caused no perceptible effect on the mechanical activity of the smooth muscle in the concentration up to 2 μM in all preparations. Figure 8 shows a representative result obtained from the guinea pig fundic strip. APP (0.2 or 1 μM) failed to inhibit the relaxation induced by transmural electrical stimulation at low frequencies (0.25–1 Hz) or by VIP (10 or 3 nM). In this case, poststimulation contraction appeared to be decreased by APP, but similar results were not obtained from other experiments. APP (up to 2 μM) had no inhibitory effect on the response to VIP and transmural electrical stimulation in the rat fundic strip and the isolated chick rectum.

Discussion

It was reported that APP inhibited vasodilatations elicited by chorda-lingual nerve stimulation and a close arterial infusion of VIP, but not those caused by ACh and bradykinin in the cat submandibular gland (19). This prompted us to study the possibility that APP acts universally as a VIP antagonist.
Quite recently, however, Karpinski et al. (28) reported that the vasodilatation induced by parasympathetic nerve stimulation in this gland was not inhibited by the dose of APP used by Lundberg et al. (19). This result is consistent with the present experiment. However, when higher doses of APP which induced slight vasoconstriction were used, APP caused a dose-dependent inhibition of an atropine-resistant vasodilatation of the cat submandibular gland in response to both chorda-lingual nerve stimulation and VIP. Furthermore, we found that APP was effective in inhibiting an atropine-resistant vasodilatation of the cat colon induced by pelvic nerve stimulation and VIP in a dose-dependent manner.

The inhibitory effect of APP on the nerve-mediated vasodilatation may result from a postsynaptic event because APP causes the dose-dependent inhibition on the VIP-mediated vasodilatation. Furthermore, there was no difference in plasma VIP concentration during pelvic nerve stimulation between the presence and absence of APP, although APP tended to decrease the peak value of VIP-like immunoreactivity appearing in the colonic venous plasma. Similar results have been reported in the cat submandibular gland (29). The reduction of the peak value of VIP output in the venous plasma may be due to the secondary effect of blood flow diminished by APP and/or of APP-mediated vasoconstriction which modified released VIP to appear in the venous plasma.

Although APP had an inhibitory effect on vasodilatation, it failed to inhibit the relaxation induced by the vago-vagal reflex or by the close arterial infusion of VIP in the dog stomach and that caused by transmural electrical stimulation or VIP in smooth muscle segments isolated from guinea pig and rat fundus and chick rectum. These results indicate that APP inhibits relaxations of vascular smooth muscle but not those of gastrointestinal smooth muscle induced by VIP and non-adrenergic, non-cholinergic inhibitory nerve stimulation.

The pancreatic peptide family (APP, BPP, NPY and PYY) was reported to cause a vasoconstriction resistant to α-adrenergic blocking agents (30). In the present experiments, APP elicited the vasoconstriction and the inhibitory effect on the VIP- and nerve-mediated vasodilatation in the same dose range in cat colon and submandibular gland, but it had no effect on the tension of the smooth muscle of the gastrointestinal tract. Therefore, the difference in the ability of APP to inhibit the relaxation of the smooth muscle induced by VIP may be attributed to a difference in the responsiveness of blood vessels and gastrointestinal tract smooth muscle to APP.

Although APP inhibited the vasodilatation induced by pelvic nerve stimulation and by VIP with the same dose range, slightly higher doses of APP also inhibited vasodilatations mediated by bradykinin and isoproterenol. Therefore, we do not think that APP is a specific VIP antagonist. Furthermore, the inhibitory effect of APP on vasodilatation in response to pelvic nerve stimulation or VIP...
was in a non-competitive manner and was always accompanied with the APP-mediated colonic vasoconstriction. These results suggest that the inhibitory effect of APP on the VIP-mediated vasodilatation is mainly a physiological competition, but not a pharmacological competition.

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