Inhibition of gastric acid secretion in rat stomach by PACAP is mediated by secretin, somatostatin, and PGE₂

P. Li, T.-M. Chang, D. Coy, and W. Y. Chey. Inhibition of gastric acid secretion in rat stomach by PACAP is mediated by secretin, somatostatin, and PGE₂. Am. J. Physiol. Gastrointest. Liver Physiol. 278: G121–G127, 2000.—Pituitary adenylate cyclase-activating polypeptide (PACAP), existing in two variants, PACAP-27 and PACAP-38, is found in the enteric nervous system and regulates function of the digestive system. However, the regulatory mechanism of PACAP on gastric acid secretion has not been well elucidated. We investigated the inhibitory action of PACAP-27 on acid secretion and its mechanism in isolated vascularity perfused rat stomach. PACAP-27 in four graded doses (5, 10, 20, and 50 µg/h) was vascularity infused to determine its effect on basal and pentagastrin (50 ng/h)-stimulated acid secretion. To study the inhibitory mechanism of PACAP-27 on acid secretion, a rabbit antisecretin serum, antisomatostatin serum, or indomethacin was administered. Concentrations of secretin, somatostatin, PGE₂, and histamine in portal venous effluent were measured by RIA. PACAP-27 dose-dependently inhibited both basal and pentagastrin-stimulated acid secretion. PACAP-27 at 10 µg/h significantly increased concentrations of secretin, somatostatin, and PGE₂ in basal or pentagastrin-stimulated state. The inhibitory effect of PACAP-27 on pentagastrin-stimulated acid secretion was reversed 33% by an antisecretin serum, 80.0% by an antisomatostatin serum, and 46.1% by indomethacin. The antisecretin serum partially reduced PACAP-27-induced local release of somatostatin and PGE₂. PACAP-27 at 10 µg/h elevated histamine level in portal venous effluent, which was further increased by antisomatostatin serum. However, antisomatostatin serum did not significantly increase acid secretion. It is concluded that PACAP-27 inhibits both basal and pentagastrin-stimulated gastric acid secretion. The effect of PACAP-27 is mediated by local release of secretin, somatostatin, and PGE₂ in isolated perfused rat stomach. The increase in somatostatin and PGE₂ levels in portal venous effluent is, in part, attributable to local action of the endogenous secretin.

PITUITARY ADENYLATE cyclase-activating polypeptide; histamine

PITUITARY ADENYLATE cyclase-activating polypeptide (PACAP) is a neuropeptide isolated from the ovine hypothalamus in 1989 (17). This neuropeptide is present in two bioactive forms, the major form with 38 amino acid residues (PACAP-38) and the other with 27 residues (PACAP-27) sharing the same 27 NH₂-terminal amino acids (18). PACAP is considered a member of the secretin/glucagon/vasoactive intestinal polypeptide (VIP) superfamily (2). It stimulates adenylate cyclase in cultured rat anterior pituitary cells and augments the release of growth hormone, ACTH, prolactin, and leuteinizing hormone from superfused rat pituitary cells (17). PACAP-like immunoreactivity has been demonstrated to exist in neural elements throughout the gastrointestinal tract of various species, including the chicken, mouse, rat, hamster, guinea pig, ferret, cat, pig, sheep, and human (33). The presence of PACAP-immunoreactive nerve cells and fibers in the gut wall suggests that PACAP may also participate in regulation of both motor and secretory functions in the digestive tract. Indeed, PACAP stimulates endocrine (9, 34) and exocrine (1, 13, 22) pancreatic secretion in dogs (19), and augments pepsinogen secretion from chief cells of the guinea pig stomach (18). Mungan et al. (23, 24) observed that PACAP-27 inhibited pentagastrin- and histamine-stimulated gastric acid secretion in conscious rats. However, the inhibitory mechanism of PACAP on acid secretion has not been clarified. It has been reported that suppression of gastric acid secretion by secretin was mediated through the release of somatostatin and PGE₂ in the rat both in vitro (7) and in vivo (31). We have recently demonstrated the presence of secretin cells and mRNA in rat antral and oxyntic mucosa (5). In contrast, Zeng et al. (37) have demonstrated that PACAP stimulates the release of histamine from isolated enterochromaffin-like (ECL) cells of the stomach and increases acid secretion only in rats pretreated with an antisomatostatin. In the present study, we investigated whether or not the inhibitory effect of PACAP-27 on gastric acid secretion involves local release of secretin, somatostatin, PGE₂, and histamine in totally isolated vascularity perfused rat stomach.

MATERIALS AND METHODS

Materials. PACAP-27 was synthesized by D. Coy. Isobutyl methylxanthine, PGE₂, and indomethacin were purchased from Sigma Chemical (St. Louis, MO), and pentagastrin was purchased from Wyeth-Ayerst (Philadelphia, PA). Rabbit antisera to somatostatin and to secretin were raised in our laboratory. The antisecretin serum (R7-5) had a titer of 1:1,000,000. The antiserum was specific for secretin and had no cross-reaction with other regulatory peptides, including gastrin, CCK-8, insulin, glucagon, VIP, motilin, peptide histi-
dine-isoleucine amide (PH1), and PACAP at a concentration as high as 0.1 μM. The antisomatostatin serum used had a titer of 1:63,000. It reacted only with somatostatin-14 (100%) and somatostatin-28 (15%). Other regulatory peptides, including CCK-8, rat secretin, PACAP, amylin, calcitonin gene-related peptide (CGRP), glucagon, insulin, and rat pancreatic polypeptide had no cross-reaction with the antisera at a concentration as high as 0.1 μM.

Experimental animals. Male Sprague-Dawley rats weighing 230–270 g were deprived of food for 36 h but allowed free access to water. They were anesthetized with 25% urethane by injecting a dose of 0.35 ml/100 g body wt both intraperitoneally and subcutaneously.

Vascular perfusion of totally isolated rat stomach. A totally isolated vascularly perfused rat stomach was prepared as previously described (5). In brief, the celiac artery was cannulated with a polyvinyl tube (PE-50, 1D 0.28 mm) for perfusion of Krebs-Ringer buffer containing 10% rat erythrocytes (KRB-RBC). No more than 60 s were permitted to elapse between ligation of the aorta and initiation of gastric vascular perfusion. The portal vein was isolated and a polyvinyl tube (PE-190, 1D 0.86 mm) was inserted to drain portal venous effluent. The mesenteric arteries, renal arteries and veins, and hepatic and pancreaticoduodenal arteries were ligated. The spleen was removed. A polyvinyl tube (ID 1.4 mm) was placed in the proximal stomach 0.5 cm aborally from the esophagogastric junction to perfuse 0.15 M NaCl, and another polyvinyl tube (ID 3.0 mm) was inserted in the distal stomach, 0.5 cm oral to the pylorus via a duodenal incision and pyloric channel for drainage of gastric luminal fluid. Both tubes were tied with 3-0 silk, and then the stomach was isolated by cutting the esophagus and the duodenum at the incision sites of these two tubes.

The totally isolated rat stomach was immediately placed in an organ bath chamber filled with KRB at 37°C. The vascular bed was continuously perfused intra-arterially with KRB-RBC at 1.4 ml/min, which contained 4% BSA, 5% freshly washed rat erythrocytes, and 50 μM isobutyl methylxanthine, a phosphodiesterase inhibitor that increases sensitivity for stimulation of acid secretion (10). The KRB-RBC was continuously gassed with a mixture of 95% O2 and 5% CO2 via a tube oxygenator bath, and KRB-RBC perfusate was maintained at 37°C throughout the experiments. The portal venous effluent was collected in 10-min intervals in ice-chilled tubes without recirculation. Samples were stored at −20°C for determination of hormone levels, including secretin, somatostatin, PGE2, and histamine. The gastric lumen was perfused with 0.15 M NaCl (pH 7.4) at a speed of 1.0 ml/min that was gassed with 100% O2. The luminal effluent was collected in 10-min intervals for measurement of acid concentration.

Experimental designs. To stabilize acid secretion, all of the experiments were performed 30 min after vascular perfusion with KRB-RBC, and luminal perfusion with 0.15 M NaCl began as follows. 1) In the study of basal acid secretion, six stomachs were continuously infused with KRB-RBC for 80 min. In another six rat stomachs, PACAP-27 dissolved in KRB in graded doses at 5, 10, 20, and 50 μg/h was infused in stepwise manner for 20 min, initiating 20 min after infusion of KRB-RBC began. 2) In the study of pentagastrin-stimulated acid secretion, six isolated stomachs were infused with pentagastrin at 50 μg/h for 50 min after 20 min of KRB-RBC infusion. In another 18 stomachs, PACAP-27 at doses of 5, 10, 20, or 50 μg/h was infused during the last 30 min of pentagastrin infusion in each group of six stomachs. 3) To investigate possible roles of secretin, somatostatin, or PGE2 in the inhibition of pentagastrin-stimulated acid secretion by PACAP-27, a rabbit antisomatostatin serum (1 ml/h), a rabbit antisecretin serum (0.2 ml/h), indomethacin (4.6 fmol/h), or a combination of rabbit antisomatostatin and antisecretin sera and indomethacin was infused for 40 min starting 10 min before PACAP-27 was infused at 10 μg/h in each group of five stomachs. A normal rabbit serum (NRS) was infused at 1 ml/h or 0.2 ml/h for 40 min as a control in five separate stomachs. 4) To examine the effect of antisomatostatin serum on acid secretion and release of histamine in response to PACAP-27, we infused the antisera or NRS (1 ml/h) for 40 min before administration of PACAP-27 in each of five stomachs, respectively. Concentrations of secretin, somatostatin, PGE2, and histamine in portal venous effluent were determined by the corresponding specific RIA. Acid concentration in the gastric luminal effluent was titrated to an end point pH 7.4 with 0.01 N NaOH, using a Fisher titrator (Fisher Scientific, Pittsburgh, PA).

RIA of secretin, somatostatin, PGE2, and histamine. RIA of secretin (3), somatostatin (7), and PGE2 (7) were carried out as described previously. RIA of histamine was carried out after acetylation using a commercial specific assay kit (Beckman/Coulter/Immunotech, Brea, CA). The assay sensitivity in this assay was 0.2 nM of histamine.

HPLC analysis of secretin-like immunoreactivity in the portal venous effluent. Thirteen milliliters of portal venous effluents collected from each isolated rat stomach infused with PACAP-27 at 10 μg/h were adjusted to pH 3.0 with dilute HCl and then extracted on two C-18 Sep-Pak cartridges. The eluates were dried in a Speed-Vac (Savant Instruments, Farmingdale, NY). Dried samples were resuspended in HPLC equilibration solvent and passed through 0.2-μm nylon filter was applied to a MCH-10 C18 HPLC analytical column. The solvents used were 0.1 M triethylammonium formate, pH 3.0 (solvent A) and 0.1 M triethylammonium formate in 2-propanol-acetonitrile-water (5/2/3 vol/vol/vol) solution (solvent B). After sample injection, the column was eluted with a gradient 36–56% solvent B in 90 min and then to 100% solvent B in 10 min. Fractions were collected in 1-min periods and dried on the Speed-Vac for determination of secretin by RIA.

Statistical analysis. Results were expressed as means ± SE. Gastric acid secretion was expressed as micromoles per 10 min or integrated acid output (μmol/min). Percentage increase over basal value was calculated by comparing acid secretion in the last 20 min of each treatment period with that in 20 min of the basal secretory period. Statistical differences were analyzed by one-way ANOVA. Tukey's test was used for multiple comparisons of the means. P values of <0.05 were considered statistically significant.

RESULTS

Effect of PACAP-27 on basal and pentagastrin-stimulated acid secretion. Basal acid secretion during the infusion of KRB-RBC was stable (Fig. 1A). The mean acid output was 5.2 ± 0.6 μmol/20 min (Fig.1B). Infusion of PACAP-27 at doses of 5, 10, 20, and 50 μg/h resulted in a dose-related decrease of basal acid secretion (in μmol/20 min) from 5.2 ± 0.6 μmol/20 min to 4.8 ± 0.4 (−7.7%), 4.0 ± 0.4 (−23.1%), 3.6 ± 0.4 (−30.8%), and 3.8 ± 0.4 (−29.5%), respectively. PACAP-27 at 20 μg/h reached the maximal inhibition of acid secretion (Fig. 1B). The decrease of acid secretion in response to infusion of PACAP-27 at doses of 10 μg/h or greater was statistically significant compared with the basal value.
Pentagastrin (50 ng/h) stimulated acid secretion within 10 min, reached a peak (5.2 ± 0.6 µmol/10 min) at 30 min, and maintained a similar magnitude of acid output for at least 20 min (Fig. 2A). The acid secretion stimulated by pentagastrin was also significantly inhibited by PACAP-27 at 10, 20, and 50 µg/h from 10.8 ± 0.8 µmol/20 min to 5.8 ± 0.3 (−46.3%), 5.1 ± 0.3 (−52.8%), and 5.5 ± 0.6 (−50.0%) µmol/20 min, respectively (Fig. 2B).

Effect of PACAP-27 on concentration of secretin, somatostatin, and PGE2. In the basal state, secretin, somatostatin, and PGE2 levels in portal venous effluent were 0.4 ± 0.1 pM, 14.1 ± 2.4 pM, and 0.8 ± 0.3 nM, respectively (Fig. 3). Secretin and PGE2 levels were not influenced by administration of pentagastrin (0.3 ± 0.1 pM and 0.9 ± 0.3 nM, respectively). Somatostatin concentration was elevated to 23.1 ± 6.2 pM after the infusion of pentagastrin, but the increase was not statistically significant (Fig. 3). In both basal and pentagastrin-stimulated states (P < 0.05–0.01), the administration of PACAP-27 at 10 µg/h resulted in marked increases in concentrations of secretin, somatostatin, and PGE2 in portal venous effluent, respectively (Fig. 3).

Effect of antisecretin and antisomatostatin sera and indomethacin on inhibition by PACAP-27 of pentagastrin-stimulated acid secretion. To further determine which hormones contribute to the inhibitory effect of PACAP-27 on acid secretion, a rabbit antisecretin or antisomatostatin serum or indomethacin was given intra-arterially 10 min before infusion of PACAP-27. As shown in Fig. 4, PACAP-27 significantly inhibited pentagastrin-stimulated acid secretion. Administration of a rabbit antisecretin serum (0.2 ml/h) partially but significantly reversed the acid inhibition by 33.3%. A rabbit antisomatostatin serum (1 ml/h) reversed the inhibition by 80.0%. However, NRS in equivalent doses to the antisecretin serum or antisomatostatin serum failed to influence the inhibitory action of PACAP-27. Indomethacin at 4.6 µmol/h blocked inhibition of acid secretion in response to PACAP-27 by 46.1%. These observations indicated that secretin, somatostatin, and PGE2 mediate the inhibition of acid secretion by PACAP-27.

Effect of antisecretin serum on somatostatin and PGE2 levels in portal venous effluent. As shown in Fig. 5, the rabbit antisecretin serum significantly reduced the release of somatostatin in response to PACAP-27 from 48.7 ± 6.7 pM to 32.8 ± 2.8 pM (P < 0.05). A similar significant change by the antisecretin serum was also observed in portal venous concentration of PGE2 (4.2 ± 1.1 vs. 2.0 ± 1.1 nM, P < 0.05) (Fig. 5). NRS, however, did not influence the release of either somatostatin or PGE2 (data not shown), suggesting that the releases of somatostatin and PGE2 by PACAP-27 are in part mediated by local secretin.

Effect of antisomatostatin serum on acid secretion and release of histamine in response to PACAP-27. Infusion of PACAP-27 at 10 µg/h significantly inhibited acid secretion (5.1 ± 0.7 vs. 3.9 ± 0.5 µmol/20 min, P < 0.05 compared with basal acid secretion).
0.05) but markedly increased the release of histamine from the stomach (246.2 ± 47.2 vs. 515.4 ± 125.6 nM). The antisomatostatin serum (1 ml/h) further increased histamine level in portal venous effluent (721.4 ± 142.2 nM) and abolished the inhibition by PACAP of basal acid secretion (Fig. 6).

Reverse-phase HPLC analysis of secretin-like immunoreactivity in portal venous effluent. The results of HPLC analysis clearly indicated that secretin-like immunoreactivity was present in portal venous effluent after infusion of PACAP-27 (10 µg/h). As shown in Fig. 7, secretin-like immunoreactivity in the extract of portal venous effluent was eluted from the HPLC column with a retention time identical to that of synthetic rat secretin. The fractions containing PACAP-27-like immunoreactivity did not exhibit any secretin-like immunoreactivity. This result indicated that elevation of secretin-like immunoreactivity in the portal venous effluent was not due to cross-reaction by PACAP-27.

DISCUSSION

In the present study, we have demonstrated that PACAP-27 dose-dependently inhibited both basal and pentagastrin-stimulated gastric acid secretion in a totally isolated vascularly perfused rat stomach model. These results are consistent with observation of Munigan et al. (24) that PACAP-27 significantly inhibited pentagastrin-induced acid secretion in conscious rats. In their studies, however, basal acid secretion was not affected by PACAP-27 in rats. It is possible that a different experimental design, including animal models and dosages of PACAP, might have influenced their results. For example, 50 nmol·kg⁻¹·h⁻¹ but not 5 nmol·kg⁻¹·h⁻¹ of PACAP-27 markedly inhibited basal acid secretion in their conscious rats with chronic gastric cannula, but not in pylorus-ligated anesthetized rats (23). Because there have been no available data regarding PACAP concentration in the systemic circulation or local tissues, the dosage range of exogenous PACAP that can mimic plasma or local tissue level that would affect physiological functions is yet to be determined.

We found that PACAP-27 significantly increased secretin level in the portal venous effluent, suggesting that PACAP-27 stimulated local release of secretin from the stomach. This observation supports our previous reports that 1) cells containing secretin-like immunoreactivity were found in the mucosa of gastric antrum and fundus (4, 6) and 2) secretin-like immunoreactivity...
as well as bioactivity were found in antral mucosal extracts of rats, dogs, and humans (6). Recently, we confirmed the presence of secretin cells in mucosa of gastric antrum and corpus of the rat. Moreover, secretin mRNA was found in the mucosae of both rat antrum and corpus (5). In addition, a rabbit antisecretin serum partially but significantly blocked the inhibition of gastric acid secretion by PACAP-27. The observation strongly suggested that the inhibitory effect of PACAP-27 be mediated, in part, by a paracrine action of secretin in gastric mucosa.

Like secretin, PACAP is a member of the secretin/glucagon/VIP family of peptides. PACAP and secretin stimulate adenylate cyclase to increase intracellular cAMP level (8, 12). One may question whether the secretin-like immunoreactivity detected in the portal venous effluent might be PACAP because it might cross-react with secretin. However, the result of HPLC clearly indicated that secretin-like immunoreactivity in portal venous effluent was eluted with a retention time the same as that of synthetic secretin but not PACAP-27. Thus secretin was released from the isolated rat stomach after administration of PACAP-27. This result supports our recent work in intact rats in which PACAP stimulates the release of secretin and CCK (13).

It has been demonstrated that somatostatin inhibits gastric acid secretion by suppressing parietal cell secretion (27) and inhibiting gastrin release from G cells in the antrum (34) as well as histamine secretion from ECL cells (28). We observed in the isolated perfused rat stomach model that PACAP-27 increased somatostatin level in portal venous effluent. The suppression by PACAP-27 of pentagastrin-stimulated acid secretion was reversed by >80% after administration of a rabbit antisomatostatin serum, indicating that the inhibition by PACAP-27 of acid secretion was significantly mediated through local release of somatostatin.

Prostaglandins are known to be potent inhibitors of gastric acid secretion (16, 25, 29). In the present study, we found that indomethacin, an inhibitor of prostaglandin synthesis, suppressed the inhibitory effect of PACAP-27 on pentagastrin-induced acid secretion by 43%. PACAP-27 also elevated PGE₂ concentration in portal venous effluent, suggesting that PGE₂ is involved in the inhibition of acid secretion by PACAP-27 in rats. Interestingly, a combination of antisomatostatin and antisecretin serums and indomethacin completely reversed the inhibition by PACAP-27 on pentagastrin-induced acid secretion. These data strongly suggest that the locally released somatostatin, secretin, and prostaglandins play pivotal roles in PACAP-induced inhibition of acid secretion in this isolated vascularly perfused rat stomach model.

We have observed that a rabbit antisecretin serum partially but significantly reduced concentration of somatostatin and PGE₂ in portal venous effluent and partially reversed the PACAP-induced inhibition of acid secretion. It supports our earlier reports in in vivo and in vitro studies (7, 30, 32) that the inhibition of gastric acid secretion by secretin is mediated by simultaneous release of both somatostatin and PGE₂ in rats. However, PACAP-27 also appeared to stimulate directly the release of somatostatin and PGE₂ from the stomach, because the antisecretin serum did not completely reverse the elevated concentrations of somato-
PACAP INHIBITS ACID SECRETION IN RAT STOMACH

statin and PGE2 in portal venous effluent. Mungan et al. (23) reported that PACAP significantly inhibited histamine-stimulated acid secretion in rats. This provided evidence that PACAP may directly affect the action of histamine on parietal cells to suppress acid secretion. Recently, Zeng et al. (36) reported that PACAP stimulates the release of both histamine and somatostatin from cultured ECL cells and D cells in rat stomach. We have also observed in the present study that PACAP-27 stimulated release of histamine, which was further enhanced by antisomatostatin. However, PACAP-27 did not increase acid secretion either at the doses tested or during administration of the antisomatostatin antibody. It is likely that PACAP may stimulate more potently the release of somatostatin from D cells than the release of histamine from ECL cells, and somatostatin not only acts on ECL cells but also on parietal cells to inhibit acid secretion. Thus it may override the stimulatory action of histamine on acid secretion. Interestingly, we failed to find that PACAP-27 stimulates acid secretion when antisomatostatin serum was given. It is possible that PACAP may have a direct effect on parietal cells to suppress acid secretion. This observation is at variance with that of Zeng et al. (37), who reported that PACAP stimulated acid secretion during immunoneutralization with an antisomatostatin antibody in anesthetized rats. However, it should be noted that, in our single-pass isolated vascularly perfused rat stomach, hormones released from the antrum do not modulate gastric acid secretion. Thus, in the intact animal, PACAP may also stimulate secretion of somatostatin from antral D cells and suppress gastric secretion from antral G cells. These effects, together with those observed in the oxyntic gland, will result in inhibition of gastric acid secretion. The release of gastrin is expected to increase on immunoneutralization with an antisomatostatin antibody. The synergistic action between gastrin and histamine on parietal cells (33) apparently was able to override the direct effect of PACAP, thereby resulting in an increase in gastric acid secretion.

Recently Moller et al. (20) reported that PACAP coexisted with CGRP and substance P in the sensory nervous system in rats. Capsaicin treatment reduced the density of PACAP-, CGRP-, and substance P-immunoreactive fibers, suggesting that PACAP is a sensory neuropeptide. A double immunostaining study revealed both PACAP- and CGRP-containing nerve fibers in the stomach of rats. We and others have found that CGRP is capable of inhibiting gastric acid secretion by local release of somatostatin (14, 15). It is possible that PACAP and CGRP may interact to modulate gastric acid secretion.

In conclusion, PACAP-27 is a new inhibitory neuropeptide of gastric acid secretion in rats. The inhibitory mechanism of PACAP-27 on acid secretion is mediated, in part, by local releases of secretin, somatostatin, and PGE2 from the stomach. Furthermore, the action of secretin locally released by PACAP on acid secretion is mediated, in part, via the release of somatostatin and PGE2. Further studies are needed to determine whether the sensory nervous system is also involved in the PACAP-induced suppression of acid secretion.

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