PACAP stimulates gastric acid secretion in the rat by inducing histamine release

ARNE K. SANDVIK,1,2 GUANGLIN CUI,1,2 INGUNN BAKKE,1,2 BJORN MUNKVOLD,1,2 AND HELGE L. WALDUM2

1Department of Physiology and Biomedical Engineering and 2Section of Gastroenterology, Department of Medicine, University Hospital of Trondheim, Faculty of Medicine, The Norwegian University of Science and Technology, N-7489 Trondheim, Norway

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Sandvik, Arne K., Guanglin Cui, Ingunn Bakke, Bjorn Munkvold, and Helge L. Waldum. PACAP stimulates gastric acid secretion in the rat by inducing histamine release. Am J Physiol Gastrointest Liver Physiol 281: G997–G1003, 2001.—Previous studies have shown that pituitary adenylate cyclase-activating peptide (PACAP) stimulates enterochromaffin-like (ECL) cell histamine release, but its role in the regulation of gastric acid secretion is disputed. This work examines the effect of PACAP-38 on aminopyrine uptake in enriched rat parietal cells and on histamine release and acid secretion in the isolated vascularly perfused rat stomach and the role of PACAP in vagally (2-deoxyglucose) stimulated acid secretion in the awake rat. PACAP has no direct effect on the isolated parietal cell as assessed by aminopyrine uptake. PACAP induces a concentration-dependent histamine release and acid secretion in the isolated stomach, and its effect on histamine release is additive to gastrin. The histamine H2 antagonist ranitidine potently inhibits PACAP-stimulated acid secretion without affecting histamine release. Vagally stimulated acid secretion is partially inhibited by a PACAP antagonist. The results from the present study strongly suggest that PACAP plays an important role in the neurohumoral regulation of gastric acid secretion. Its effect seems to be mediated by the release of ECL cell histamine.

PITUITARY ADENYLATE CYCLASE-ACTIVATING polypeptide (PACAP) is a neuropeptide that was first isolated from the ovine hypothalamus by Miyata and et al. (19). It belongs to the secretin/glucagon/vasoactive intestinal polypeptide family of regulatory peptides and has two bioactive forms, with 38 (PACAP-38) and 27 (PACAP-27) amino acid residues (20). It has diverse biological effects and is widely distributed in the enteric nervous system in many species including the rat and man (13). Among many other effects, PACAP stimulates pancreatic exocrine and endocrine secretion (1, 12), releases gastric somatostatin (17), stimulates intestinal fluid secretion (6), and inhibits contraction in gastrointestinal smooth muscle (11). PACAP binds to the vasoactive intestinal peptide type 1 and 2 (VPAC1 and VPAC2, respectively) receptor subtypes (formerly regarded as vasoactive intestinal peptide receptors) but has much higher affinity to a third receptor subtype, the PACAP type 1 (PAC1) receptor (16).

The effect of PACAP on gastric acid secretion is still disputed. The enterochromaffin-like (ECL) cell possesses the PAC1 receptor (30), and several studies show that PACAP potently stimulates histamine release from isolated rat ECL cell preparations (18). The effect of PACAP on histamine release from isolated ECL cells is comparable with that of gastrin (18), and gastrin is generally considered to stimulate the parietal cell via ECL cell histamine (29). PACAP, however, has been reported to inhibit acid secretion when administered to isolated, vascularly perfused rat stomachs (17). Other studies indicate that PACAP administered to intact animals has no effect on basal acid secretion but inhibits acid secretion induced by pentagastrin or histamine (21). Yet another study suggested that PACAP augments histamine and carbachol-induced acid secretion in isolated parietal cells (8), indicating that the parietal cells also possess a PACAP receptor.

The present study was done to reach a more comprehensive understanding of the effect of PACAP on gastric acid secretion and histamine release by using experimental systems of different complexity. The models were isolated and enriched parietal cells, isolated vascularly perfused rat stomachs, and awake, vagally stimulated fistula rats. The aim of the studies with isolated and enriched parietal cells was to examine whether PACAP has a direct effect on the parietal cell alone or works by potentiating the effect of histamine. In the acid-secreting, isolated vascularly perfused rat stomachs, the relations between the different cells in the acid secretory pathways of the oxyntic mucosa are preserved, enabling experiments that elucidate the relations between the different steps in the humoral and paracrine acid secretory pathways. In the awake gastric fistula rat with vagally stimulated acid secretion...
alone, or in combination with the PACAP antagonist PACAP-(6–38), the effect of endogenous PACAP in physiological concentrations may be examined.

MATERIALS AND METHODS

Materials. PACAP-(1–38) (PACAP-38), PACAP-(6–38), and human gastrin-(1–17) (G-17) were obtained from Bachem (Bubenberg, Switzerland). Histamine radioimmunoassay kits were purchased from Immunotech (Marseille, France), dimethylamine-[14C]aminopyrine was from New England Nuclear (Boston, MA), and dextran-T70 was from Pharmacia (Uppsala, Sweden). Immunocytochemical reagents were anti-histidine decarboxylase antibody (no. B260-1) from Eurodiagnostics (Malmö, Sweden) and anti-β-H⁺/K⁺-ATPase antibody (no. MA3-923) from Affinity Bioreagents (Golden, CO). Vectastain peroxidase rabbit ABC kit (PK-4001) and a peroxidase substrate kit (AEC, SK-4200) were both from Vector Laboratories (Burlingame, CA). All other chemicals were of analytical grade and were obtained from Sigma (St. Louis, MO). Male Wistar rats weighing 240–250 g were purchased from Møllegaard Breeding Centre (Skensved, Denmark).

Animals. The animal experiments were approved by the Animal Welfare Committee at the University Hospital of Trondheim. The rats were housed in wire-mesh cages at 24°C with a 12:12-h light-dark cycle and free access to tap water and a commercial rat diet. The animals were anesthetized before all surgical procedures with 0.2 ml/100 g body wt of a composition of (per ml) 2.5 mg fluanison, 0.05 mg fentanyl, and 1.25 mg midazolam.

Protocol-dispersed gastric parietal cells. Gastric mucosal cells were dispersed using pronase, and parietal cells were enriched by elutriation in a Beckman J-6M/E centrifuge with a JE-6B rotor (3). The purity of the cell preparations was estimated by differential counting of stained (H⁺/K⁺-ATPase antibody for parietal cells and histidine decarboxylase antibody for ECL cells) cell smears, as previously described (3). Total histamine content in the elutriated fractions was examined by boiling of cells for 15 min in distilled water and measurement of histamine in the supernatant. Parietal cell acid secretion was assessed using the aminopyrine accumulation method as described in detail previously (5). Aminopyrine accumulation studies were done in the basal state and during stimulation with the phosphodiesterase inhibitor isobutyl methylxanthine (IBMX) alone (100 μM) or combined with histamine in 500 μM or 750 μM (maximally effective in pilot studies) concentrations. The effect of PACAP-38 was studied in 100 μM, 1 nM, 10 nM, 100 nM, or 1 μM concentrations; PACAP was administered either with IBMX only or with IBMX and 500 or 750 μM histamine. Five or six cell preparations were studied for each stimulant or combination of stimulants.

Protocol-isolated vascularly perfused stomachs. After a 36-h fast, isolated vascularly perfused rat stomachs were prepared as previously described (14), and the stomachs were removed from the animal and transferred to an organ bath filled with Krebs-Ringer buffer. The vascular bed was perfused through a catheter in the aorta with 2 ml/min of a Krebs-Ringer buffer with an ionized calcium concentration of 1.12 mM (at pH 7.25), dextran-T70 at 40 g/l as colloid, glucose at 5 mM, and pyruvate at 5 mM. BSA was added in a 0.1% (wt/vol) concentration to prevent adhesion of peptide to the polypropylene arterial line. Washed ovine erythrocytes were added to 10% (vol/vol) final concentration. The vascular perfusate was gassed with O₂ (96%) and CO₂ (4%) using a membrane oxygenator. The vascular effluent was not recirculated, thus excluding any effect of endogenous gastrin on the oxyntic mucosa. The gastric lumen was perfused with distilled water titrated to pH 7.0 with NaOH and gassed with 100% O₂. All perfusates and the organ bath were kept at 37°C. The stomach perfusions started with a 20-min stabilization period, and 50 μM IBMX was added at 20 min with PACAP-38 and/or G-17 or ranitidine added at 40 min. The experiments included one group receiving IBMX only (basal), one group receiving IBMX and 520 pM G-17 (which induces maximal gastrin-stimulated histamine release and acid secretion in this model), and five groups receiving IBMX and PACAP-38 in 10 pM, 100 pM, 1 nM, 10 nM, and 100 nM concentrations, respectively. One group received 520 pM G-17 combined with the maximally effective PACAP-38 concentration of 10 nM, and one group received 10 nM PACAP and 2 μM ranitidine (found in pilot experiments to be the maximally effective concentration for suppressing gastrin-induced acid secretion). The number of stomachs studied was six or seven for each group. The venous and luminal effluents were collected in 10-min batches throughout the 100-min perfusion period. In one subgroup, the immediate histamine response to 520 pM G-17 and 10 nM PACAP-38 was studied by collection of the venous effluent in 1-min periods immediately before and after administration of the peptides. The venous effluent was collected on ice and immediately centrifugated and stored at −20°C until analysis for histamine. Acid output was measured by titration with 1 mM NaOH. Histamine analysis was done with a previously evaluated histamine radioimmunoassay kit that is very specific, with a sensitivity for histamine of 0.5 nM (25) and a coefficient of variation of 6.4%.

Protocol-intact animals. In four groups of five rats each, the animals were fitted with stainless steel gastric fistulas with the use of standard surgical techniques. One week later, a jugular vein catheter was inserted. After the second operation, the animals were fasted with free access to tap water for 24 h before the start of experiments. The secretion studies were done with the animals in Bolllmann-type cages. The stomach was rinsed with normal saline through the fistula, and gastric juice was collected by passive drainage. After an initial 30-min washout period, gastric secretions were collected for 30 min and then for six 15-min periods during which the animals received one of the following treatments: 1) a bolus injection (1 ml) followed by intravenous infusion (1 ml/h) of saline with 0.1% BSA (control); 2) a bolus injection (1 ml) followed by intravenous infusion (1 ml/h) of saline with 0.1% BSA and, after 60 min, PACAP-(6–38) in a bolus dose of 20 nmol/kg followed by continuous infusion with 20 nmol/kg h; 3) a bolus dose of 2-deoxyglucose (2-DG, 100 mg/kg) followed by infusion with 2-DG at 100 mg/kg h; and 4) a bolus dose of 2-DG followed by infusion of 2-DG as described above for 60 min, after which PACAP-(6–38) was administered in a bolus dose of 20 nmol/kg followed by continuous infusion with 20 nmol/kg h combined with 2-DG. Acid output was measured by titration with 1 mM NaOH.

Analyses. Differences between groups were evaluated using analysis of variance with the Dunnett or Bernoulli posttests as appropriate. A P value < 0.05 was considered significant.

RESULTS

Aminopyrine uptake in enriched parietal cells. Parietal cells were enriched from dispersed mucosal cells to 72% purity. Total histamine content in the parietal cell fractions after cellular lysis never exceeded 72 nM when distributed to the parietal cell incubation vol-

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ume, and the number of ECL cells in the parietal cell fractions varied from 0.5 to 1.7%. Histamine (500 μM) with IBMX increased aminopyrine uptake significantly. When basal aminopyrine uptake was set to 100%, the uptake with 500 μM histamine was 541 ± 4% and with 750 μM histamine 704 ± 80% (both P < 0.01, means ± SE). PACAP in the 100 pM to 1 μM concentration range, alone or with or without IBMX, had no effect on aminopyrine uptake. PACAP administered with 500 μM histamine and IBMX significantly increased aminopyrine uptake from 541 ± 17% (histamine and IBMX alone) to a maximum of 996 ± 101% with 1 nM PACAP (Fig. 1A). When coadministered with 750 μM histamine, on the other hand, PACAP did not augment the effect of histamine (Fig. 1B).

Histamine release in isolated rat stomachs. PACAP (0.01–100 nM) induced a concentration-dependent increase in histamine output from baseline 3.1 ± 0.6 nmol/60 min (mean ± SE) to a maximum of 20.9 ± 4.2 nmol/60 min as shown in Fig. 2. When we combined the maximally effective concentration of G-17 (520 pM) with 10 nM PACAP (this concentration was chosen, since it gave maximal acid secretion), histamine output was 45.8 ± 8.8 nmol/60 min, which is significantly (P < 0.01) higher than with PACAP alone (18.1 ± 2.9 nmol/60 min). Although the combined stimulation showed a trend toward increased histamine output compared with G-17 alone, this difference was not statistically significant (Fig. 3). Ranitidine at 2 μM did not significantly change histamine output stimulated by 10 nM PACAP (24.8 ± 3.7 vs. 18.1 ± 2.9 nmol/60 min, respectively). The studies on the immediate effect of PACAP and gastrin on histamine release show that both peptides induce an instantaneous increase in venous histamine output (Fig. 4); gastrin increased venous histamine concentration from 69.3 ± 16.3 to 751.3 ± 158.7 and PACAP from 74.7 ± 14.6 to 724.0 ± 209.8 nM (means ± SE).

Acid output in isolated rat stomachs. PACAP (0.01–100 nM) induced a dose-dependent increase in acid output from baseline 4.7 ± 0.9 μmol/60 min (mean ± SE) to a maximum of 65.8 ± 11.3 μmol/60 min with 10 nM PACAP, as shown in Fig. 5. A trend toward in-

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**Fig. 1.** A: accumulation of [14C]aminopyrine in enriched parietal cells stimulated with histamine alone (500 μM) or histamine combined with pituitary adenylate cyclase-activating peptide (PACAP). Results are expressed as means ± SE in percent basal accumulation. Background stimulation with 100 μM isobutyl methylxanthine (IBMX) was given in all incubations. *Significance of differences from histamine alone (P < 0.05). B: accumulation of [14C]aminopyrine in enriched parietal cells stimulated with histamine alone (750 μM) or histamine combined with PACAP. Results are expressed as means ± SE in percent basal accumulation. Background stimulation with 100 μM IBMX was given in all incubations. PACAP did not significantly augment histamine-stimulated [14C]aminopyrine uptake.

**Fig. 2.** Venous histamine output from isolated rat stomachs in response to increasing concentrations PACAP-38. Results are given as means ± SE (n = 6 or 7). **Significance of differences from basal (P < 0.01).

**Fig. 3.** Venous histamine output from isolated rat stomachs in response to 10 nM PACAP-38 or 520 pM gastrin, alone or in combination. Results are given as means ± SE (n = 6). Significance of differences from basal (##P < 0.01) and between stimulants (##P < 0.01) is indicated.
creased secretion was found with the 0.1 nM concentration of PACAP, and acid output was significantly increased from 1 nM PACAP. Combining G-17 (520 pM) with 10 nM PACAP (this concentration was chosen, since it gave maximal acid secretion) augmented acid secretion compared with PACAP alone, but the difference was not statistically significant (Fig. 6). Ranitidine (2 mM) decreased the acid response to PACAP (10 nM) to 14.5 ± 4.8 μmol/60 min (P < 0.01), which was not significantly different from baseline (Fig. 7).

Acid output in fistula rats. The baseline, 2-DG, and 2-DG/PACAP-(6–38) acid output time courses are shown in Fig. 8. Baseline acid output was unchanged during the study period, varying from 25.0 ± 1.8 to 28.8 ± 0.7 μmol/15 min (means ± SE). With PACAP-(6–38) alone, acid output varied from 28.7 ± 6.6 to 20.9 ± 2.3 mmol/15 min (not significantly different from baseline during any collection period). In both 2-DG groups, acid output increased significantly and to a similar degree. Moreover, PACAP-(6–38) attenuated 2-DG-stimulated acid secretion. In the final fraction (90–105 min), the acid output was 40.2 ± 8.8 μmol/15 min in the 2-DG/PACAP-(6–38) group, which was 44% inhibited (P < 0.05) compared with that in the 2-DG-alone group (72.0 ± 7.6 μmol/15 min).

**DISCUSSION**

Our understanding of the physiological role of the neurohumoral system in the regulation of gastric acid secretion is incomplete. The vagus nerve clearly influences ECL cell function (7, 23), whereas acetylcholine plays only a minor, if any, role in the stimulation of the ECL cell (18, 23). On the other hand, PACAP-immunoreactive nerve fibers are abundant in the gastric mucosa of both rat and humans (13), the ECL cell possesses PAC1 receptors (30), and PACAP potently induces histamine release from this cell (18). At least in the rat, the potent acid secretagogue effect of gastrin may be fully explained by ECL cell histamine release. It is quite unexpected that PACAP, which induces a histamine release of the same magnitude as gastrin, should act as an inhibitor of acid secretion (17, 21).

Several groups have already shown that PACAP acts directly on ECL cells via the PAC1 receptor, and that PACAP potently stimulates histamine release from those cells (18). This effect of PACAP is not disputed. However, because the existing data on PACAP and acid secretion are more conflicting, we studied the...
direct effect of this peptide on isolated oxyntic mucosal cells enriched in parietal cells. With 500 μM histamine, PACAP slightly but significantly augmented histamine-stimulated aminopyrine accumulation. With 750 μM histamine, on the other hand, addition of PACAP had no significant effect on histamine-stimulated aminopyrine accumulation. The latter concentration of histamine was maximally effective. Thus, even if the number of contaminating ECL cells in the parietal cell fractions was low, it seems that those cells release enough histamine to stimulate acid secretion. This is in accordance with previous studies from our group (5) that showed that histamine released from contaminating ECL cells by gastrin may induce aminopyrine uptake in parietal cells. Furthermore, in a recent study, Athmann et al. (2) found that PACAP evoked an H2 receptor antagonist-sensitive increase in intracellular calcium ion concentration in adjacent parietal cells (2). This indicates that also in isolated parietal cell-enriched gastric mucosal preparations, PACAP acts solely on the ECL cell. Thus our studies cannot confirm previous observations (8) that PACAP has a direct effect on the parietal cell.

The results from this study also show that PACAP-38 stimulates histamine release and acid secretion in the isolated, vascularly perfused rat stomach in the dose range from 0.1 to 100 nM. The vascular perfusate is not recirculated in this experimental model, excluding the possibility that endogenous gastrin acts on the ECL cell. Ranitidine significantly attenuated the acid secretory response to PACAP, suggesting that histamine has a central role in the acid secretagogue effect of PACAP. Moreover, combining gastrin with PACAP gave only a slight, nonsignificant increase in gastrin-stimulated acid output. This indicates that PACAP and gastrin mainly act via the same acid stimulatory pathway, i.e., the release of ECL cell histamine that secondarily stimulates the parietal cell. Taken together with previous studies, these results show that neurohumoral stimulation of acid secretion acts via a direct muscarinic effect on the parietal cell (15, 24) and by an indirect mechanism via histamine release.

It has recently been reported that PACAP inhibits basal and pentagastrin-stimulated acid secretion in the isolated vascularly perfused rat stomach (17); and, although measured for only one concentration of PACAP-27, this peptide also increased venous histamine output significantly. The experiments on isolated rat stomachs described by Li et al. (17) can be directly compared with those presented here. When recalculating the concentrations of PACAP given by Li et al. to molar, the concentration range is 19–190 nM, whereas the results we report here concern the 10 pM to 100 nM concentration range, with significant stimulation of histamine and acid outputs from 1 nM and higher concentrations. For the highest dose given in our study, there is a tendency (nonsignificant) toward attenuated acid secretion. It is possible that high doses of PACAP activate acid inhibitory pathways like, for instance, as Li et al. suggest, PACAP-induced somatostatin release. The discrepancy between the results in the two studies is, however, too marked to be explained by a difference in the dose range only. It is possible that the complexity of this regulatory system is such that minor differences in experimental design may induce markedly different results in the end-point parameter of acid secretion. An alternative explanation is that PACAP-27 as used by Li et al. has effects different from PACAP-38 on the processes studied here. However, this seems unlikely, since the two forms of PACAP have very similar actions in all systems studied until now (18, 22).

Because PACAP is a neuropeptide, the physiological concentration of this substance on its target cell(s) is unknown. The problem of finding a physiological concentration-response range for such peptides in isolated cells or stomachs or by exogenous administration to whole animals prompted us to study the effect of endogenous PACAP in the regulation of gastric acid secretion in fistula rats with 2-DG-induced acid secretion. The gastric secretagogue effect of 2-DG is considered to be vagally mediated (9). In these studies, 2-DG clearly stimulated acid secretion, and this effect was partially inhibited by the PAC1 receptor antagonist PACAP-(6–38). To our knowledge, these results are the first suggesting that endogenous PACAP takes part in vagally stimulated acid secretion. The observation that the PACAP antagonist only partially inhibited 2-DG-stimulated acid secretion probably reflects that other signal substances are involved in the vagal stimulation of acid secretion as well. Acetylcholine is an established direct stimulant of the parietal cell (10, 26), and ECL cell histamine release is indisputably induced via the PAC1 receptor (30), although the direct effect of acetylcholine on the ECL cell is more questionable (18). Furthermore, numerous previous studies show that atropine completely abolishes 2-DG-stimu-
lated acid secretion (4, 9). These observations may be explained by atropine blocking either the release of PACAP from postganglionic enteric neurons and/or the potentiating effect on the parietal cell of acetylcholine and histamine (27, 28). The precise nature of this permissive cholinergic pathway for parietal cell function remains to be fully explored. Another question is whether PACAP-(6–38) could act as a partial agonist on the receptor at the doses used. Our studies infusing the antagonist alone showed no effect on baseline acid output in the fistula rats. Thus there is no reason to believe that there are significant effects other than PAC₁ antagonism relevant to the interpretation of those studies.

Previous studies have shown that PACAP infused intravenously inhibits pentagastrin- and histamine-stimulated acid secretion in the fistula rat (21). This effect was, however, observed only at the highest dose of PACAP given, and the lower doses showed either no effect or a slight (although nonsignificant) increase in acid output. The discrepancy between the results obtained by Mungan et al. (21) and our findings may be explained by PACAP-induced somatostatin release (17). Because somatostatin inhibits acid secretion via several different mechanisms, a biphasic dose response can be expected. Because the present results using 2-DG to stimulate acid secretion reflect the effect of endogenous PACAP, we feel that they represent a more physiological situation and strongly indicate that PACAP acts as a gastric acid secretagogue in vivo.

In conclusion, the present study shows that endogenous PACAP partially mediates vagally stimulated gastric acid secretion and that PACAP acts via the release of ECL cell histamine.

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