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

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Sandvik, Arne K., Guanglin Cui, Ing Gunn Bakke, Bjørn 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.

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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 combination 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 pM, 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 collected, thus excluding any effect of endogenous gastrin on the gastric 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 nM 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 hatches 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 Bellmann-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 nmol NaOH.

Analyses. Differences between groups were evaluated using analysis of variance with the Dunnett or Bonferroni 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-
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 ± 17% 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-

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 mmol/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 mmol/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 mmol/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 mmol/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
PACAP antagonist (P)**
cells enriched in parietal cells. With 500
before administration of 2-DG. Differences from basal (*P
15-min periods, and time point 0 reflects 15-min basal secretion
before administration of 2-DG. Differences from basal (*P < 0.05 or
**P < 0.01) and between 2-DG alone and 2-DG in combination with
PACAP antagonist (#P < 0.05) are indicated.

Fig. 8. Acid output from awake gastric fistula rats (n = 5 in each
group) in response to 2-deoxyglucose (2-DG) alone or combined with
PACAP antagonist (PACAP-(6–38)). Acid output was measured in
5 min in each group) in response to 2-DG-stimulated acid secretion in
animals, excluding the possibility that endogenous gastrin may induce aminopyrine up-
take 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-en-
riched 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 se-
cretion 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 gas-
trin acts on the ECL cell. Ranitidine significantly at-
tenuated the acid secretory response to PACAP, sug-
gest 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 indi-
cates 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 his-
tamine output significantly. The experiments on isolated rat stomachs described by Li et al. (17) can be directly
compared with those presented here. When recalcul-
ing 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
centration range, with significant stimulation of histamine and acid outputs from 1 nM and higher
centration. For the highest dose given in our study, there is a tendency (nonsignificant) toward at-
tenuated acid secretion. It is possible that high doses of
PACAP activate acid inhibitory pathways like, for in-
stance, as Li et al. suggest, PACAP-induced somatostat-
in 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 con-
centration-response range for such peptides in isolated
cells or stomachs or by exogenous administration to
whole animals prompted us to study the effect of en-
dogenous PACAP in the regulation of gastric acid se-
cretion in fistula rats with 2-DG-induced acid secre-
tion. 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 antago-
nist PACAP-(6–38). To our knowledge, these results
are the first suggesting that endogenous PACAP takes
part in vagally stimulated acid secretion. The observa-
tion that the PACAP antagonist only partially inhib-
ited 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 ques-
tionable (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 PAC_{1} 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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