REGULATION BY VASOACTIVE INTESTINAL PEPTIDE OF CYCLIC AMP ACCUMULATION IN GASTRIC EPITHELIAL GLANDS

A characteristic of human stomach

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Received 19 February 1980

1. Introduction

Cyclic AMP production in gastric epithelium is regulated by numerous substances. In the different species studied, histamine stimulated the production of cyclic AMP only in the fundic part of gastric epithelium ([1,2], C.G. et al., in preparation). This effect was shown to be related to the regulation by histamine of the acid secretion by parietal cells [3,4]. In the other cells of the stomach, namely the pepsinogen and mucous-secreting cells, which occur both in fundus and in antrum [5], secretin was described as the sole peptide stimulating cyclic AMP production at low physiological doses ([4], C.G. et al., in preparation). When a stimulating effect of vasoactive intestinal peptide (VIP) was found, concentrations of VIP 200-times higher were needed to exert the same effect as secretin (C.G. et al., in preparation), strongly suggesting that VIP, a natural analog of secretin was a positive agonist of this peptide.

This study was carried out in humans, using an original preparation of pure gastric epithelial glands. We demonstrate that in the human, the peptide stimulating at physiological doses the cyclic AMP system of gastric epithelium is not secretin but VIP, that acted at as low as 10^{-11} M both in fundus and in antrum epithelial glands.

2. Materials and methods

2.1. *Preparation of human gastric epithelial glands*

Sections (10 cm^2) of the healthy fundic and antral parts of the stomach were obtained during subtotal gastrectomy for cancer. It was verified that none of the patients underwent any treatment by histamine H_2 receptor blocking agent. The fragment was sewed in order to make a sac exposing mucosa. Mucous was removed with absorbant paper. Epithelial cells were isolated and harvested using an EDTA procedure, as described for the isolation of human colonic epithelial crypts [6]. Glands were rinsed and resuspended in Krebs-Ringer phosphate (pH 7.5). Microscope examination showed entire gastric epithelial glands, keeping their structure after separation from the *lamina propria*. Single cells were scarce. We verified that the preparation was not contaminated by cells from blood or from the connective tissue of the submucosal layer. DNA [7] and proteins [8] were measured: 1 mg DNA corresponding to 56.26 ± 13.95 mg protein (n = 10).

2.2. *Extraction and measurement of VIP*

Extraction and assay of VIP from the cellular preparation and from the whole thickness fragments of stomach were done as in [6,9].

2.3. *Measurement of cyclic AMP accumulation*

Unless otherwise mentioned, in a standard assay, cells (~2–4 μg DNA/ml) were incubated under continuous agitation at 150 osc./min in a shaking bath (SB_4, Techne, Duxford, Cambridge) for 30 min at 37°C in 0.5 ml Krebs-Ringer phosphate, containing 1.4% bovine serum albumin (w/v), 0.5 mM 3-isobutyl-1-methylxanthine (iBuMeXan) (pH 7.5). Cells were preincubated for 10 min before addition of effectors. The incubation was stopped by adding 50 μl 11 N $HClO_4$. After centrifugation for 10 min at 4000 × g

the cyclic AMP present in the supernatant was succinylated and measured by radioimmunoassay as in [6].

2.4. *Peptides and reagents*

Highly purified porcine VIP was generously supplied by V. Mutt (GIH Laboratory, Stockholm) through the Gastrointestinal Hormones Resources Committee of the National Institute of Arthritis, Metabolism and Digestive Diseases (USA) and synthetic porcine secretin by E. Wünsch (Max Planck Institute, Munich). Porcine pancreatic glucagon was purchased from Novo Research Inst. (Copenhagen), bovine serum albumin (fraction V) from Miles Labs. (Elkhart, IN), calf thymus DNA from Sigma Chemical Co. (St Louis, MO). Other chemicals and biochemicals, all of highest purity grade, were purchased from standard suppliers.

3. Results

3.1. *VIP concentrations in human stomach*

VIP concentrations in fragments of human gastric fundus and antrum amounted to 607 ± 214 ng/g tissue wet wt (mean ± SEM, $n = 3$), and compared well with those in [10]. No VIP was found in gastric epithelial glands indicating that the VIP measured in gastric fragments was confined to the submucosal and the muscular layers of the stomach.

3.2. *Stimulation of cyclic AMP production in human gastric epithelial glands by peptides and histamine*

VIP (10^{-8} M) increased cyclic AMP levels in all conditions tested (fig.1). In the absence of phosphodiesterase inhibitor, the maximal rise in a preparation of fundus epithelial glands represented 2 ± 0.02-times the basal level ($p < 0.05$) and was followed by a gradual decrease. In the presence of phosphodiesterase inhibitor, the effect of VIP was potentiated and remained stable from 30–90 min incubation. The % of increase above basal of the VIP-induced cyclic AMP rise was maximal in the presence of 0.5 mM iBuMeXan (fig.1).

The effect of different concentrations of VIP in promoting a cyclic AMP rise in fundus and antrum gastric epithelial glands was studied in optimal conditions (fig.2). VIP induced a significant cyclic AMP rise at as low as 10^{-11} M ($p < 0.05$ and $p < 0.01$ in fundus and antrum, respectively); maximal stimula-

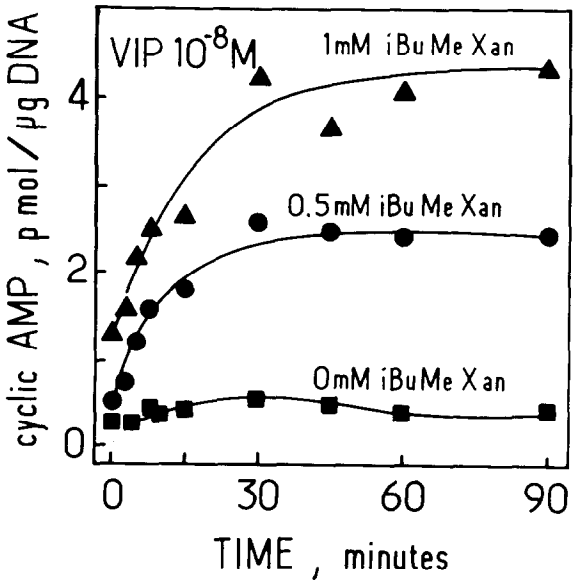

Fig.1. Stimulation by VIP of cyclic AMP accumulation in epithelial glands isolated from human gastric fundus. Epithelial glands (3 µg DNA/ml) were incubated at 37°C in the presence of the indicated concentrations of phosphodiesterase inhibitor iBuMeXan as otherwise in section 2. Reaction was started after a 10 min preincubation by addition of VIP and was stopped at the indicated time by addition of 11 N $HClO_4$. Values are mean of a single experiment performed in triplicate, 2 other experiments gave similar results.

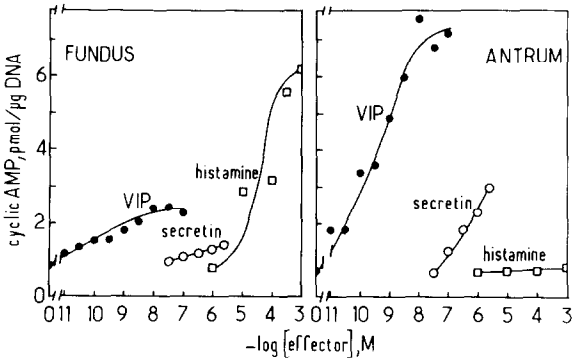

Fig.2. Cyclic AMP production in epithelial glands isolated from fundus and antrum from the same human stomach in response to increasing concentrations of VIP, secretin and histamine. Cells (2.3 µg DNA/ml) were incubated 30 min at 37°C in the presence of 0.5 mM iBuMeXan. Reaction was started after a 10 min preincubation by addition of the effector and was stopped by addition of 11 N $HClO_4$. The experiment was performed in triplicate, 2 other experiments gave similar results.

Table 1
Cyclic AMP production in human gastric epithelial crypts in response to VIP and histamine

	Cyclic AMP production (% basal)	
	Fundus	Antrum
Basal	100	100
VIP 10^{-8} M	450 ± 140	1770 ± 550[a]
Histamine 10^{-3} M	560 ± 140	123 ± 13[a]
VIP 10^{-8} M + histamine 10^{-3} M	1125 ± 190	1750 ± 460

[a] $p < 0.05$ for difference between results observed in fundus and antrum

Values are means ± SEM of values obtained in 5 different stomachs. Each experiment was done in triplicate. Basal level of cyclic AMP was 1.36 ± 0.24 and 1.26 ± 0.36 pmol cyclic AMP/μg DNA in fundus and antrum, respectively

tion of cyclic AMP production was observed at 10^{-8} M VIP, half-maximal stimulation being observed at 3×10^{-10} M VIP. As indicated in table 1, the % of increase above basal of the VIP-induced cyclic AMP rise was significantly lower in fundus where parietal cells account for 30% of the total cell population [11] otherwise composed of mucous cells and chief cells, than in antrum composed mainly of mucous and pepsinogen-containing cells [5].

Secretin was also able to stimulate cyclic AMP formation in human gastric epithelium (fig.2). However, the potency of secretin remained 10^4-times lower than that of VIP since a significant cyclic AMP rise was observed only at 3×10^{-7} M and 10^{-7} M in fundic and antral epithelial glands, respectively ($p < 0.05$). Addition of 10^{-7} M VIP together with 5×10^{-6} M secretin elicited no additive effect (not shown)

Histamine determined a cyclic AMP rise only in epithelial glands isolated from fundus (fig.2), being ineffective in antrum. These data, in agreement with observations of adenylate cyclase activities in human biopsy specimen [2,12,13], indicate that the histamine-sensitive cyclic AMP system is present only in the fundic part of the human stomach, where parietal cells are localized. Incubation of maximally active concentrations of VIP (10^{-8} M) together with histamine (10^{-3} M) showed that their effect on cyclic AMP accumulation in human fundic epithelial glands were additive (table 1).

4. Discussion

Our data demonstrate that, in human, gastric epithelial glands isolated from fundus and antrum express a cyclic AMP system highly sensitive to VIP. Indeed, VIP was used in a range of concentrations (10^{-11}–10^{-8} M) that was 10^4-times lower than that reported to activate adenylate cyclase activity in homogenates of human gastric mucosa [14]. Secretin was considerably less potent than VIP in stimulating cyclic AMP production in human gastric epithelial glands and presumably acted only through the VIP-sensitive cyclic AMP system as shown by the absence of additive effect of VIP and secretin.

Comparison of the effects of VIP and secretin on cyclic AMP accumulation in gastric epithelium evidences a narrow species specificity: in epithelial cells isolated from dog fundus and in epithelial glands prepared from rat fundus and antrum, secretin stimulated cyclic AMP production at 10^{-9}–10^{-10} M; furthermore concentrations of VIP 200-times higher than those of secretin were needed to achieve the same stimulating effect on cyclic AMP production in rat gastric epithelial glands (C.G. et al., in preparation). Thus, the sensitivity of cyclic AMP production in gastric epithelium to secretin and VIP differs in man with that observed in dog and rat.

It must be stressed that the VIP-sensitive cyclic AMP system of human gastric epithelium as well as the secretin-sensitive system of the other mammals studied [3,4] do not involve the parietal cell population, as suggested by:

(1) The occurrence of a VIP-induced stimulation in fundus as well as in antrum;
(2) The lower magnitude of the VIP-induced stimulation in fundus;
(3) The additivity of the effects of histamine and

VIP in stimulating cyclic AMP production in fundus epithelial glands.

Therefore, the VIP-sensitive cyclic AMP system is likely to operate in human mainly in pepsinogen-secreting cells and/or mucous cells as does secretin in dog [4] and rat (C.G. et al., in preparation).

The sensitivity to VIP of the cyclic AMP production in epithelial glands together with the important amounts of VIP present in the gastric wall strongly suggest that, in human, VIP plays a physiological role in regulating the function of gastric epithelium.

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