Histamine interaction on surface recognition sites of H_2-type in parietal and non-parietal cells isolated from the guinea pig stomach

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Received 1 October 1982

In gastric cells isolated by pronase digestion from the guinea pig, histamine stimulated cAMP production in 3 fundic cell fractions ($EC_{50} = 1.6–2 \times 10^{-4}$ M) enriched in parietal (94%), peptic (63%) and mucous cells (87%) as well as in antral cells ($EC_{50} = 4 \times 10^{-4}$ M) that are devoid of parietal cells. Histamine stimulations were completely inhibited by the H_2 antagonist cimetidine ($K_i = 0.27–0.57 \times 10^{-6}$ M) or by the H_1 antagonist diphenhydramine, but at 100-times lower potency ($K_i = 22–45.7 \times 10^{-6}$ M), indicating the presence of histamine H_2 receptors in parietal and nonparietal cells of the guinea pig gastric mucosa.

Histamine *Fundic, antral cell* *H_2 receptor* *Cyclic AMP* *Gastric secretion*

1. INTRODUCTION

In vivo, histamine regulates endocrine [1] and exocrine secretions of acid and pepsin [2,3] by the stomach. In gastric mucosa, histamine is stored in mast cells as well as in endocrine-like cells [4] and could have an endocrine–paracrine role on these gastric secretions. We have demonstrated that histamine interacts with typical H_2 receptors mediating cellular cAMP production, adenylate cyclase [5] and cAMP-dependent protein kinase [6] activation in gastric glands isolated from the guinea pig fundus or antrum. Since it is now well accepted that histamine stimulates gastric acid secretion through H_2 receptors linked to cAMP generation in parietal cells [7], we have suggested [6] that gastric cells other than parietal cells bear an histamine-sensitive cAMP system. In order to explore this possibility, we have studied the effect of histamine and its H_1 or H_2 antagonists on cAMP levels in antral and in fundic cell fractions [8] enriched in parietal (94%), peptic (63%) and mucous cells (87%). Gastric cells isolated by the same method from the antral mucosa (devoid of parietal cells) were also analyzed as control, without further separation.

Here, we show that not only parietal cells, but also non-parietal cells isolated from the guniea pig gastric mucosa (peptic and mucous cells from the fundus) as well as antral cells bear histamine H_2 receptors linked to the cAMP generating system. A preliminary description of these experiments has been presented in abstract form [9].

2. MATERIALS AND METHODS

2.1. *Materials*

Histamine dihydrochloride, diphenhydramine (DPH), cAMP and 3-isobutyl-1-methylxanthine (IBMX) were from Sigma Chemicals (St Louis MO); and carrier-free $Na^{125}I$, IMS 300, from the Radiochemical Center (Amersham). Cimetidine was a generous gift from Dr Brimblecombe of Smith, Kline and French Laboratories, Ltd (Welwyn Garden City, Hertfordshire). Highly purified natural porcine VIP (lot 79.4.25) was obtained from Professor V. Mutt (GIH Laboratory, Stockholm) and synthetic ovine cyclic somatosta-

Published by Elsevier Biomedical Press
001457593/82/0000—0000/$2.75

tin-14 (lot B 00926) was purchased from Beckman (Switzerland). All other chemicals were of analytical grade.

2.2. *Isolation and separation of epithelial cells from guinea pig stomach*

Male Hartley Dunkin guinea pigs (300–450 g), maintained on *ad libitum* diet during the week preceding the experiments were killed by cervical dislocation and exsanguination. Isolated epithelial cells were prepared as in [8]. Cells were dispersed by incubation at 37°C, under gassing with 95% O_2–5% CO_2 in a solution of 0.15% pronase in Krebs Ringer bicarbonate buffer (pH 7.5) for 60 or 90 min to obtain the isolated fundic (fraction F) or antral (fraction A) cells, respectively. Fraction F was further separated by velocity sedimentation at unit gravity in a solution of Ficoll 70 (Pharmacia, Uppsala) in Krebs Ringer bicarbonate buffer containing 0.03% bovine serum albumin. A linear 1–3% Ficol 70 gradient was formed under the cell layer, and the cells were sedimented during 1 h at 4°C. Then, 10 ml samples were collected from the chamber of sedimentation and pooled in 3 fractions according to the repartition of cell diameters [8]. Fraction I contained 87% mucous-secreting cells (11.2 ± 1.6 μm), fraction II contained 63% chief cells (15.8 ± 2.2 μm) and fraction III contained 94% parietal cells (22.0 ± 2.8 μm). The samples from the fundic mucosa as well as fraction A were centrifuged at $200 \times g$ for 2 min and cell pellets were resuspended and washed twice in Krebs Ringer phosphate buffer (pH 7.5) to a final concentration of $0.3–0.5 \times 10^6$ cells/ml (fraction III) or $1–1.5 \times 10^6$ cells/ml (fraction A, I and II). About 96% of cells excluded trypan blue and their ATP content, measured by bioluminescence, remained stable over a 3 h period when incubated under the conditions described for cAMP analysis (vide infra). Cell protein concentration was measured as in [10] using bovine serum albumin as standard.

2.3. *Morphological studies on guinea pig gastric cells*

For electron microscopy, cells were fixed and processed as in [8]. Semi-thin sections were cut from Epon blocks and stained with toluidine blue and examined by light microscopy. Under our experimental conditions, no parietal cells were found in the preparations of isolated antral cells.

2.4. *Cyclic AMP analysis*

The incubation of gastric cells from the guinea pig fundus or antrum was performed in 0.5 ml Krebs Ringer phosphate buffer (pH 7.5), containing 1% bovine serum albumin and 0.5 mM IBMX, a competitive inhibitor of the cAMP-phosphodiesterase activity. In a standard assay, cell suspensions were incubated (20°C, 1 h) with continuous gentle agitation. After 10 min preincubation, the reaction was initiated by the addition of 100 μl reagents. The incubation was stopped at the time indicated by the addition of 50 μl 11 N $NClO_4$. Cyclic AMP production was determined by radioimmunoassay [11], by the use of ^{125}I-tyrosyl–succinyl–cyclic AMP and the antibody 301-8, as in [12,13]. All determinations were performed in duplicate or triplicate.

2.5. *Processing of the data and statistical analysis*

The data were normalized as the percentage of the response to a given concentration of stimulant, at a given incubation time. These calculations accounted for the variability of the cAMP response in the different cellular preparations and therefore permitted the comparison between individual experiments. Absolute values are given as pmol cAMP produced/10^6 cells. The apparent EC_{50} and IC_{50} values were the doses required to produce, respectively, 50% of the maximal stimulation or inhibition produced by the test agents. Antagonism by H_1 and H_2 antihistamines was analyzed assuming competitive inhibition [14–16], according to the equation in [17]

$$K_i = IC_{50}/(1 + S/EC_{50})$$

where:

K_i = the inhibition constant of antagonist;

S = the histamine concentration

Results were analyzed by standard methods using Student's paired *t*-test.

3. RESULTS

3.1. *Effects of time and histamine on cAMP levels in fundic and antral cells*

In the 4 cellular fractions incubated 1 h at 20°C in the presence of 0.5 mM IBMX, basal cAMP levels ranged from 4.45 ± 1.13–6.73 ± 2.3 pmol

Table 1

Basal and histamine-stimulated cAMP levels in cell fractions separated from the guinea pig fundus or antrum

Cell fractions	Major cell type (%)	cAMP (pmol/10^6 cells)			Parietal cells (%)	Protein (μg/10^6 cells)
		Basal	Histamine	% of maximum		
III	Parietal (94%)	5.5 ± 0.5	457 ± 51	100%	94	700 ± 27
II	Peptic (63%)	5.2 ± 0.3	124 ± 21	27 ± 4%	16	254 ± 11
I	Mucous (87%)	4.45 ± 0.4	73 ± 18	16 ± 3%	2.3	198 ± 20
A	(antral)	6.73 ± 0.8	21 ± 2	5 ± 1%	0	227 ± 13

The relative efficacies of histamine in stimulating cAMP generation in epithelial cells separated from the guinea pig fundus were compared to the percentage of parietal cells present in the cell fractions. In each of 7 preparations of cells, the cAMP response to 10^{-3} M histamine was calculated as pmol cAMP/10^6 cells. The percent of maximum was obtained in each cell fraction by ascribing the value of 100% to the cAMP production observed in parietal cells

cAMP/10^6 cells and histamine (10^{-3} M) stimulated cAMP generation in all the isolated gastric cell fractions (table 1). The maximal response of cAMP to 10^{-3} M histamine occured at 30 min in fractions A and III, at 60 min in fractions I and II, and plateaued up to 90 min in the 4 cell fractions (fig. 1). No change in basal cAMP levels was observed in the 4 cell fractions, according to the time of incubation. Histamine (10^{-3} M) produced 3-, 16-, 24- and 83-fold stimulation in cAMP accumulation in cell fractions A, I, II and III, respectively (table 1). Under basal conditions and after the addition of 10^{-3} M histamine, we have verified that cAMP levels in those 4 fractions were linearly correlated with the number of cells in a wide range from $0.5–4 \times 10^6$ cells/ml (fractions A, I, II) and from $0.17–0.7 \times 10^6$ cells/ml (fraction III). In view of the remarkable efficacy of histamine in stimulating cAMP production in the parietal cell fraction, it was conceivable that the histamine effect observed in cell fractions I, II and A might be due to a 'contamination' by parietal cells. The relative efficacies of histamine on cAMP production in these different cell fractions are therefore presented in table 1 to explore this possibility. For each individual preparation, maximal cAMP generation induced by 10^{-3} M histamine in the parietal cell fraction was assigned to a value of 100% and the relative efficacies for histamine in other cell populations was then calculated. The protein content in the cell fractions, which is a function of the cell diameter and function of the parietal cell concentration [8] is also presented in

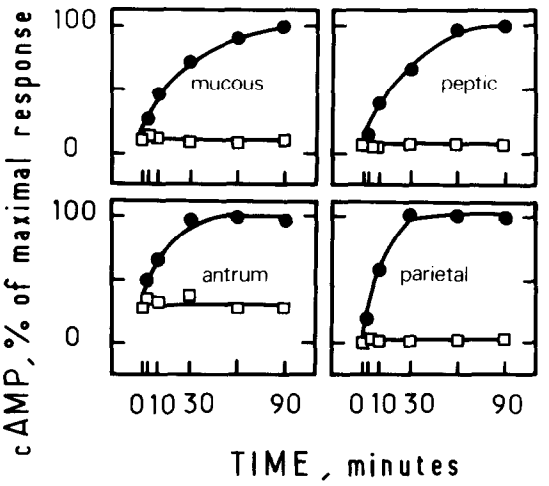

Fig. 1 Time course of histamine-induced cAMP accumulation in cell fractions isolated from the guinea pig fundus or antrum. Cells were suspended in standard solution containing 0.5 mM IBMX and were incubated at 20°C in the absence (control, □) or in the presence of 10^{-3} M histamine (●). Data are the mean of 2–3 expt performed in duplicate or triplicate.

table 1. These values agreed with those in [8]. Our results clearly indicate that the relative efficacies of histamine on cAMP in fractions I, II, III and A did not correlate linearly with the percentage of parietal cells present in the cell preparation, suggesting the presence of histamine receptors mediating cAMP synthesis in non-parietal cells. Indeed, 16% of parietal cells in fraction II (enriched in chief

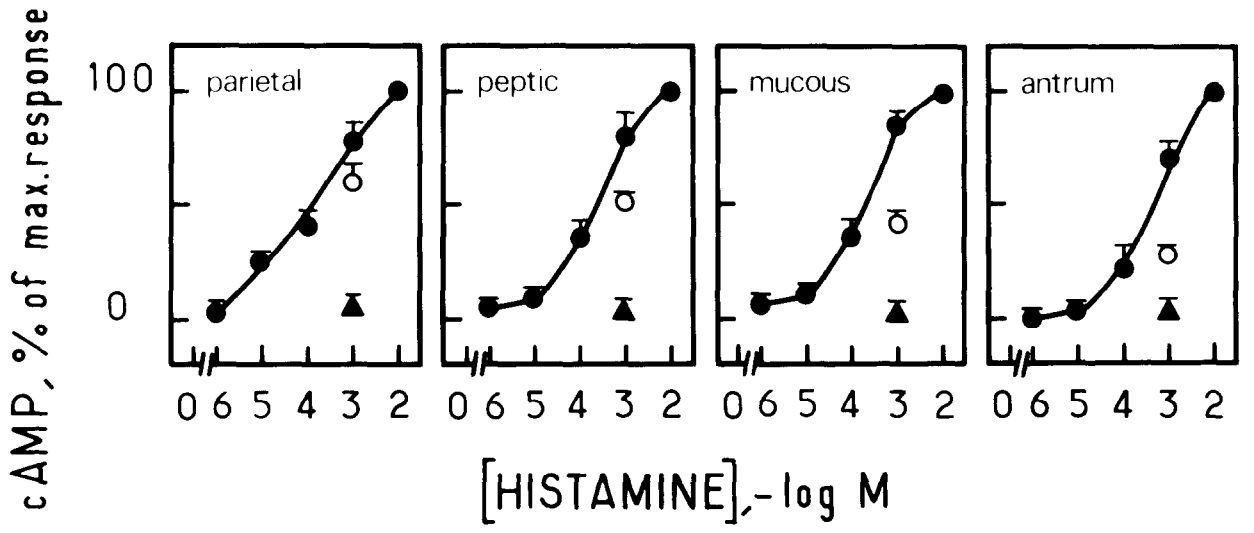

Fig. 2. Stimulation by various concentrations of histamine and inhibition by cimetidine or diphenhydramine of cAMP levels in cell fractions separated from the guinea pig fundus or antrum. Results were normalized to the 100% maximal response to 10^{-2} M histamine (●). Cimetidine (▲) or diphenhydramine (○) at 10^{-4} M were tested in combination with 10^{-3} M histamine. Each point is the mean ± SEM of the results of 4–5 expt performed in duplicate or triplicate.

cells) and only 2% of parietal cells in fraction I (enriched in mucous-secreting cells) were able to produce respectively 27% and 16% of the amount of cAMP synthetized by fraction III which contained ~94% of parietal cells.

3.2. *Chemical characterization of the histamine-induced cAMP accumulation in cell fractions isolated and separated from the fundus or antrum*

The stimulatory effect of histamine was dose-dependent over 10^{-6}–10^{-2} M (fig. 2). Half-maximal increase of cAMP caused by histamine was observed at 1.6×10^{-4} M (fraction III), at 2×10^{-4} M (fraction I and II) and at 4×10^{-4} M (fraction A). This figure also indicates that addition of the H_2 receptor antagonist cimetidine at 10^{-4} M in combination with 10^{-3} M histamine in the 4 fractions resulted in a 100% inhibition of the increase caused by histamine. When the same experiment was conducted in the presence of the H_1 receptor blocker diphenhydramine at 10^{-4} M, cAMP levels were only reduced by 23, 35, 51 and 62% in fractions III, II, I and A, respectively. Here, we assigned the value of 100% to the cAMP rise observed in the presence of 10^{-3} M histamine. Cyclic AMP production stimulated by 10^{-3} M histamine in fractions I, II and III was gradually and completely inhibited by increased concentrations of the H_1 receptor antagonist DPH (10^{-5}–10^{-2} M) or by the H_2 receptor antagonist cimetidine and 22, 26.6 and 45.7×10^{-6} M diphenhydramine in parietal, peptic and mucous cell fractions, respectively.

4. DISCUSSION

These data indicate that histamine can directly stimulate cAMP production, through H_2 receptors, in non-parietal cells isolated from the guinea pig fundus or antrum.

In gastric cells separated from the fundus, it was shown that the efficacy of histamine on parietal cells is 3.7- and 6.3-times higher than that of the peptic or mucous cell fractions, respectively. Accordingly, the efficacy of histamine on cAMP production, adenylate cyclase [5] or cAMP-dependent protein kinase [6] activations was 2–4-times higher in fundic than in antral glands (where parietal cells are absent). In contrast, histamine had no effect on enterocytes isolated from the guinea pig duodenum [5], indicating the cellular specificity of the cAMP stimulations by histamine in the 4 gastric cell frac-

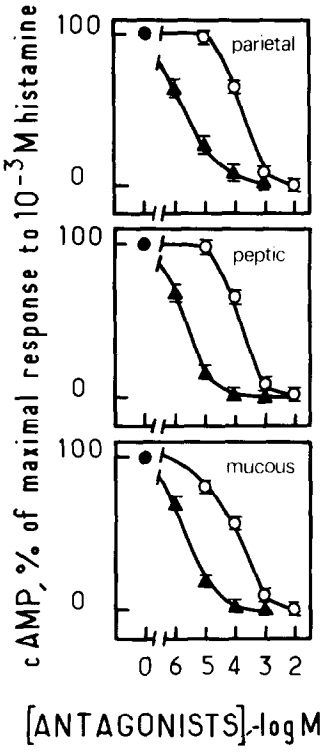

Fig. 3. Dose–response curves for the inhibition by cimetidine or by diphenhydramine of cAMP production induced by 10^{-3} M histamine (●) in cell fractions separated from the guinea pig fundus. Histamine and cimetidine (▲) or diphenhydramine (○) were added to the cells simultaneously and the incubation continued for 60 min at 20°C in the presence of 0.5 mM IBMX. Data are mean ± half-range of two separate experiments performed in duplicate. For these two preparations, protein concentrations were: 666–675, 270–225 and 184–168 $\mu g/10^6$ parietal, peptic and mucous cells, respectively.

tions studied. However, we cannot exclude the presence of undifferentiated parietal cells sedimenting with the fundic cell fractions enriched in peptic cells or mucous cells during the density gradient separation. Indeed, surface receptors for histamine could be considered in stem cells before morphological or functional differentiation of the gastric cells, since this amine can increase cAMP production in gastric glands isolated from the human [18] or rat [19] fetuses.

The pharmacological specificity of the histamine receptors evidenced here was determined by the use of the selective H_2 antagonist cimetidine and H_1 antagonist diphenhydramine. The measured IC_{50} and calculated K_i values for these two antihistamines indicate that diphenhydramine was ~100-times less potent than cimetidine on cAMP production induced by histamine (fig. 3). The inhibition constants obtained for cimetidine and diphenhydramine in these 3 cellular fractions isolated from the fundus are in agreement with the values of K_i determined for the histamine H_2 receptor characterized in guinea pig fundic and antral glands [5,20] or in gastric [21] or non-gastric cells [15,16] containing histamine H_2 receptors. Our results are similar to those obtained in the piglet gastric mucosa [22]: these investigators showed that surface epithelial cells isolated from the fundus contain an histamine-sensitive cAMP system that has 10-fold lower activity than that of the parietal cell-rich fraction. However, they further showed that the activation of adenylate cyclase by histamine in the surface cells could be blocked by the H_1 antagonist promethazine but not by the H_2 blocker cimetidine. This difference could be explained by the loss of the H_2 specificity of the histamine receptor during cellular or adenylate cyclase preparations, as observed in [23]. Here we have observed that preparations of fundic or antral cells (even without subsequent separation) by the enzymatic digestion with pronase [8] caused a loss of the cAMP regulations by VIP or somatostatin-14, shown in gastric glands isolated by means of EDTA from the guinea pig [5,6]. The differential effects of pronase digestion on the expression of cell surface receptors for VIP, somatostatin and histamine in the system indicate that the regulatory components of the receptor cAMP systems sensitive to VIP or to somatostatin-14 on one hand and to histamine on the other were chemically different. This hypothesis can be directly tested by binding studies using [^{3}H]histamine [24] or the ^{125}I-labelled peptides.

The presence of recognition sites for histamine in non-parietal cells in this study correlates with the in vivo regulation by histamine of pepsin [3,25] and somatostatin [1] secretions by the stomach since in both cases, stimulation or inhibition are H_2 receptor-mediated effects. Finally, our results suggest that muco-peptic and somatostatin secretions by the stomach could be regulated, via cAMP, after cell-surface stimulation of histamine H_2 receptors.

ACKNOWLEDGEMENTS

This study was supported by grant CRL 81 30 25 from INSERM. The secretarial aid of Mrs Denise Lhenry is gratefully acknowledged.

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