SELECTIVE INHIBITION BY SOMATOSTATIN OF CYCLIC AMP PRODUCTION IN RAT GASTRIC GLANDS

Demonstration of a direct effect on the parietal cell function

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1. Introduction

Somatostatin, originally isolated from the hypothalamus as an inhibitor of growth hormone secretion [1], is released from somatostatin-containing D cells of the stomach [2] and pancreas [2,3] during the gastric phase of a meal [4]. In vivo, somatostatin is reported to inhibit the pepsinogen secretion induced by a meal [5] and the gastric acid secretion stimulated by histamine [6,7]. The presence of specific receptors for somatostatin on isolated gastric cells from the rat fundus [8] strongly suggests that somatostatin exerts a direct effect on these exocrine secretory processes: We have shown that secretin which stimulates pepsinogen [9] and mucous [10] secretions is highly potent in stimulating cyclic AMP production in gastric glands isolated from the rat fundus and antrum, while histamine is only effective on the acid-secreting fundic preparation [11]. Here we have examined therefore the ability of somatostatin to inhibit both secretin and histamine-induced cyclic AMP formation in our system. The effect of cimetidine, a specific H_2-receptor antagonist [12] was also investigated since it has been shown that histamine stimulates gastric acid secretion by interacting with an H_2-receptor cyclic AMP system [13] present in parietal cells [14].

Our data demonstrate that somatostatin (10^{-9}–10^{-6} M) reduces considerably the effect of histamine on the cyclic AMP production in a non-competitive way, while cimetidine (10^{-8}–10^{-4} M) inhibits competitively the histamine stimulation. In contrast, no effect of somatostatin was found on the cyclic AMP system highly sensitive to secretin in both fundic and antral regions of the rat stomach.

2. Materials and methods

2.1. *Experimental conditions*

Gastric glands were isolated from the stomach of male Wistar rats (200–250 g) using EDTA [15] as in [11]. In a standard assay, cyclic AMP production was measured in gastric glands (5–15 μg cell DNA/ml) incubated at 20°C in 0.5 ml KRP (pH 7.5), containing 2% BSA and 0.5 mM 3-isobutyl-1-methylxanthine (iBuMeXan) as a phosphodiesterase inhibitor. The reaction was started by the addition of 100 μl agents after 10 min preincubation of the other reaction constituents. The incubation was stopped after 60 min by the addition of 50 μl 11 N $HClO_4$. Cyclic AMP was determined using the radioimmunoassay technique in [16]. DNA and protein contents were measured in homogenates of gastric glands as in [17] and [18], respectively. Data were expressed in pmol cyclic AMP/μg cell DNA. The suspension of gastric glands contained 13.5 ± 1.1 μg DNA or 4.4 ± 1.6 μg protein per 10^6 cells. In the presence of 0.5 mM iBuMeXan, basal cyclic AMP production averaged 2.64 ± 0.1 and 3.8 ± 0.2 pmol cyclic AMP per 10^6 cells at 20°C and 37°C, respectively ($p < 0.001$, $n = 6$). Those values are consistent with those obtained in mucosal preparations from the canine [19] and guinea pig [20] fundus.

2.2. *Statistical analysis*

Regression lines were calculated by the least squares method. The significance of the differences between paired values were calculated by the Student's *t*-test.

2.3. *Chemicals*

Synthetic porcine secretin (lot S19) was generously supplied by Professor E. Wünsch (Abteilung für Peptidchemie, Martinsried) and highly purified natural VIP was a generous gift from Professor V. Mutt (GIH Lab., Stockholm) through Gastrointestinal Hormones Resources (NIHMD). Cimetidine (SKF 92334, lot 8R 118) was kindly supplied by Mrs D. M. Francis (Smith, Kline and French Labs., Welwyn Garden City, Hertfordshire). The synthetic cyclic somatostatin (lot E 0535) was purchased from Beckman (Switzerland). All other chemicals were of analytical grade.

3. Results

3.1. *Effect of somatostatin on basal and secretin-induced cyclic AMP formation in rat gastric glands*

The effect of somatostatin was studied at concentrations of secretin producing maximal and half-maximal stimulation of cyclic AMP production [11]. Somatostatin 10^{-6} M had no significant effect on basal and secretin-stimulated cyclic AMP production in rat gastric glands (table 1). In the same conditions, somatostatin was also unable to cause a detectable effect on the VIP-induced cyclic AMP formation which was mediated by the secretin-sensitive cyclic AMP system [11] in gastric glands isolated from either the rat fundus or antrum (data not shown).

3.2. *Effect of somatostatin on histamine-induced cyclic AMP formation in rat gastric glands*

The optimal conditions for the histamine stimulation are shown in fig.1. The addition of 0.5 mM iBuMeXan, as a phosphodiesterase inhibitor, caused an ~2.3-fold increase over basal cyclic AMP production (0.196 ± 0.007 vs 0.083 ± 0.015 pmol cyclic AMP/μg cell DNA) and a maximal potentiation of the effect of histamine (fig.1A). Maximal and half-maximal effects were obtained by incubating fundic glands for 1 h at 20°C with $5 \cdot 10^{-3}$ and $5 \cdot 10^{-4}$ M histamine, respectively (fig.1B). In accordance with [21], histamine had no effect on cyclic AMP production in gastric glands isolated from the antrum (table 1). In gastric fundus, the addition of somatostatin 10^{-6} M produced a 64 and 61% inhibition of the effect produced by $5 \cdot 10^{-4}$ and $5 \cdot 10^{-3}$ M histamine, respectively (table 1).

Table 1
Effect of 10^{-6} M somatostatin on basal and cyclic AMP production stimulated by secretin (A) or histamine (B) in rat gastric glands

Additive	Cyclic AMP (pmol/g DNA)	
	Fundus	Antrum
A. Control	0.184 ± 0.009 (8)	0.080 ± 0.006 (9)
Somatostatin	0.165 ± 0.010	0.083 ± 0.013
Secretin 10^{-9} M	2.36 ± 0.070[b] (13)	3.52 ± 0.287[b] (9)
Secretin + somatostatin	2.43 ± 0.108	4.13 ± 0.188
Secretin 10^{-8} M	5.49 ± 0.307[b] (8)	8.18 ± 0.397[b] (6)
Secretin + somatostatin	5.55 ± 0.479	7.26 ± 0.461
B. Histamine $5 \cdot 10^{-4}$ M	2.59 ± 0.092[b] (6)	0.065 ± 0.028 (5)
Histamine + somatostatin	0.937 ± 0.087[a]	
Histamine $5 \cdot 10^{-3}$ M	5.69 ± 0.171[b] (6)	0.103 ± 0.006 (5)
Histamine + somatostatin	2.26 ± 0.343[a]	

[a,b] $p < 0.05$ or better from control[b] or from corresponding value in absence of somatostatin[a] by paired Student's *t*-test

Gastric glands were incubated under the conditions in section 2. The values given are the means + SE for the no. determinations shown in parentheses

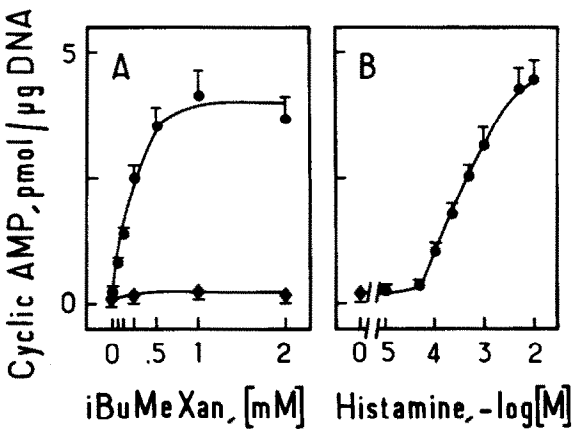

Fig.1. Cyclic AMP production in rat fundic glands: (A) effect of iBuMeXan as a phosphodiesterase inhibitor in the absence (♦) or in presence of 10^{-3} M histamine (●); (B) effect of various concentrations of histamine (●), in the presence of 0.5 mM iBuMeXan. Mean values ± SEM of 3 separate expt performed in duplicate or triplicate.

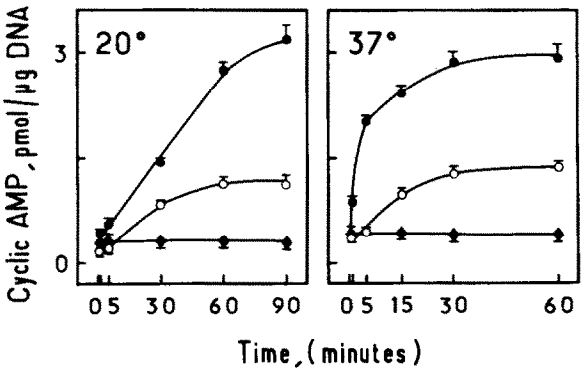

Fig.2. Time course of cyclic AMP production in rat fundic glands incubated at 20 or 37°C in the absence of effector (♦) and in presence of 10^{-3} M histamine (●) or histamine plus 10^{-6} M somatostatin (○). Values are mean ± SEM of triplicates. A second experiment gave similar results.

3.3. *Effect of time and temperature on the inhibitory effect of somatostatin on cyclic AMP production induced by histamine in rat fundic glands*

As shown in fig.2, histamine 10^{-3} M increased the production of cyclic AMP within 1 min at 20°C; the effect was maximal (9-fold increase over control) at 60 min and cyclic AMP levels became constant for the 90 min incubation. When somatostatin 10^{-6} M (a dose that is maximally effective in inhibiting cyclic AMP production induced by histamine, vide infra) was added to histamine, cyclic AMP levels were significantly reduced for the entire experimental period ($p < 0.001$). At 37°C the initial velocity of cyclic AMP production induced by histamine was higher, but the extent of the maximal stimulation (6-fold increase over control) was lower than that observed at 20°C (fig.2). At this physiological temperature, somatostatin also reduced cyclic AMP accumulation during the 60 min incubation with histamine. The addition of somatostatin does not alter the time course of the increase in cyclic AMP caused by histamine, since cyclic AMP became maximal and constant in the presence or in the absence of somatostatin by 60 min and 30 min at 20 and 37°C, respectively (fig.2).

3.4. *Effect of various concentrations of somatostatin and histamine on cyclic AMP production in rat fundic glands*

Since histamine 10^{-3} M produced a maximal increase in cyclic AMP production after a 60 min incubation at 20°C, the characteristics of the inhibitory effect of somatostatin were studied under these experimental conditions. Cyclic AMP formation induced by 10^{-4} and 10^{-3} M histamine was decreased by 10^{-9} M somatostatin; maximal inhibition was obtained with 10^{-7} and 10^{-6} M somatostatin, respectively (fig.3A). At this concentration of inhibitor,

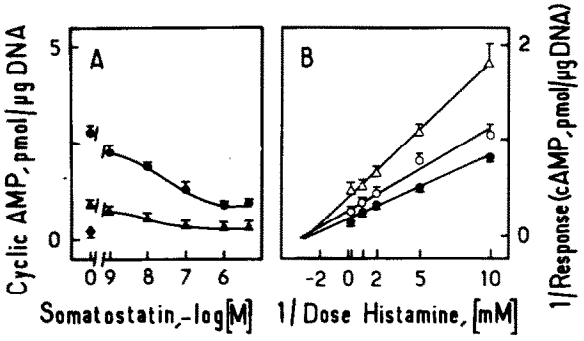

Fig.3. (A) Effect of various concentrations of somatostatin on cyclic AMP production induced by 10^{-4} M (▲) or 10^{-3} M (●) histamine in rat fundic glands. Mean values ± SEM of 2 separate expt performed in triplicate. (B) Lineweaver-Burk analysis of the cyclic AMP production induced by 10^{-4} to $5 \cdot 10^{-3}$ M histamine (●) and by histamine plus 10^{-8} M (○) or to 10^{-7} M (△) somatostatin. Equations of the regression lines and correlation coefficients were as follows: $y = 0.065x + 203$, $r = 0.981$ ($p < 0.001$) for histamine alone; $y = 0.081x + 0.305$, $r = 0.931$ ($p < 0.001$) and $y = 0.138x + 0.433$, $r = 0.937$ ($p < 0.001$) for histamine plus 10^{-8} and 10^{-7} M somatostatin, respectively.

cyclic AMP production induced by histamine was again significantly elevated compared to control ($p < 0.001$). Half-maximal inhibition of the increase in cyclic AMP (ID_{50}) caused by 10^{-4} and 10^{-3} M histamine occurred with 10^{-8} M somatostatin. As shown in fig.3B, the Lineweaver-Burk analysis [22] of our data shows that somatostatin inhibits histamine-induced cyclic AMP production by changing the maximal response while the apparent half-maximal stimulation ($3 \cdot 10^{-4}$ M histamine) was not changed in the presence of the inhibitor: this is typical of a non-competitive type of inhibition.

3.5. *Effect of cimetidine on basal, secretin and histamine-induced cyclic AMP formation in rat fundic glands*

We found that cimetidine had no effect on basal or secretin-stimulated cyclic AMP production in gastric glands isolated from the rat fundus or antrum (data not shown). The inhibitory effect of cimetidine on cyclic AMP formation induced by histamine 10^{-4} or 10^{-3} M could be detected at 10^{-8} M cimetidine, was half-maximal at $3 \cdot 10^{-7}$ and $8 \cdot 10^{-7}$ M and was maximal at 10^{-5} and 10^{-4} M cimetidine, respectively (fig.4A). At these concentrations of inhibitor, the stimulatory effect produced by histamine (10^{-4} or 10^{-3} M) was completely blocked. Application of

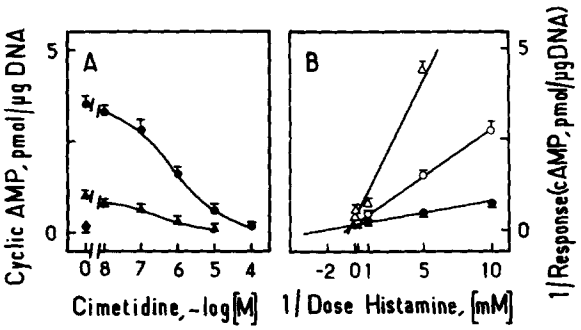

Fig.4. (A) Effect of various concentrations of cimetidine on cyclic AMP production induced by 10^{-4} M (▲) or 10^{-3} M (●) histamine in rat fundic glands. Mean values ± SEM of 2 separate expt performed in triplicate. (B) Lineweaver-Burk analysis of the cyclic AMP production induced by 10^{-4} to 10^{-2} M histamine (●) and by histamine plus 10^{-7} M (○) or 10^{-6} M (△) cimetidine. Equations of the regression lines and correlation coefficients were as follows: $y = 0.056x + 0.251$, $r = 0.898$ ($p < 0.001$) for histamine alone; $y = 0.257x + 0.227$, $r = 0.984$ ($p < 0.001$) and $y = 0.826x + 0.305$, $r = 0.992$ ($p < 0.001$) for histamine plus 10^{-7} and 10^{-6} M cimetidine, respectively.

the Lineweaver-Burk transformation of our data in fig.4B indicates that cimetidine inhibits histamine-induced cyclic AMP production by changing the apparent half-maximal stimulation with no modification of the maximal response, i.e., in a competitive way. Thus, our results with cimetidine characterized the action of histamine on gastric glands as an H_2 receptor activation implicated in gastric acid secretion since this H_2 receptor antagonist could inhibit both histamine-induced cyclic AMP formation [23] and gastric acid secretion in man [24].

4. Discussion

These data suggest that the inhibitory effect of somatostatin on histamine-stimulated cyclic AMP production in rat gastric glands accounts for the direct inhibition by somatostatin on the release of hydrochloric acid by the stomach. In vivo and in vitro, somatostatin inhibits histamine-induced gastric acid secretion in a variety of species [6,7,25] by a non-competitive mechanism [7,25], similarly to the non-competitive inhibition of cyclic AMP production by somatostatin observed in our system. This mechanism excludes that somatostatin might interact directly with the H_2 receptors for histamine [26] and suggests that the somatostatin binding sites shown in parietal cells [8] are biologically functional in the rat fundus. Indeed, the app. K_d ($4.5 \cdot 10^{-9}$ M) of these sites is consistent with the ID_{50} (10^{-8} M) of the somatostatin inhibition in rat gastric glands (section 3). Furthermore, the elevation of somatostatin in venous effluents to $\sim 10^{-9}$ M in response to nutrients or HCl administration in dogs [4,27] and rats [28] compares well with the potency of somatostatin on the histamine-sensitive cyclic AMP system here. The concentration of somatostatin in contact with parietal cells is also dependent on the paracrine function [29] of the gastric D cells that secretes somatostatin [30,31], in the vicinity of parietal cells [32,33].

On the other hand, we found that somatostatin had no significant effect on the cyclic AMP system highly sensitive to secretin in gastric glands isolated from the rat fundus and antrum. The stimulation of cyclic AMP production by secretin was localized in non-parietal cells in rat [11] and dog stomach [19] and correlated with the distribution of pepsinogen in isolated canine mucosal cells [19]. Thus,

although somatostatin inhibits the pepsinogen secretion in man [5] and dog [34], it did not seem effective in inhibiting the secretin-sensitive cyclic AMP system in the rat gastric mucosa. The biological significance of the somatostatin binding sites shown in chief and/or mucous cells of the rat fundus [8] remains unknown.

In conclusion, it appears that somatostatin is capable of a direct inhibition of the histamine-sensitive cyclic AMP system involved in the gastric acid secretion but fail to inhibit the secretin-sensitive cyclic AMP system in pepsinogen- and/or mucous-secreting cells. This does not exclude that somatostatin can inhibit gastric acid secretion indirectly by lowering the gastrin release as shown in vivo [35,36] and in vitro [37]. Our results may clarify the mode of action of regulatory peptides on gastric secretion: To date, the absence of any evidence for a direct effect of secretin or VIP on parietal cells is surprising in regard to their inhibition on gastric acid secretion in vivo [38,39]. Therefore, the stimulation of gastric and pancreatic somatostatin release by those peptides [40–42], together with the demonstration of the direct regulation by somatostatin of the histamine–H_2 receptor activation in rat gastric glands, could be an important scheme by which peptides normally modulate gastric acid secretion.

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