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Distribution of adenyl cyclase sensitive to histamine in rabbit gastric mucosa

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Evidence has accumulated for the involvement of adenosine-3′,5′-cyclic monophosphate (cAMP) in gastric acid secretion [1–6], although there are some conflicting reports [7, 8]. On the other hand, it has been suggested that guanosine-3′,5′-cyclic monophosphate (cGMP) rather than cAMP stimulates gastric acid secretion [9, 10]. However, the studies on 3′,5′-phosphodiesterase [11] and adenyl and guanyl cyclase [12] indicate that, compared with cGMP, cAMP has an important role to play in gastric acid secretion. It has been shown also that the gastric mucosa of guinea pig [13], rat [14] and rabbit [12] contains a histamine-sensitive adenyl cyclase, whereas in dog the histamine-sensitive enzyme is absent [15].

To determine the role of cAMP in gastric acid secretion, it is important to find which cells contain secretagogue-sensitive adenyl cyclase, since the gastric mucosa is composed of several different cells, i.e. an heterogenous organ. Although in the gastric mucosa of rabbits, parietal cells (acid secreting cells) are present in almost all parts of the gland, they are most dense close to the neck region as in other mammals [16, 17]. The purpose of this study was to investigate both the histological distribution of adenyl cyclase in rabbit gastric mucosa using serial tissue sections and the effect of secretagogues on it.

Glandular stomach was obtained from 17 hr-fasted rabbits immediately after slaughtering by cervical dislocation. The tissue, having been pinned flat, was frozen by using dry-ice. A tissue cylinder of 4 mm diameter was prepared from the frozen tissue using a cork borer. The tissue cylinder was homogenized with 2 ml of ice-cold 10 mM Tris–HCl (pH 7·6) containing 1 mM EDTA and 1 mM dithiothreitol (DTT). The homogenate was centrifuged at 2,000 *g* for 15 min and the pellet was resuspended in the same buffer to be 10 mg protein/ml. Twenty μl of this suspension were used for the assay of adenyl cyclase.

Serial tissue sections were made from the frozen cylinder. Sections of 16 μ thick were cut parallel to the mucosal surface in a cryostat at −22° according to the procedure described earlier [18]. The first complete section was obtained usually 80–96 μ below the mucosal surface. The first 9 sections were discarded, since they contained some secreted mucous substances. Then the samples for analysis were taken as follows: six sections from the tenth section were taken for determination of adenyl cyclase and the subsequent four sections were mounted on a glass slide and stained with 0·025% toluidine blue for histological identification. The same procedure was repeated until the mucosa was exhausted. The sections were homogenized with 200 μl of ice-cold 10 mM Tris HCl (pH 7·6) containing 1 mM EDTA and 1 mM DTT in a glass homogenizer fitted with a Teflon pestle. The homogenate was centrifuged at 2,000 *g* for 15 min and the supernatant was discarded. The pellet was homogenized with 30 μl of the same buffer by vigorous vibration and 20 μl of the suspension were used for the assay of adenyl cyclase. Five μl of the sample were taken for the determination of protein according to the method of Lowry *et al.* [19]. The final protein concentration of each sample was about 100 μg (range, 60–120 μg).

Adenyl cyclase activity was determined basically according to the method of Salomon *et al.* [20] with a slight modification as follows. The reaction mixture contained 50 mM Tris–HCl (pH 7·5), 5 mM $MgCl_2$, 20 mM creatine phosphate, creatine phosphokinase (100 U/ml), 1 mM cAMP and 1 mM [^{32}P]ATP (10–15 cpm/pmole) in the absence and presence of histamine phosphate, tetragastrin or NaF. The total volume was 100 μl. Incubation was carried out at 30° for 10 min. The reaction was terminated by adding a solution (pH 7·5) containing 2% sodium dodecyl sulfate, 10 mM ATP and 1·4 mM cAMP. The cAMP formed was isolated by using two successive columns (0·4 × 3 cm) of neutral alumina (Sigma, activity I) and Dowex 50 AG WX 2 resin (200–400 mesh, H^+), and determined by its radioactivity. The value was corrected for the recovery of cAMP checked by using [^{3}H]cAMP (approximately 7,000 cpm).

The production of cAMP by the preparation obtained from the whole frozen tissue cylinder was linear both with time up to 10 min of incubation and with protein concentration up to 200 μg/100 μl. Table 1 summarizes the effect of histamine, tetragastrin and NaF on adenyl cyclase in this preparation. The sensitivity to histamine of this preparation is similar to that of fresh unfrozen tissue [12]. Tetragastrin (0·1 mM) did not increase the enzyme activity. The presence of fluoride, an almost universal stimulator of adenyl cyclase activity, increased the enzyme activity 14-fold.

The histological distribution of adenyl cyclase and the effect of secretagogues on the enzyme activity are represented in Fig. 1. The diagram at the top of the figure shows the qualitative proportions of the cell types estimated from the stained sections, adjacent to those used for the enzyme assay. Adenyl cyclase activity was low in the epithelial cell layer, high in the region about 0·4 mm from surface (rich in mucous neck cells), low in the region about 0·5–0·7 mm from surface (rich in parietal cells), and again quite high in the region about 1·0 mm from surface (rich in chief cells). The addition of 1 mM histamine caused a marked increase in adenyl cyclase activity in the region about 0·5–0·7 mm from surface. On the other hand, there was an insignificant stimulation in the enzyme activity, on addition of 0·1 mM tetragastrin. The addition of 10 mM NaF resulted in the

Table 1. Effect of histamine, tetragastrin and NaF on adenyl cyclase in rabbit gastric mucosa

Additions	cAMP formed (nmoles/mg protein/hr)	
Control	0·62 ± 0·11	(5)
Histamine (1 mM)	1·80 ± 0·31	*(5)
Tetragastrin (0·1 mM)	0·76 ± 0·14	(5)
NaF (10 mM)	8·64 ± 1·47	*(3)

Results are expressed as mean ± S.D. The number of experiments is shown in parenthesis.

* Statistically significant from the control. $p < 0{\cdot}001$ (Student's *t*-test).

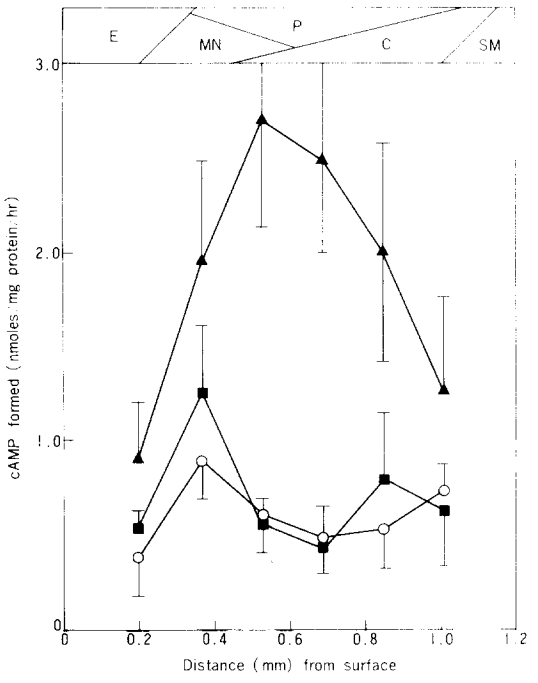

Fig. 1. Effect of histamine and tetragastrin on adenyl cyclase contained in serial sections of rabbit gastric mucosa. Twenty μl of the sample were incubated in the reaction mixture (total vol. 100 μl) containing 50 mM Tris-HCl (pH 7·5), 5 mM $MgCl_2$, 20 mM creatine phosphate, creatine phosphokinase (100 U/ml), 1 mM cAMP and 1 mM [^{32}P]ATP (10–15 cpm/pmole) in the absence and presence of histamine phosphate or tetragastrin. Incubation was carried out at 30° for 10 min. ○, control; ▲, control + 1 mM histamine; ■, control + 0·1 mM tetragastrin. Vertical bars represent standard deviations. Histological regions are indicated by letters: E, epithelial cell; MN, mucous neck cell; P, parietal cell; C, chief cell; SM, submucosa.

stimulation of the activity in each of the above samples. From these results, it is concluded that histamine-sensitive adenyl cyclase is related to parietal cells.

It has been reported that gastrin oligopeptide elevated the cAMP level in the gastric mucosa of rats when administered with theophylline [6], and this increased cAMP is suggested to be accumulated mainly in parietal cells [21]. Since gastrin oligopeptides are unable to stimulate adenyl cyclase obtained from the gastric mucosa of mammals, as evidenced by this and other studies [13, 14], while histamine can stimulate it, it seems reasonable to assume that histamine interacts directly with adenyl cyclase of parietal cells, while gastrin or its oligopeptides stimulate gastric acid secretion *via* indirect processes. The results described in this report, therefore, support the hypothesis proposed by Kahlson *et al.* [22], Bersimbaev *et al.* [14], and Rangachari [23] that histamine is the mediator for gastrin in stimulating gastric acid secretion.

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