Pentagastrin activation of adenylate cyclase in human gastric biopsy specimens[1]

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Summary. Adenylate cyclase activity of human fundic mucosa is log-normally distributed and equally stimulated by pentagastrin and histamine. Cimetidine inhibits the histamine, but not the pentagastrin effect, which is even intensified by H_2-receptor blockade. The results indicate that pentagastrin and histamine activate adenylate cyclase via distinct receptors.

Histamine and gastrin both stimulate hydrochloric acid secretion, but only histamine is a proven stimulant of gastric mucosal adenylate cyclase (AC) in vitro of various mammalian species including man. Gastrin, or its C-terminal pentapeptide pentagastrin, apparently act upon this enzyme system only under in vivo conditions[2-4]. However, since recent findings showed the existence of gastrin receptors[5] and a gastrin sensitive AC[6,7] in the rat gastric mucosa, a general in vitro ineffectiveness of the peptide hormone seems to be equivocal. The present study examined the situation in the human gastric mucosa. The influence of pentagastrin, histamine and of the histamine H_2-receptor antagonist cimetidine on the activity of human gastric mucosal AC was investigated.

Experimental. Biopsy specimens (0.5–3.0 mg wet wt) of fundic gastric mucosa from 37 individuals endoscoped after an over-night fast for diagnosis of the upper gastrointestinal tract were used. From the same region of the fundic stomach, the specimens were taken for histological examination, or immediately frozen and stored at −20 °C for later estimation of AC-activity. For the measurements with NaF, pentagastrin, histamine and cimetidine, only biopsy specimens histologically identified as normal fundic gastric mucosa were chosen. The biopsy was homogenized by hand in a glass-teflon homogenizer and the AC-assay was done as described previously[8] using Gilman's protein binding test[9] for the estimation of the newly synthesized cAMP. Since AC-activity in the biopsy specimens of the different individuals followed a logarithmic distribution, the calculation of the mean ±SEM and the statistical analysis with the t-test for paired comparison were done after logarithmic transformation of the values.

Results. The cumulative frequency plot (figure 1) represents log-normal distribution of the AC-activities stimulated by pentagastrin (range: 46–310 pmoles cAMP/mg protein/20 min) or NaF (375–3323 pmoles cAMP/mg protein/20 min). Basal and histamine activated AC showed the same pattern of distribution. Figure 2 shows a similar concentration - response relationship of pentagastrin and histamine in fundic mucosal homogenates from biopsies of 8 persons. Maximal activation was obtained by a concentration of 10^{-3} moles/l pentagastrin and by 10^{-4} moles/l histamine. Basal activity of 128 pmoles cAMP/mg protein/20 min increased to 210 cAMP/mg protein/20 min. Half maximal activation required a concentration of approximately 10^{-5} moles/l of either stimulant. 10^{-6} moles/l cimetidine did not change basal AC. This H_2-receptor antagonist in concentrations of 10^{-7}, 10^{-5} and 10^{-3} moles/l

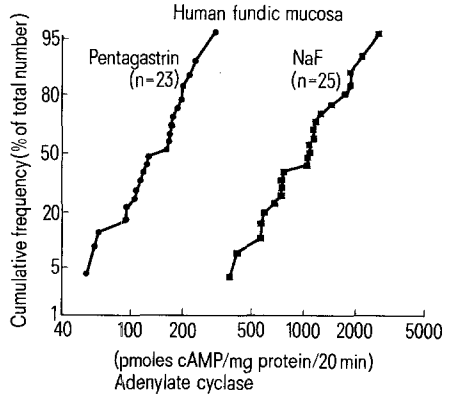

Fig. 1. Adenylate cyclase in human fundic biopsy specimens activated by 10^{-5} moles/l pentagastrin (●) or 10^{-2} moles/l NaF (■). Ordinate: cumulative frequency in percent of total number, abszissa: log scale of enzyme activity.

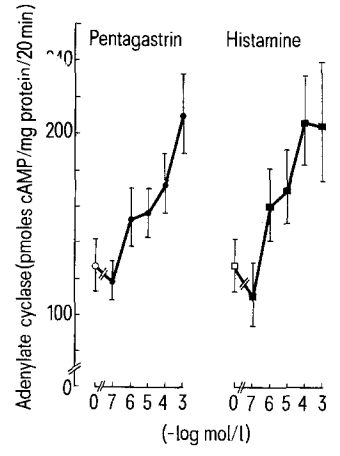

Fig. 2. Concentration response curves of pentagastrin and histamine on human fundic adenylate cyclase activity. Biopsy specimens from 8 persons. All calculations were done with the logarithms of the data. In this and figure 3, each value represents the antilogarithm of the mean ±SEM.

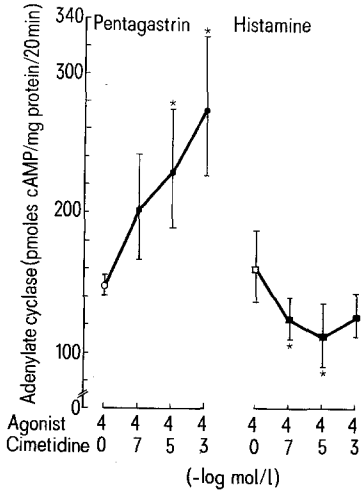

Fig. 3. Effect of different concentrations of cimetidine on pentagastrin (10^{-4} moles/l) and histamine (10^{-4} moles/l) stimulated adenylate cyclase in 4 human fundic biopsy specimens. For further information see legend of figure 2. + $p < 0.05$.

intensified the pentagastrin effect progressively ($p < 0.05$) and inhibited the action of histamine ($p < 0.05$; figure 3).

Discussion. Activities of AC of human fundic gastric mucosa were log-normally distributed and this should be considered to avoid misinterpretations. In our experiments with human gastric mucosa, pentagastrin activated AC in vitro. A stimulating action of histamine has been reported previously by us[10]. Cimetidine antagonized the histamine effect on AC, whereas the pentagastrin activation was even enhanced. These results support the view that pentagastrin and histamine act upon distinct receptors in stimulating AC and confirm recent findings in the rat and dog gastric mucosa.

Náfrády and Wollemann[7] showed in vitro stimulation of rat gastric mucosal AC by pentagastrin, an activation which could not be inhibited by histamine H_2-receptor blockade with cimetidine. Soumarmon et al.[5] showed 'high affinity gastrin receptor sites' in isolated parietal cells; and they detected furthermore a gastrin-sensitive AC in purified plasma membranes[6]. Brown and Gallagher[11] reported a specific and concentration-dependent binding of gastrin to rat gastric mucosal particles. Again, H_2-receptor blockade with cimetidine did not interfere with the binding. Soll[12,13], using isolated parietal cells of the dog gastric mucosa, presented findings indicating that the gastrin effect on oxygen consumption, uptake of ^{14}C-aminopyrine and cAMP production was not influenced by cimetidine. Although our results with human gastric mucosa fit well the data of these animal experiments, they offer no plausible explanation to the effect of pentagastrin increased through the presence of cimetidine in the incubation medium. A methodical origin of that phenomenon can be excluded, because cimetidine did not influence basal AC. However, one could assume a reciprocal inhibiting interaction between gastrin and histamine receptors, as already reported for adrenergic alpha and beta receptors and cAMP and cGMP production in rat gastric mucosa[14].

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Brain stem projections to the cerebral cortex in the rat

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Summary. By means of the HRP method it was shown that the entire cerebral cortex, but in greater proportion the frontal and posterior temporo-parietal regions, receive fibres from the dorsal and medial raphe nuclei and from the locus coeruleus. In contrast, the pars compacta substantiae nigrae and the tegmental area send projections to the motor and cingular areas respectively.

In the past few years, great importance has been given to the projections from the brain stem to the cerebral cortex, since the cortical activity is largely influenced by these afferences. In this preliminary study, several facts stressing the influence of some mesencephalic nuclei on the telencephalon are reported.

Material and methods. 50% horseradish peroxidase solution (HRP) was injected (0.02–0.05 µl) into different areas of the cerebral cortex of rats (51 animals). The injection was performed under nembutal anesthesia and under the control of a stereoscopic microscope by means of a 1-µl Hamilton syringe provided with a glass capillary.

After a survival of 24–36 h the rats were perfused and processed according to the Llamas and Martínez-Moreno's[1] technique, modification of the La Vail's one[2] for demonstration of the HRP labelled neurons.

Results. The HRP deposited in the cerebral cortex did not reach the white matter, except in 2 cases. The animals were divided according to the location of the HRP injections, in 5 groups: 1. frontal (areas 8 and 10 of Brodman); 2. motor (6 and 8); 3. parietal (1, 2, 3 and 7); 4. posterior temporo-parietal (20, 39, 40); 5. occipital and cingular (17, 18 and 29).

In all cases, labelled neurons were found in the medial and dorsal raphe nuclei, mainly in ipsilateral side. A significant number of marked neurons were also present, bilaterally, in the locus coeruleus (figure 2). The number of marked neurons in these nuclei was different depending on the area where HRP was deposited, the labelled neurons were more abundant when the HRP injection was performed in the frontal and in the posterior temporo-occipital cortex (figure 1, A and B). The rats of both groups (1 and 4) showed also labelled neurons in the nucleus linearis (pars caudalis et intermedia) and in the periaqueductal grey matter, although in sporadic form. No somatotopic order of the marked neurons was found in any of these nuclei.

The animals of group 2 (HRP injection in motor areas) showed labelled neurons in addition to the nuclei, mentioned above, in the pars compacta of the homolateral substantia nigra (figure 1, C). When the HRP was deposited in the medial surface of the motor cortex, the labelled neurons were located in the medial portion of the pars compacta. In contrast, when the HRP was injected in the convexity of the motor cortex, the labelled neurons could be seen in the lateral portion of the pars compacta substantiae nigrae.