Histamine Metabolism in Human Gastric Mucosa
Effect of Pentagastrin Stimulation

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The metabolism of histamine in the human gastric mucosa was studied in the basal state and during pentagastrin stimulation. Studies were made in healthy volunteers and in patients with peptic ulcer disease. Mucosal biopsies were taken from antral and oxyntic gland areas whereupon histamine content, histidine decarboxylase activity, and histamine methyltransferase activity were simultaneously assayed. Histamine content of the oxyntic gland mucosa was decreased as a consequence of pentagastrin administration in all groups studied, and this decrease was numerically largest in patients with duodenal ulcer disease. Pentagastrin induced a significant increase in histidine decarboxylase activity of the oxyntic gland mucosa with the most profound increase seen in patients with duodenal ulcer. The highest rates of histamine formation were present in the oxyntic mucosa of patients with Zollinger–Ellison syndrome. The activity of histamine methyltransferase was the same in all groups studied and was not changed by pentagastrin. In conclusion, pentagastrin administration in humans is followed by a significant mobilization of histamine only from the oxyntic gland mucosa, an effect that is more pronounced in patients with duodenal ulcer disease.

Much of the evidence for a physiological role of gastric mucosal histamine in the process of activation of the parietal cells has been gained from experiments in rats, where histamine is stored mainly in enterochromaffinlike (ECL) cells, from which it is released and newly synthesized at different rates depending on the degree of stimulation (1-4). In other species, the role of mucosal histamine for acid secretion is far from settled. However, gastrin injection and vagal stimulation have been shown to increase histidine decarboxylase activity in the guinea pig (5), and in isolated glands from the rabbit gastric mucosa, pentagastrin is capable of releasing histamine (6), although no information is available concerning alterations in histamine content and histamine-forming capacity in the intact rabbit mucosa. In addition, when given to dogs, pentagastrin has recently been shown to induce a transient but significant decrease of histamine content in the oxyntic mucosa, accompanied by an increase in histidine decarboxylase activity (7). These observations suggest that gastric mucosal histamine can be mobilized by gastrin stimulation in all species studied with appropriate techniques. In humans, changes in histamine content of the gastric mucosa during fasting in health and under different clinical conditions have been observed (8), while information on the dynamics of the metabolism of the amine is meagre.

We have recently developed and evaluated a technique allowing studies of the overall metabolism of gastric mucosal histamine in humans, providing detailed information on storage, rate of synthesis, and catabolism in the basal state and in response to stimulation (9). The aim of the present study was to characterize the intramucosal metabolism of histamine in the human stomach in the basal state and in response to pentagastrin with special reference to quantitative as well as qualitative differences between healthy controls and patients with peptic ulcer disease.

Abbreviations used in this paper: ANOVA, analysis of variance; BAO, basal acid output; ECL, enterochromaffinlike; HDC, histidine decarboxylase; HMT, histamine methyltransferase; PAO, peak acid output.
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Materials and Methods

Patients

Seventeen healthy volunteers (5 female, 12 male, mean age 38 yr), 18 patients with duodenal ulcer (9 female, 10 male, mean age 46 yr), 10 patients with gastric ulcer disease (5 female, 5 male, mean age 63 yr), and 6 patients with Zollinger-Ellison syndrome (1 female, 5 male, mean age 47 yr) have been investigated. The ulcer patients were receiving histamine H₂-blocker therapy for different periods of time, and all were on the waiting list for surgical treatment. Three of the 6 patients with Zollinger-Ellison syndrome had an intact, unoperated stomach, one had been operated on with a proximal gastric vagotomy, one patient had undergone antrectomy, and one had previously been operated on with a selective gastric vagotomy and antrectomy. All gastrinoma patients were on long-term antisecretory drugs. Two patients had omeprazole, the others ranitidine in high doses.

Biopsy Collection

The endoscopic procedures were carried out between 8 and 12 AM after an overnight fast. In the ulcer patients, antisecretory drugs had been postponed for 3-5 days before the endoscopy, but only for 24 h in patients with a gastrinoma. No premedication was used except for local Xylocain anesthesia (Astra, Södertälje, Sweden) applied to the posterior pharynx. After entry of the endoscope into the stomach, the macroscopic border at the major curvature separating the oxyntic and antral gland area was located with the stomach both exsufflated and maximally insufflated with air. The endoscope was thereafter retracted 4-5 cm and biopsies were taken from that site. Antral mucosal biopsies were obtained at the major curvature 3-4 cm aboral to the pyloric sphincter. The endoscopes used were Olympus XQ and XQ 10 (Axel Johnson Instrument AB, Stockholm, Sweden) with standard biopsy forceps FB 24 K. At least two biopsy samples were taken from each site for each analysis. All biopsy specimens were immediately frozen on dry ice and stored at -70°C until further processing.

Pentagastrin Stimulation

Biopsies were performed immediately before and after 90 min of continuous intravenous infusion of pentagastrin (Peptavlon, Macclesfield, Great Britain), 90 pg/h for another 75 min. Pentagastrin infusion was continued until all biopsy specimens had been collected.

Gastrin

Blood samples were drawn before the endoscopic investigation and serum gastrin was determined using a double-antibody polyethylene glycol-assisted radioimmunoassay [Diagnostic Products Corporation, Los Angeles, Calif.] using human gastrin 17 for calibration (10). The method had been standardized against research standard A (69-439, synthetic human nonsulphated gastrin 17)(11).

Gastric Acid Secretion

A gastric acid secretory test was performed in all ulcer patients and in 9 healthy volunteers, whereupon basal acid output (BAO) (mmol per 30 min) and peak acid output (PAO) (mmol per 30 min) were determined after administration of pentagastrin (Peptavlon, 6 pg/kg body weight).

Determination of Histamine Content

Histamine content was determined as described by Snyder (12), a method using the enzymatic transfer of the ¹³C methyl group of ¹³C-S-adenosylmethionine to tissue histamine to form ¹³C-methylhistamine. A tracer amount of ³H-histamine was added to the endogenous histamine and the final product formed being ¹³C-³H-methylhistamine. The amount of formed methylhistamine was measured by counting both ¹³C and ³H, and the ratio of ¹³C to ³H was directly proportional to the amount of endogenous histamine in the mucosa.

In detail, the analysis included the following steps. The frozen biopsies were quickly weighed and homogenized in 0.01 M sodium phosphate buffer (pH 7.4). The homogenate was boiled for 10 min in a water bath and then centrifuged at 600g for 20 min to release bound histamine. Supernatant (100 µl) was transferred into 10-ml polyethylene tubes and 800 µl of 0.01 M sodium phosphate buffer was added in addition to 50 µl of ³H-histamine (New England Nuclear, Boston, Mass.; specific activity, 7.8 &i/mmol). The reaction was started by adding 50 µl of a solution of S-¹⁴C-adenosylmethionine (New England Nuclear; sp act 58.6 &i/mmol) and histamine-N-methyltransferase, previously prepared from guinea pig brains. The incubations were run for 60 min at 37°C and the reaction was stopped by adding 0.5 ml 4 M NaOH. After saturation with NaCl (0.5 g), formed methylhistamine was extracted with 4 ml of chloroform. After centrifugation, the supernatant was discarded and the organic phase was washed with 1 ml 2 M NaOH. After a second centrifugation, the supernatant was again discarded and 2 ml of the organic phase was transferred to scintillation vials. The chloroform phase was evaporated overnight, scintillation fluid (Luma Gel, Lumac, Landgraaf, Netherlands) was added, and the ratio between ¹³C and ³H was counted in a LKB Rackbeta liquid scintillation spectrometer (Wallac Oy, Åbo, Finland). In blanks, 100 µl 0.01 M sodium phosphate buffer was used instead of tissue extract, the blanks and histamine standards were treated accordingly. All assays were run in duplicate.
mM glucose, 0.25 ml 1.2 mM aminoguanidine (Sigma Chemical Company, St. Louis, Mo.), 0.98 ml 0.01 M sodium phosphate buffer (pH 7.4), and 0.02 ml 14C-ring-labeled histamine (Amersham, England; specific activity, 56 μCi/mM glucose, 0.25 ml) and 0.2% wt/vol glucose; and 0.02 ml 14C-ring-labeled histidine. This method has been adapted for use in our laboratory (16). Mucosa from two biopsies was pooled, minced, and incubated for 3 h at 37°C under nitrogen in beakers containing 20 μg 2-ring-14C-labeled L-histidine (Amersham, England; sp act 9.8 mCi/mmol); 10^-4 M aminoguanidine; 10^-5 M pyridoxal phosphate; 10^-4 M sodium phosphate buffer (pH 7.4); and 0.2% wt/vol glucose; the total incubation volume made up to 1.5 ml. In the blanks, 10^-4 M semicarbazide was used to inhibit histidine decarboxylase activity; the incubation was arrested by adding carrier histidine (40 mg base] and perchloric acid to a final concentration of 0.4 M. Histamine was separated from histidine on an ion exchange resin (Dowex 50 W-X 4, 100–200 mesh; Pharmacia, Upsala, Sweden) and converted to pipisy histamine, the radioactivity of which was determined at infinite thickness in a gas-flow counter. For further details over the specificity of the enzyme assay, see reference 9.

Statistics and Ethics

For the analysis of differences between groups, one-factor analysis of variance (ANOVA) was computed. For differences within groups, Wilcoxon signed rank test was used together with a double-sided paired Student’s t-test. To test correlations, regression analyses was performed. The study was approved by the local ethics committee. Informed consent to participate in the study was obtained from each subject.

Results

Mean BAO in the control subjects was 1.5 mmol per 30 min, which was almost identical to that of the patients with gastric ulcer (1.4 mmol per 30 min). The duodenal ulcer patients had a higher mean BAO (2.6 mmol per 30 min). These patients also had the highest peak acid outputs following pentagastrin administration, the mean being 19.9 mmol per 30 min (Table 1).

Gastrin

Basal gastrin concentrations differed slightly between the study groups (Table 1), with the greatest variation among patients with gastric ulcers. Patients with Zollinger-Ellison syndrome had extremely high gastrin concentrations (mean, 658.5; range, 83–2400).

Histamine content of the gastric mucosa in the oxyntic and in the pyloric gland areas is shown in Figure 1A and B. In the oxyntic mucosa, histamine content in the basal state did not differ between the study groups. We measured higher histamine content in the oxyntic mucosa than in the pyloric mucosa; the difference was most evident among the healthy volunteers. Pentagastrin infusion was never followed by any change in the amine content of the antral mucosa. However, pentagastrin administration induced a significant decrease in histamine content of the oxyntic mucosa in all groups studied. This decrease in histamine content was most forcibly substantiated statistically in patients with duodenal ulcer disease (p < 0.001).

In the basal state, histidine decarboxylase (HDC) activity of the oxyntic and the pyloric mucosa did not differ between controls and ulcer patients. However, the HDC activity of the oxyntic mucosa exceeded that of the pyloric gland mucosa except in the gastric ulcer patients (Figure 2). Pentagastrin exerted no effect on antral mucosal HDC of control subjects or gastric ulcer patients. However, a small but significant (p < 0.05) increase in enzyme activity was observed in the pyloric gland mucosa of patients with duodenal ulcer. On the other hand, pentagastrin administration was followed by a dramatic increase in HDC activity of the oxyntic mucosa of all groups (Figure 2B). The magnitude of the increase in HDC activity induced by pentagastrin was significantly larger in duodenal ulcer patients than in control subjects (p < 0.05). However, by far the highest rates of histamine formation encou-

Table 1. Basal Acid Output (mmol per 30 min), Peak Acid Output (mmol per 30 min), and Basal Serum Gastrin Levels (pmol/L) in Healthy Volunteers and Patients With Peptic Ulcer Disease

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Duodenal ulcer</th>
<th>Gastric ulcer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 9)</td>
<td>(n = 8)</td>
<td>(n = 9)</td>
</tr>
<tr>
<td>BAO</td>
<td>1.45 ± 0.4</td>
<td>2.56 ± 0.7</td>
<td>1.42 ± 0.3</td>
</tr>
<tr>
<td>PAC</td>
<td>18.4 ± 1.0</td>
<td>19.9 ± 3.5</td>
<td>10.9 ± 2.3</td>
</tr>
<tr>
<td>Gastrin</td>
<td>18.4 ± 3.1</td>
<td>30.1 ± 3.2</td>
<td>51.2 ± 15.4</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD.
Histamine methyltransferase (HMT) activity in the oxyntic mucosa and in the pyloric gland mucosa was similar, and no difference in enzyme activity was observed between control subjects and patients with peptic ulcer disease. Pentagastrin caused no demonstrable change in enzyme activity (Figure 4).

No correlation was found between BAO or PAO and the corresponding values for mucosal histamine content or HDC activity. In addition, no relationship was found between PAO and the increase in HDC activity or the magnitude of decrease in mucosal histamine content.

Discussion

Recently, histamine-containing endocrine cells have been demonstrated in the gastric mucosa of dogs and humans, but these cells were found to be notably few (17). By applying a different histological technique, Simonsson et al. (18) found a substantial number of the endocrine cells of the human oxyntic mucosa, which they considered to be ECL cells. Using a refined immunocytochemical method, we have recently been able to confirm the existence of histamine-containing endocrine cells in the human oxyntic mucosa; these cells appear to be more frequent (19). It is not presently known whether the density of histamine-containing endocrine cells differs between healthy
Figure 2. Histidine decarboxylase activity (ng/g x h) in the oxyntic (A) and pyloric (B) gland areas in the basal state and after pentagastrin stimulation. Data were collected from 17 healthy volunteers and 17 duodenal and 10 gastric ulcer patients. Mean and SD are given; *p < 0.05; **p < 0.01; ****p < 0.0001.

Apparenty, two pools of histamine exist in the oxyntic gland mucosa, i.e., a mucosal mast cell pool (21) and an endocrine cell population. We found a slightly higher histamine content in this area than in the pyloric gland area. On the other hand, we were unable to demonstrate any difference in mucosal histamine content between healthy volunteers and patients with peptic ulcer disease. Our results contrast to those previously presented (22-24). However, it should be pointed out that we compared our results in peptic ulcer patients with results in perfectly healthy controls, which has not been done previously. In addition, long-term treatment with antisecretory drugs, particularly H₂-receptor antagonists, induces changes in the mucosal content of the amine that may contribute to the differences observed (8,25). More important, however, is to study the changes in histamine content, reflecting the magnitude of release of the amine upon appropriate stimulation. We observed a significant decrease in histamine content of the oxyntic gland mucosa after pentagastrin administration in healthy volunteers as well as in patients with peptic ulcer disease. It is interesting to note the tendency for patients with peptic ulcer disease to release more histamine in response to pentagastrin. It is not currently possible to know whether pentagastrin exerts its effect on one or both of the histamine pools within the oxyntic gland mucosa. However, the hypothesis that gastrin excerts its effect on the pool of histamine
in the endocrine cells is reasonable, especially since no effect was seen on the stored amounts of histamine in the antral mucosa.

The histamine-forming enzyme (histidine decarboxylase) has been shown to reside in the ECL cells of the rat stomach (26). In species in which the oxyntic gland mucosa contains histamine in ECL cells, it is also easy to measure HDC activity. Previous investigators have experienced considerable difficulties in measuring this enzyme activity in the mucosa of the dog and human stomach (27). However, a "specific" HDC in the canine fundic mucosa has recently been shown to exist (7). The same kinetic properties of the HDC of the human oxyntic mucosa has been demonstrated (9). As has repeatedly been shown to occur in the rat stomach, gastrin accelerates histamine formation in the oxyntic gland mucosa of the dog and guinea pig (5,9). Again, gastrin does not exert this stimulatory effect only in certain species; we have now also shown a corresponding effect of pentagastrin on the rate of histamine formation in the human oxyntic gland mucosa. Interestingly, we observed a more pronounced effect of pentagastrin on the rate of histamine formation in the human oxyntic gland mucosa. The importance of gastrin in the regulation of HDC activity was further substantiated by the exceedingly high values measured in the gastric mucosa of patients with Zollinger-Ellison syndrome. The difference in responsiveness between healthy volunteers and patients with duodenal ulcer disease. The importance of gastrin in the regulation of HDC activity was further substantiated by the exceedingly high values measured in the gastric mucosa of patients with Zollinger-Ellison syndrome. The difference in responsiveness between healthy volunteers and patients with duodenal ulcer disease may be caused by factors such as a high density of enzyme-containing cells (presumably ECL cells), larger amounts of the enzyme per cell, or a higher responsiveness of the enzyme to a certain stimulus or a combination of these suggested mechanisms. The small effect of pentagastrin on the HDC activity of the pyloric gland mucosa of patients with duodenal ulcer may be caused by the appearance of endocrine cells containing histamine. Studies are in progress to obtain more detailed information regarding the histamine-containing endocrine cell pool in patients with peptic ulcer disease.

Histamine methyltransferase is the main metabolizing enzyme in the human stomach (8). Apart from being metabolized, histamine is also released into gastric juice and its effluent (24-28). Previous studies have shown decreased HMT activity in patients with duodenal ulcer disease (13,29), an observation we were unable to corroborate. Again, differences in prestudy H₂-receptor therapy (8) and the character of the control population may explain these differences. However, we were unable to demonstrate any difference in HMT activity on pentagastrin infusion, either in healthy volunteers or in patients with peptic ulcer disease.

The pathogenesis of duodenal ulcer disease is still not fully understood. Patients with duodenal ulcer are known to be hypersecretors of acid in the basal state as well as after pentagastrin or histamine stimulation (30). In addition, the parietal cells are more sensitive not only to gastrin but also to histamine (31-33). In isolated gastric glands from patients with duodenal ulcers, the secretory response to histamine is also increased (34). In the present study, we have shown that pentagastrin is capable of mobilizing histamine from the oxyntic gland mucosa more effectively in patients with duodenal ulcer disease than in healthy volunteers. Consequently, the parietal cells of duodenal ulcer patients would be reached by larger amounts of histamine following gastrin stimulation. The observed lack of correlation between histamine mobiliza-
Figure 4. Histamine methyltransferase activity (μg/g x h) in the oxyntic (A) and pyloric (B) gland areas. Data were collected from 11 healthy volunteers and 12 duodenal and 7 gastric ulcer patients. Mean and SD are given.

Figure 4. Histamine methyltransferase activity (μg/g x h) in the oxyntic (A) and pyloric (B) gland areas. Data were collected from 11 healthy volunteers and 12 duodenal and 7 gastric ulcer patients. Mean and SD are given.

tion and basal as well as pentagastrin-stimulated acid output should not be interpreted as evidence against a role of histamine in the regulation of acid secretion. On the other hand, it should be borne in mind that the data presented identify histamine metabolism only over the time period for biopsy collection, whereas acid secretion is collected continuously over a long period.

References