Antral gastrin-producing G-cells and somatostatin-producing D-cells in different states of gastric acid secretion*

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SUMMARY The number of G- and D-cells per area and the ratio of G/D-cells were investigated in biopsy specimens of the pyloric antrum from normochlorhydric subjects without peptic ulcer, from patients with duodenal ulcer, gastrinoma, pernicious anaemia, and after selective proximal vagotomy. Compared with normochlorhydric subjects antral G-cell density was significantly raised in pernicious anaemia, unchanged in duodenal ulcer, and diminished in gastrinoma patients. After vagotomy G-cell density was found to be raised if compared with patients with duodenal ulcer. D-cell density was significantly increased in gastrinoma patients, unchanged in duodenal ulcer, and diminished in pernicious anaemia and after vagotomy. The G/D-cell ratio was increased in pernicious anaemia and after vagotomy, unchanged in duodenal ulcer, and decreased in gastrinoma patients. It is concluded that the antral pH governs the ratio of G- and D-cells. Therefore, the G/D-cell ratio increases in states of reduced acid secretion and decreases in massive hyperchlorhydria. Hypergastrinaemia as such does not affect the G/D-cell ratio.

A physiological role of the somatostatin-producing D-cells in the gastric mucosa has not been established. Regulation of gastric acid secretion has been suggested, because exogenous somatostatin inhibits basal and stimulated gastric acid secretion and somatostatin is released into veins draining the antrum and the fundus. As D-cells have been shown to contact and sometimes even to embrace neighbouring antral G-cells and fundic parietal cells by long slender cytoplasmic processes emerging from their basal pole, the suggested regulatory potency on parietal cell function could be mediated by direct effects on parietal cells and indirectly by inhibition of gastrin release from antral G-cells. If antral D-cells are involved in the regulation of gastric acid secretion by controlling or modulating the function of neighbouring G-cells, gastric secretion might depend on function and number of D-cells. In turn, D-cell function and number could depend on G-cells. Thus, not only the total mass of endocrine cells is relevant but also the ratio of antagonistic cells. This study describes the G/D-cell ratio in the human antral mucosa in normochlorhydric subjects and in duodenal ulcer, in states of massive hyperchlorhydria (gastrinoma) and achlorhydria respectively hypochlorhydria (pernicious anaemia, vagotomy). It will be shown that the G/D-cell ratio depends on gastric acid secretion—that is, increases in hypo- and achlorhydric patients and decreases in states of massive hyperchlorhydria.

METHODS

SUBJECTS Antral mucosal samples were investigated from 60 patients with duodenal ulcer, 12 patients with gastrinoma, six patients with pernicious anaemia, 18 patients after selective proximal vagotomy performed three to 12 months before examination and 16 control subjects. The mean age of duodenal ulcer patients (43 males and 17 females) was 37 years (range 20–49 years), of gastrinoma patients (seven males and five females) 46 years (range 28–59 years), of pernicious

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anaemia patients (two males and four females) 64 years (range 47–71 years), of selective proximal vagotomy patients (16 males and two females) 48 years (range 36–61 years) and of controls (nine males and seven females) 31 years (range 12–54 years). All duodenal ulcer patients had relapsing ulcer disease and an active ulcer proven by gastroscopy at the time of investigation. The diagnosis of a gastrinoma was made in patients with intractable ulcer disease by the demonstration of excessive hyperchlorhydria, hypergastrinaemia, and the inapproative finding of a tumour shown later to be a gastrinoma by immunohistology. Some of the duodenal ulcer patients and most of the gastrinoma patients had been treated with cimetidine. However, as 1·2 g cimetidine per day for six weeks and a bedtime dose of 400 mg for another 12 weeks did not alter antral G-cell densities, duodenal ulcer and gastrinoma patients with and without cimetidine pretreatment were considered as one group. Diagnosis of pernicious anaemia was based on achlorhydria due to fundic atrophic gastritis, megaloblastic anaemia, and vitamin B₁₂ malabsorption. The patients had been treated with cyanocobalamin injections at regular intervals. Selective proximal vagotomy was performed because of relapsing duodenal ulcer disease. The control group consisted of 13 normochlorhydric volunteers without known diseases of the gastrointestinal tract who were fully informed about the object and the risk of the investigation and of three patients with dyspeptic complaints. As in the latter group no pathological findings could be obtained by extensive gastroenterological diagnostic procedures, the results have been pooled together with those of the healthy volunteers in one control group. Basal and pentagastrin-stimulated acid secretion and basal serum gastrin were examined in all subjects.

TISSUE SAMPLING
Antral mucosa specimens were obtained by forceps biopsy during gastroscopy. Usually three to four biopsies were excised from 1 to 3 cm proximal to the pylorus at the lesser and greater curve and from the anterior wall. To investigate the overall distribution of G- and D-cells seven antra were obtained during operation and multiple mucosal specimens were excised at 2 cm intervals from the pylorus towards the fundic region from the lesser and greater curves and from the anterior wall. Antrectomy was performed for the following reasons: duodenal ulcer (two), gastric ulcer (two), Whipple’s procedure (chronic pancreatitis, one, pancreatic carcinoma, one), total gastrectomy for gastrinoma (one).

IMMUNOHISTOLOGY
Tissue samples were immediately fixed in Bouin’s fluid and embedded in paraffin wax. Sections (5 µ thick) were cut vertically to the mucosa surface, deparaffinised and stained for gastrin and somatostatin using the PAP-technique. The method used for evaluating the number of antral G- and D-cells has been described in detail elsewhere. In short, the area used for counting cell density encompassed the mid-zone of the antral mucosa where antral G- and D-cells are situated in man. The size of the area (0·35×0·23 mm) corresponds to a field visible with a Zeiss-Photomicroscope II using an ocular magnifying ×8 and an objective magnifying ×25. Every cell irrespective of its shape or whether a nucleus was present or not was identified as a G- or D-cell if a dark-brown granular reaction was produced by the PAP-technique which was absent on a serial section treated with normal rabbit serum instead of antigastrin- or antisomatostatin-serum. At least 8–10 adjacent areas from two to three different sections were counted and the mean number of cells per area was calculated. Because of either inappropriate size of biopsies, inadequate fixation, or sections which were not cut vertically to the surface, biopsies from another 50 patients could not be evaluated.

Antisera against synthetic human gastrin I (2–17) (ICI Ltd, Macclesfield, Cheshire, England) and against synthetic cyclic somatostatin (Serono Company, Freiburg, FRG) were raised in rabbits. The gastrin antibody used in this study could be completely absorbed by G-34, G-17, and G-4 but not by somatostatin, the somatostatin antibody by addition of cyclic somatostatin but not of G-17 and G-4. Neither antibody cross-reacted with insulin, glucagon, pancreatic polypeptide, GIP, VIP, and neuromedin. For immunohistology, gastrin antiserum was diluted 1:3000, somatostatin antiserum 1:1000. Unlabelled sheep anti-rabbit-IgG and the peroxidase-antiperoxidase (PAP) complex were purchased from Dakkopatt (Copenhagen).

HISTOLOGY
To investigate the effect of inflammatory cell infiltration on the G- and D-cell density, haematoxylin and eosin stained sections from the antral mucosa of 23 patients (11 controls, 12 duodenal ulcer patients) were examined and scored according to the following system:

Grade 1 No inflammatory cell infiltration or only mild superficial chronic gastritis of the antral mucosa.
Grade 2 Moderate chronic inflammatory cell infiltration of the antral mucosa. No inflammatory cell infiltration or mild superficial gastritis of the fundic mucosa.
Grade 3 Moderate chronic inflammatory cell infiltration of the antral and fundic mucosa.
Table 1  G- and D-cells per area and G/D-cell ratio at different sites of antrum in seven patients

<table>
<thead>
<tr>
<th>Origin of biopsies and distance from pylorus (cm)</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
<th>Patient 6</th>
<th>Patient 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior wall</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2</td>
<td>28.4  6.1</td>
<td>4.7</td>
<td>13.2  3.0</td>
<td>4.4</td>
<td>65.4  15.7</td>
<td>4.2</td>
<td>29.4  10.2</td>
</tr>
<tr>
<td>4–6</td>
<td>28.3  4.2</td>
<td>6.7</td>
<td>24.5  5.3</td>
<td>4.6</td>
<td>72.7  12.8</td>
<td>5.8</td>
<td>35.4  12.5</td>
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<tr>
<td>8–10</td>
<td>25.9  7.8</td>
<td>3.3</td>
<td>17.6  4.2</td>
<td>4.1</td>
<td>27.8  10.1</td>
<td>2.7</td>
<td>28.7  14.0</td>
</tr>
<tr>
<td>Lesser curve</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2</td>
<td>30.1  6.3</td>
<td>4.8</td>
<td>11.5  2.1</td>
<td>5.5</td>
<td>14.8  7.8</td>
<td>1.9</td>
<td>15.0  3.3</td>
</tr>
<tr>
<td>4–6</td>
<td>19.2  4.1</td>
<td>4.7</td>
<td>19.9  2.7</td>
<td>7.4</td>
<td>37.5  18.1</td>
<td>2.1</td>
<td>25.9  5.5</td>
</tr>
<tr>
<td>8–10</td>
<td>23.1  5.0</td>
<td>4.6</td>
<td>17.5  3.7</td>
<td>4.7</td>
<td>11.0  6.8</td>
<td>1.6</td>
<td>20.5  5.7</td>
</tr>
<tr>
<td>Greater curve</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2</td>
<td>32.9  7.3</td>
<td>4.5</td>
<td>12.1  2.8</td>
<td>4.3</td>
<td>43.4  7.4</td>
<td>5.9</td>
<td>29.1  9.9</td>
</tr>
<tr>
<td>4–6</td>
<td>41.0  7.8</td>
<td>5.2</td>
<td>20.3  6.1</td>
<td>3.3</td>
<td>46.8  13.0</td>
<td>3.6</td>
<td>35.7  9.0</td>
</tr>
<tr>
<td>8–10</td>
<td>20.4  4.2</td>
<td>4.7</td>
<td>11.2  2.5</td>
<td>4.5</td>
<td>22.0  6.8</td>
<td>3.2</td>
<td>8.0   0.8</td>
</tr>
</tbody>
</table>

Fundic type mucosa

Patient 1: 48 years, Whipple's procedure, chronic pancreatitis.
Patient 2: 73 years, Whipple's procedure, pancreatic carcinoma.
Patient 3: 26 years, Billroth I, duodenal ulcer.
Patient 4: 46 years, Billroth II, gastric ulcer.
Patient 5: 40 years, Billroth II, duodenal ulcer.
Patient 6: 35 years, Billroth I, gastric ulcer.
Patient 7: 71 years, total gastrectomy, gastrinoma.
STATISTICS
The results are presented as means±SEM. Differences between means were assessed by the Wilcoxon-Mann-Whitney test. Values of α-error lower than 0.05 were considered as significant. Correlation between different variables was estimated by linear regression analysis.

Results

DISTRIBUTION OF G- AND D-CELLS WITHIN ANTRAL MUCOSA
The distribution of antral G- and D-cells and the G/D-cell ratio was investigated in seven patients at comparable sites of the anterior wall, the lesser and the greater curve. The results, which are summarised in Table 1, indicate considerable inter- and intra-subject variations of G- and D-cell densities and of the cell ratios. However, within one individual there was a fairly good correspondence of G- and D-cell numbers evaluated 1–2 cm next to the pylorus at the anterior wall and the greater curve, whereas at other sites greater variations were observed. A trend to lower cell density existed in five out of seven subjects at the lesser curve. Usually fluctuation of one cell type was followed by corresponding changes in number of the other cell type.

G- AND D-CELL DENSITIES IN DIFFERENT STATES OF GASTRIC ACID SECRETION
The inter- and intraindividual variation of the G- and D-cell density shown in the whole antrum explains the wide scattering of both cell types if pyloric biopsies are investigated which were obtained from controls, duodenal ulcer patients, gastrinoma patients, pernicious anaemia patients, and selective proximal vagotomy patients. However, the mean G- and D-cell numbers estimated in gastrinoma patients, in patients after selective proximal vagotomy and in those with pernicious anaemia differed significantly from results obtained in controls and duodenal ulcer patients despite a considerable overlap of single values (Table 2). Thus, G-cell density decreased significantly in patients with marked gastric acid hypersecretion (gastrinoma) and increased significantly in patients with reduced gastric acid secretion or achlorhydria (selective proximal vagotomy and pernicious anaemia).

In contrast, D-cells increased significantly in gastrinoma patients and decreased after selective proximal vagotomy if compared with controls and duodenal ulcer patients (Table 2). When compared with controls but not with duodenal ulcer patients significantly fewer D-cells have been observed in pernicious anaemia patients. Thus, D-cell density was higher in a state of massive acid hypersecretion and low in hypochlorhydric and achlorhydric patients. No significant differences were found between G- and D-cell numbers in controls and duodenal ulcer patients.

There was a significantly positive correlation between the number of G-cells and that of D-cells evaluated in antral biopsies of controls, duodenal ulcer and gastrinoma patients but not of patients with pernicious anaemia and after selective proximal vagotomy (Figure). Within the different patient groups no relationship existed between both cell types and one of the following parameters: basal acid output, pentagastrin-stimulated acid output, basal serum gastrin.

G/D-CELL RATIO
The ratio of G/D-cells was within the same range in controls and duodenal ulcer patients (Table 2). In states of increased or reduced gastric acid secretion, however, remarkable differences appeared as a result of the changes described above in the G- and D-cell density. A significantly diminished G/D-cell ratio was found in gastrinoma patients. This resulted from the increase in D-cells and simultaneous decrease in G-cells in this group of patients with massive gastric acid hypersecretion. This is also illustrated by

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Age, basal and pentagastrin-stimulated acid secretion (BAO, MAO), G- and D-cell number per area, G/D-cell ratio in controls and different groups of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>Duodenal ulcer</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>31 (12–54)</td>
</tr>
<tr>
<td>(range)</td>
<td></td>
</tr>
<tr>
<td>BAO (mmol/h)</td>
<td>3.0±0.4</td>
</tr>
<tr>
<td>MAO (mmol/h)</td>
<td>24.8±3.8</td>
</tr>
<tr>
<td>G-cells/area</td>
<td>44.7±4.1</td>
</tr>
<tr>
<td>D-cells/area</td>
<td>11.8±2.0</td>
</tr>
<tr>
<td>G/D-cell ratio</td>
<td>4.4±0.4</td>
</tr>
<tr>
<td>n</td>
<td>16</td>
</tr>
</tbody>
</table>

Results are presented as mean±SEM.

*Significantly different vs controls.
†Significantly different vs duodenal ulcer patients.

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Table 3. Effect of different grades of non-atrophic gastritis on antral G- and D-cell number per area and on G/D-cell ratio (mean±SEM)

<table>
<thead>
<tr>
<th>Grade of gastritis</th>
<th>G-cells/area</th>
<th>D-cells/area</th>
<th>G/D-cell ratio</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48.9±5.1</td>
<td>13.7±3.0</td>
<td>4.1±1</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>42.7±5.4</td>
<td>10.1±1.4</td>
<td>4.8±1</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>47.0±4.6</td>
<td>12.7±1.1</td>
<td>4.2±1</td>
<td>3</td>
</tr>
</tbody>
</table>

Discussion

This study demonstrates a marked inter- and intra-individual variation of the G- and D-cell densities in the antral mucosa. G- and D-cells tended to be lower at the lesser curve than at the anterior wall and the major curve, thus confirming recent reports on the distribution of G-cells.7–9 No clear-cut decrease in the density of both cell types was found with 6 cm distance from the pylorus; this differs from the findings of others who have described a decrease in antral density from the pylorus to the body region.8–11 In spite of the considerable variation of the antral G- and D-cell number within the groups, mean G-cell density was found to be significantly increased in pernicious anaemia patients, thus confirming earlier results,12–14 and also after selective proximal vagotomy. On the other hand, there was a striking increase in D-cell density in gastrinoma patients and a significant decrease after selective proximal vagotomy and in pernicious anaemia. As described by several15–17 but not all authors7 no dependence of G- and D-cell densities upon the degree and the extent of non-atrophic chronic gastritis could be found in this study.

The raised G-cell density in vagotomised patients corresponds to the significantly greater antral gastrin concentration reported after truncal vagotomy and pyloroplasty in man18 and to the higher G-cell density and total antral G-cell mass described in rats after truncal vagotomy and pyloroplasty19 20 and in dogs after selective proximal vagotomy.21 The mechanism of G-cell hyperplasia in patients after selective proximal vagotomy and in pernicious anaemia is not completely settled. It has been suggested that achlorhydria in pernicious anaemia and hypochlorhydria after vagotomy induced antral G-cell hyperplasia, as in the case of the 'excluded antrum'.21a It was recently claimed that by vagotomy an inhibitor of gastrin release situated in the proximal stomach is removed.22 This would explain the raised serum gastrin levels after vagotomy and may indicate a dependence of G-cell growth on vagal innervation.

As was found by earlier investigators,6 15 23 G-cell density in duodenal ulcer patients did not differ from that in controls. The finding that gastrinoma patients...
have significantly fewer G-cells per area than healthy subjects and duodenal ulcer patients, however, contrasts with a recently published study in which no differences in the G-cell density between four gastrinoma patients and three controls were detected.\textsuperscript{11} This difference may be due to the small number of cases in the latter study.

The finding of a normal number of D-cells per area in duodenal ulcer patients corresponds with the normal antral somatostatin concentration found in these patients in this laboratory.\textsuperscript{24} The latter finding is at variance with another report describing a lower antral somatostatin concentration in duodenal ulcer patients.\textsuperscript{25} It may be concluded from the present study that a defective inhibition of gastrin release at low pH\textsuperscript{26} or the exaggerated postprandial gastrin release\textsuperscript{27} of duodenal ulcer patients cannot be due to a lack of somatostatin-producing D-cells, while a functional defect cannot be ruled out.

The G/D-cell ratio of 4:4/1 in controls and 4:8/1 in duodenal ulcer patients as found in this study is slightly lower than published values. A ratio of 7:1 was described in healthy subjects and of 6:1 in duodenal ulcer patients by one group\textsuperscript{28} and of 8:1 by others.\textsuperscript{29} The methods used, however, were different.

The demonstration of a linear relationship between the number of antral G- and D-cells in controls, duodenal ulcer patients and gastrinoma patients and the striking changes of the G/D-cell ratio in states of hypo-achlorhydria are in keeping with the contention of a functional relationship between D- and G-cells and between D- and parietal cells. Morphological investigations have demonstrated that a single D-cell touches in the antral mucosa several neighbouring G-cells and in the fundic area several parietal cells.\textsuperscript{4} Biochemically it has been shown that pentagastrin stimulates somatostatin release,\textsuperscript{30} whereas exogenous somatostatin inhibits gastrin release\textsuperscript{1} and that infusion of a somatostatin antiserum increases gastrin secretion.\textsuperscript{31}

Bearing in mind these apparent functional relationships between antral G- and D-cells, it is difficult to explain the marked differences in the ratio of G/D-cells in patients with gastrinoma, pernicious anaemia, and after selective proximal vagotomy because, in all these instances, serum gastrin levels are raised, while the G/D-cell ratio increases in pernicious anaemia and after selective proximal vagotomy and decreases in gastrinoma patients. A direct inhibitory effect of raised serum gastrin levels on the D-cell density, therefore, can be excluded. On the other hand, gastric secretion is markedly different in these three hypergastrinaemic groups. It appears that hypo- or achlorhydria is followed by a high G/D-cell ratio, whereas hyperchlorhydria is found together with a low G/D-cell ratio—that is, the G/D-cell ratio is dependent on the state of gastric acid secretion.

This contention is supported by experiments indicating that somatostatin release from D-cells of the stomach is enhanced in the presence of a low intra-gastric pH.\textsuperscript{3} A low intragastric pH may not only stimulate somatostatin secretion but also the growth of the D-cells; this would result in a decrease in the G/D-cell ratio in gastrinoma patients—that is, a relative preponderance of D-cells over G-cells possibly in order to inhibit antral G-cell activity. A lack of the growth stimulus in the case of a high gastric pH leads to an increase in the G/D-cell ratio in pernicious anaemia and after selective proximal vagotomy—that is, to a relative lack of D-cells. In these states of reduced or abolished acid secretion the inhibitory action of D-cells on G-cells is not needed and the relative D-cell deficiency may contribute to the raised serum gastrin levels.

We acknowledge the excellent technical assistance of Mrs A Nesslinger.

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