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# Gastrin and Somatostatin in Patients with Hyperchlorhydric Duodenal Ulcer

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## ABSTRACT

Hormonal and morphological studies were conducted to ascertain the role played by gastrin and somatostatin in the pathophysiology of duodenal ulcer, in particular hyperchlorhydric duodenal ulcer, using 35 patients with duodenal ulcer, of whom 15 were hyperchlorhydric and 20 were normochlorhydric. Twenty normal subjects with normochlorhydria were used as a control. In patients with hyperchlorhydric duodenal ulcer following significant findings were observed:

1. Basal and stimulated hyperchlorhydria,
2. Parietal cell hyperplasia,
3. Basal hypergastrinemia,
4. Increased concentration of gastrin and large number of G cells (G cell hyperplasia) in the antral mucosa.
5. Mucosal concentration of somatostatin and D cells in the antrum was reduced, but the former in patients with hyperchlorhydric duodenal ulcer was not different from that in patients with normoacidic duodenal ulcer.
6. A significant correlation in mucosal concentration was demonstrated between gastrin and somatostatin in control subjects but not in patients with duodenal ulcer.
7. There was a significant correlation in maximal acidity in gastric secretion and mucosal concentration of antral somatostatin in control subjects but not in patients with duodenal ulcer.
8. Concentration of plasma somatostatin in patients with duodenal ulcer was not different from that in control subjects.

These findings indicate that gastrin and somatostatin may participate in the pathophysiology of duodenal ulcer, at least in the subgroup of duodenal ulcer associated with hyperchlorhydria, and the subgroup of duodenal ulcer may be an endocrine disorder.

Gastrin and somatostatin are considered to be the most important peptides which are involved in the pathophysiology of duodenal ulcer. A vast number of reports have been published on the pathophysiology of duodenal ulcer from the view of gastrointestinal hormones, especially gastrin and somatostatin[8,24] There is, however, some skepticism whether gastrin and somatostatin actually play a role in the pathophysiology of duodenal ulcer. In the present study, the authors, therefore, attempted to demonstrate a close relationship of the pathophysiology of duodenal ulcer, especially hyperchlorhydric duodenal ulcer, to both of these peptide hormones in the changes in the blood and in the number of G as well as D cells and their mucosal contents.

## MATERIALS AND METHODS

1. Materials

Thirty five patients of both sexes with duodenal ulcer were selected for this study. Their mean age was 39.4 years. Twenty normal subjects with a mean age of 20.3 years volunteered to serve as controls. Their stomach and duodenum having no lesions were normal in function as well as in morphology (histology). Therefore, the age of patients with duodenal ulcer was not matched with that of the control.

Gastric acid analysis

Gastric acid secretion test was performed on all patients after overnight fast with the test being started at 9 o'clock in the morning. Levin tube was positioned in the stomach. The patients maintained a left supine position to aspirate as much as possible during the test. After the gastric juice was discarded before the test, basal acid secretion was determined by aspirating gastric sample every ten minutes for one hr. Tetragastrin at 4 μg/kg was then administered intramuscularily. Gastric samples were aspirated every ten minutes for the next one hr. Volume and acidity of the gastric juice were determined. Basal acid output (BAO) and maximal acid output (MAO) were calculated as the sum of six 10 min basal collections and six 10 stimulated collections, respectively, and they were expressed in milliequivalent per hour. The normal range at the age of about 30 years in our laboratory is 2.37 ± 0.524 mEq per hour for mean BAO and 13.4 ± 1.18 mEq per hour for mean MAO[14].

Endoscopy and biopsy

All patients underwent upper gastroduodenal endoscopy, using an Olympus GIF-Q10 or GIF-B3 endoscope. Biopsies were obtained from the anterior and posterior wall of the body, lesser curve of the gastric angle, and lesser curve of the gastric antrum, as arranged before the study. Mucosal biopsies were oriented in a fresh state on a small cardbord square with the luminal surface facing upward.

These were immediately fixed in 10% buffered formalin and processed into paraffin wax blocks in a standard fashion. Serial sections of the tissue were cut three microns in thickness on a Jung microtome, mounted on glass slides, and stained with hematoxylin and eosin.

The number of parietal cells

Using stained tissue samples, the number of parietal cells was counted per unit square (0.25 $mm^2$) in 3 to 4 different visual fields under a light microscope and the mean number was shown per unit area.

Histopathological evaluation was also performed on these stained tissue samples.

Radioimmunoassay of gastrin and somatostatin[7,22,23]

Gastrin in the blood and antral mucosa was determined by a specific radioimmunoassay system in our laboratory. Mucosal concentrations of gastrin were determined as follows. Biopsied tissue specimens obtained from the antrum were immediately frozen and kept at −70°C in a freezer until the assay. The tissue specimens were heated to boiling point, homogenized, and centrifuged and the supernatant thus obtained was diluted in Beronal buffer before the assay. Gastrin was then determined in the diluent by radioimmunoassay.

Plasma somatostatin determination was performed by a step extraction method and somatostatin in the extracted plasma was then determined by a specific radioimmunoassay system in our laboratory. For somatostatin determination in the mucosa, three biopsied tissue specimens each taken from the gastric body and antrum were immediately frozen in liquid nitrogen. After weighing the frozen tissue specimens, they were homogenized in a solution of 2N acetic acid in ice cold water. The homogenate was then boiled in hot water for 10 min and then centrifuged. The supernatant was freezed-dried and mucosal somatostatin was measured by a specific radioimmunoassay system in our laboratory. Antisera against gastrin and somatostatin used in this study were from Yokohama (gastrin) and our own (somatostatin).

Unlabeled antibody enzyme (peroxidase antiperoxidase) method for identification of G and D cells[21]

Prior to application of primary antisera, the sections were first treated with hydrogen peroxide to exhaust the endogenous peroxidase activity and then were incubated with normal goat

serum to block nonspecific background staining. The specific antisera (against gastrin or somatostatin) were applied for 12-18 hr at 4°C. The conjugated antiglobulins were used at room temperature. The following peroxidase antiperoxidase complexes were then applied: horseradish peroxidase and rabbit anti-horseradish peroxidase (Dakopatt A/S) at 1:300 at room temperature. The peroxidase activity was revealed by incubation for 2-3 min in a freshly prepared solution of 0.05% of 3, 3-diaminobenzidine tetrahydrochloride in phosphate buffered saline pH 7.2, containing 0.01% hydrogen peroxide.

The number of immunoreactive G and D cells was counted per unit area under a light microscope in the same fashion as the number of parietal cells.

Control for immunocytochemistry

In order to demonstrate that the immunocytochemical reactions were specific, the following tests were performed: 1) Prior to immunostaining, the diluted antisera were absorbed with samples of synthetic gastrin or somatostatin, 2) Normal rabbit serum was used instead of the primary antiserum as the first layer, 3) The PAP complex was applied alone and developed by the unlabeled antibody technique.

Statistical analysis

For statistical analysis, student's t test for nonpaired data (comparisons between groups) was employed and p values of 0.05 or less were considered to be significant.

## RESULTS

Gastric acid secretion

In the control group, BAO was 4.40 ± 5.32 mEq/hr, MAO was 16.53 ± 68 mEq/hr, MA was 110.30 ± 35.14 mEq/liter and SVR was 165.20 ± 61.02 ml/hr. Gastric acid secretion of patients with duodenal ulcer was divided into the following two groups. In one group, BAO was 2.57 ± 2.50 mEq/hr, MAO was 16.74 ± 2.31 mEq/hr, MA was 116.63 ± 14.75 mEq/liter, and SVR was 170.90 ± 35.43 ml/hr (normochlorhydric group, n=20), and in the other group, BAO was 9.41 ± 4.59 mEq/hr, MAO was 35.24 ± 2.37 mEq/hr, MA was 136.10 ± 15.66 mEq/liter, and SVR was 286.9 ± 67.58 ml/hr (hyperchlorhydric group, n=15). Statistically significant differences in BAO, MAO, MA and SVR were demonstrated between the hyperchlorhydric and normochlorhydric groups as well as normal control group. These data are shown in Fig. 1.

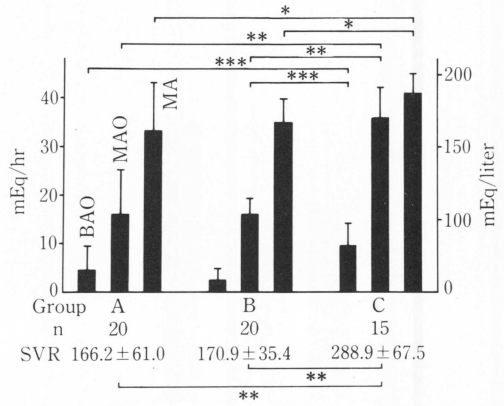


**Fig. 1.** Gastric acid secretion in patients with duodenal ulcer. A: normal control, B: normochlorhydric duodenal ulcer, C: hyperchlorhydric duodenal ulcer. BAO: basal acid output (mEq/hr), MAO: tetragastrin-stimulated maximal acid output (mEq/hr) MA: maximal acidity (mEq/liter), SVR: stimulated volume rate (ml/hr).

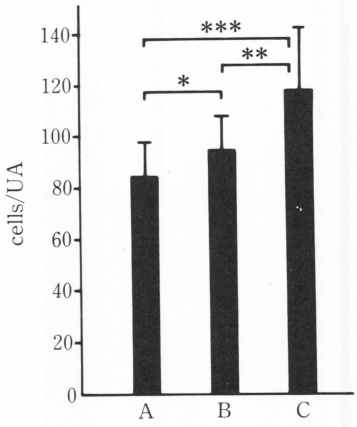


**Fig. 2.** The number of parietal cells per unit area is significantly increased in patients with hyperchlorhydric duodenal ulcer (C) when compared to that in patients with normochlorhydric duodenal ulcer (B) and normal control (A).

The number of parietal cells

The number of parietal cells per unit area was 84.1 ± 14.9 cells/UA in the normal control, 94.6 ± 14.9 cells/UA in the normochlorhydric group, and 118.7 ± 24.8 cells/UA in the hyperchlorhydric group (Fig. 2). The difference in number was statistically significant between the

normal control and the hyperchlorhydric group, and between the normochlorhydric and the hyperchlorhydric group.

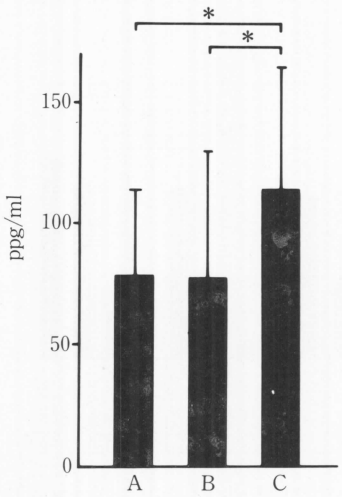


**Fig. 3.** A significant increase in basal levels of serum gastrin is observed in patients with hyperchlorhydric duodenal ulcer (C).

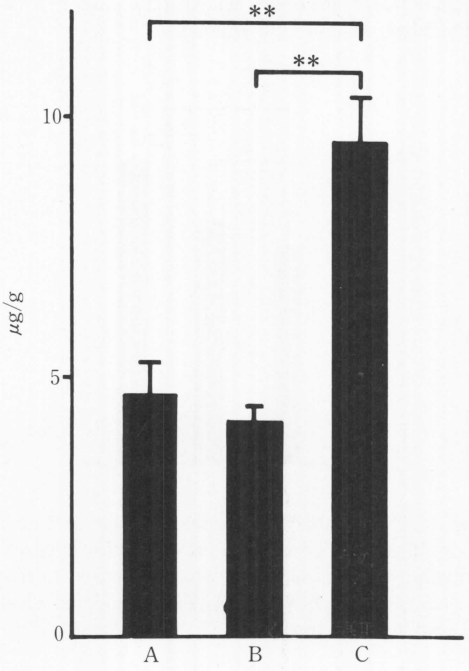


**Fig. 4.** A significantly elevated concentration of gastrin in the antral mucosa in patients with hyperchlohydric duodenal ulcer (C).

Gastrin

The serum gastrin assay revealed that patients in the hyperchlorhydric group had an elevated basal serum gastrin concentration (114.03 ± 50.52 pg/ml) when compared to the normal serum gastrin concentration of the patients in the normochlorhydric group (77.79 ± 42.07 pg/ml) and the normal control group (78.15 ± 36.45 pg/ml)(Fig. 3). The difference was statistically significant between the hyperchlorhydric and the normochlorhydric groups as well as the normal control group. Patients in the hyperchlorhydric group also had a significantly elevated mucosal gastrin concentration (9.25 ± 1.00 μg/g) at fasting when compared to the normal gastrin concentration of the patients in the normochlorhydric group (4.13 ± 0.52 μg/g) and the normal control group (4.80 ± 0.76 μg/g, Fig. 4).

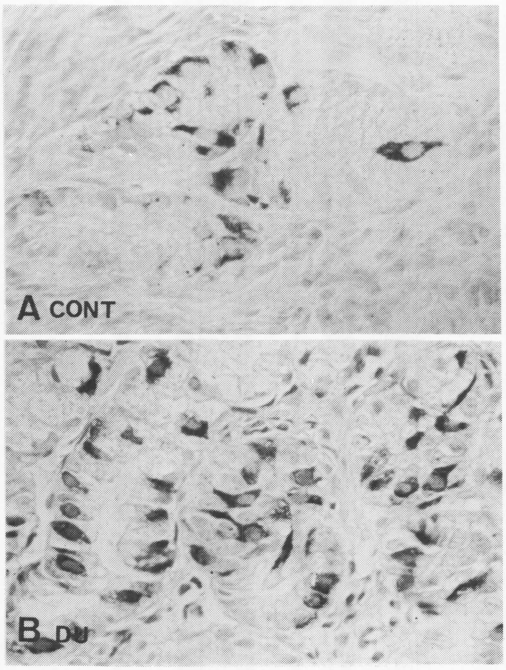


**Fig. 5.** Immunoreactive G cells in the antrum of a normal control subject (A), and a large number of immunoreactive G cells in the antrum of a patient with hyperchlorhydric duodenal ulcer (B), (PAP, original magnification × 400).

The mean number of antral gastrin G cells per unit area was significantly higher in patients in the hyperchlorhydric group (25.11 ± 7.68 cells/UA) than in the normochlorhydric group (9.90 ± 4.58 cells/UA) and the normal control group (7.84 ± 3.81 cells/UA, Fig. 5 A and B, Fig. 6).

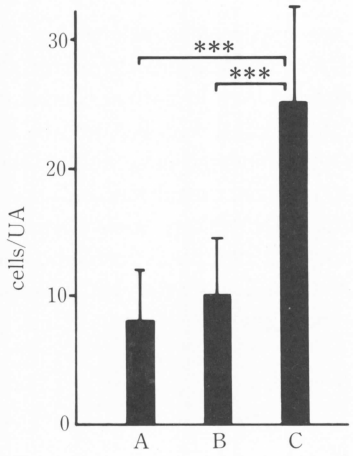


**Fig. 6.** A significant increase in the number of antral immunoreactive G cells per unit area is observed in patients with hyperchlorhydric duodenal ulcer (C).

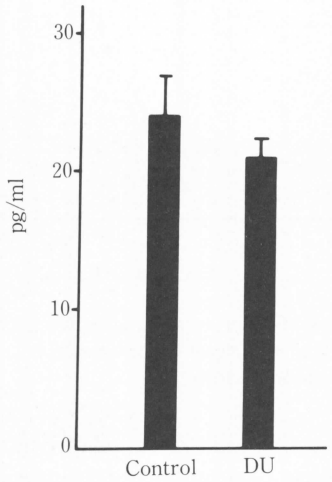


**Fig. 7.** Plasma somatostatin concentration in patients with duodenal ulcer is not different from that in normal control subjects.

Somatostatin

No difference was observed in the basal plasma somatostatin concentration between patients with duodenal ulcer (20.8 ± 1.5 pg/ml) and normal control subjects (23.8 ±2.6 pg/ml, Fig. 7). Mucosal somatostatin concentration in the antral mucosa at fasting was significantly lower in patients in the hyperchlorhydric group (0.57 ± 0.09 ng/ml) and normochlorhydric group (0.53 ± 0.04 ng/mg) than in normal control subjects (2.06 ± 0.21 ng/mg, Fig. 8), while the concentration in the gastric body was not significantly different between patients with duodenal ulcer (0.62 ± 0.16 ng/ml) and normal control subjects (1.11 ± 0.26 ng/ml, Fig. 8).

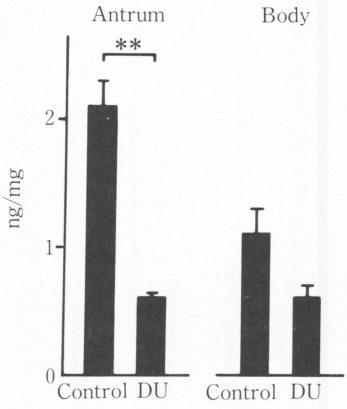


**Fig. 8.** Mucosal concentration of antral somatostatin is significantly reduced in patients with hyperchlorhydric and normochlorhydric duodenal ulcer when compared to that in normal control subjects. A significant difference in mucosal concentration of somatostatin in the gastric body could not be demonstrated between patients with duodenal ulcer and normal control subjects.

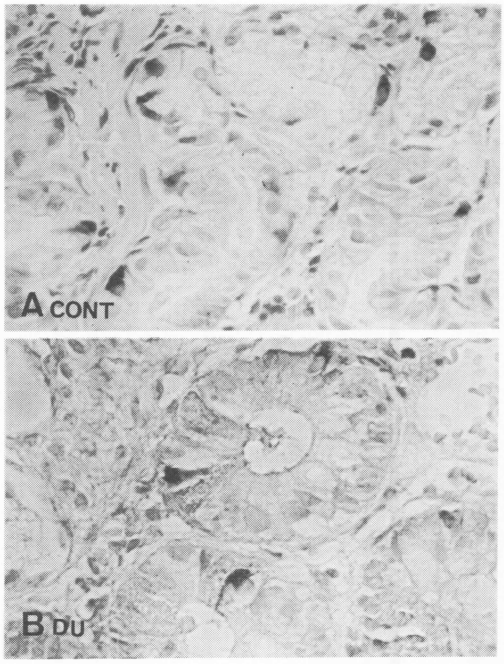


**Fig. 9.** Immunoreactive D cells in the antral mucosa of a normal control subject (A) and increase of immunoreactive D cells in the antrum of a patient with hyperchlorhydric duodenal ulcer (B)(PAP, original magnification × 400).

The mean number of somatostatin D cells was significantly lower in the antral mucosa of pa-

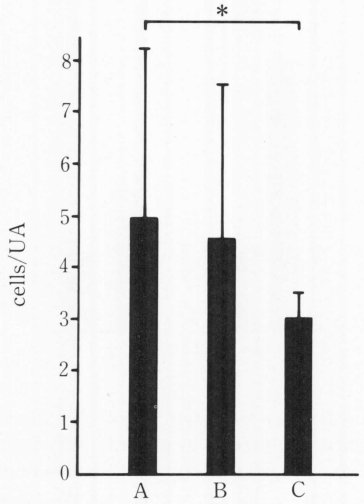


**Fig. 10.** A significant decrease in the number of immunoreactive D cells is observed in the antral mucosa of patients with hyperchlorhydric duodenal ulcer (C).

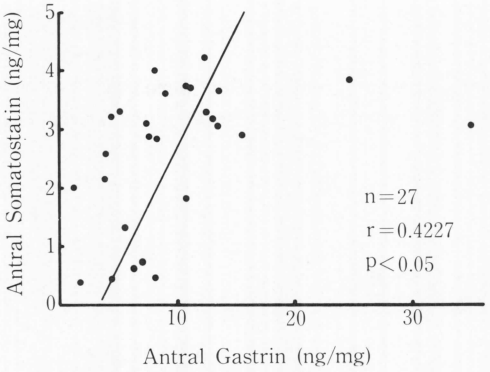


**Fig. 11.** There is a significant correlation between mucosal concentration of gastrin and somatostatin in normal control subjects.

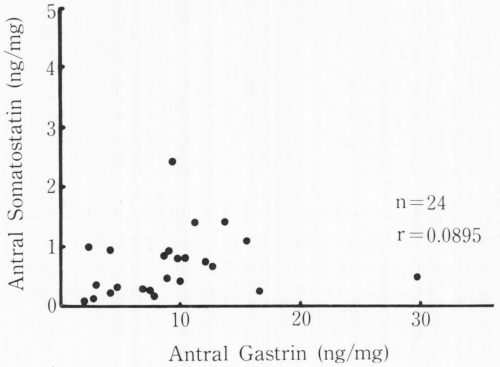


**Fig. 12.** Correlation between mucosal concentration of gastrin ad somatostatin in the antrum in patients with duodenal ulcer.

tients in the hyperchlorhydric group (3.00 ± 1.41 cells/UA) and normochlorhydric group (4.68 ± 3.43 cells/UA) than in normal control subjects (4.93 ± 3.23 cells/UA, Fig. 9 A and B, Fig. 10).

Patients with duodenal ulcer did not show any relationship between antral mucosal gastrin and somatostatin concentration, while normal control subjects showed a significant correlation between antral gastrin and somatostatin concentration (Figs. 11 and 12).

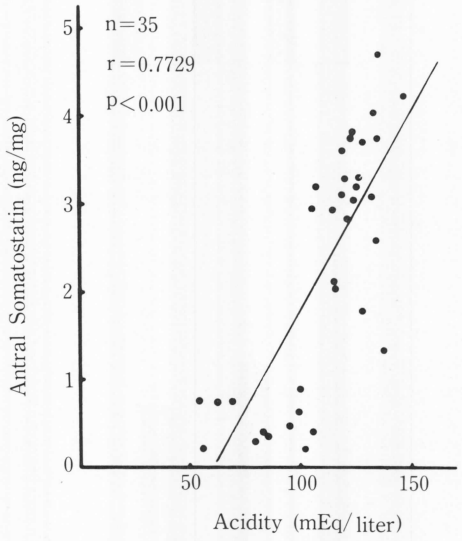


**Fig. 13.** A significant correlation between maximal acidity (MA) and mucosal concentration is observed in normal control subjects.

There was a significant correlation in normal control subjects between MA and antral mucosal somatostatin concentration (Fig. 13). However, a significant correlation between MA and antral mucosal somatostatin concentration could not be observed in patients with duodenal ulcer (Fig. 14).

## DISCUSSION

In the present study, patients with stimulated hyperchlorhydric duodenal ulcer had a large number of parietal cells per unit area (parietal cell hyperplasia). The ability to secrete acid is directly correlated with the total number of parietal cells in the gastric fundic mucosa of duodenal ulcer patients[5]. Therefore, stimulated hyperchlorhydria observed here in patients in this group of duodenal ulcer reflected a large number of parietal cells. The increased number

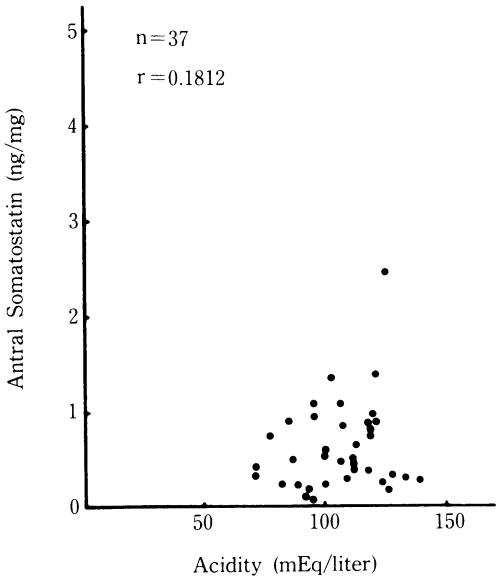


**Fig. 14.** There is no correlation between MA and mucosal concentration of antral somatostatin in patients with duodenal ulcer.

of parietal cells can be shown in the intact stomach by measuring maximal acid secretion by histamine or gastrin[9]. A good correlation was seen between maximal acid output and the number of parietal cells in patients with hyperchlorhydric duodenal ulcer in the present study. Maximal acid secretion in duodenal ulcer patients can be said to be twice the rate in normal subjects[25]. In the present study, maximal acid output in patients with hyperchlorhydric duodenal ulcer was also approximately twice that in patients with normochlorhydric duodenal ulcer and normal control subjects. Therefore, it might be correct to say that only in the subgroup of duodenal ulcer patients the increased secretion was due to the increased 'parietal cell mass' proposed by Card and Marks[3].

The pathogenesis of the increased number of parietal cells has been assumed to result from the trophic stimulus provided by excessive neural activity or by excessive amounts of circulating gastrin[24]. In the present study, basal hypergastrinemia and increased concentration of antral mucosal gastrin were present in patients with hyperchlorhydric duodenal ulcer. This is attributable to excessive antral synthesis and release of gastrin in patients in the subgroup (hyperchlorhydria) with duodenal ulcer. An increase in the number of antral gastrin G cells observed here could also contribute to this type of hypergastrinemia[15]. Gastrin has been shown to induce hypertrophic gastric mucosa in patients with Zollinger-Ellison syndrome associated with marked hypergastrinemia. However, marked hypergastrinemia may not always be necessary in inducing parietal cell hyperplasia in ordinary duodenal ulcer cases[20]. Slight but significant hypergastrinemia observed in patients with hyperchlorhydric duodenal ulcer may, therefore, contribute to the excessive number of parietal cells.

Significant basal hyperchlorhydria was also present in patients with hyperchlorhydric duodenal ulcer. It may be easily understood that the slight but significant hypergastrinemia led to a higher spontaneous secretion of acid. Basal hyperchlorhydria may have reflected the increased number of parietal cells. It has been indicated that in many patients with duodenal ulcer the increased basal acid secretion was strictly proportional to the increased maximal secretory capacity[10,11,26]. Basal hyperchlorhydria was, therefore, assumed to reflect an increase in the number of parietal cells as in the case of stimulated hyperchlorhydria (maximal acid secretion) also observed in patients with hyperchlorhydric duodenal ulcer.

Although the pathogenesis of basal hypergastrinemia remains to be elucidated in the present study, the excessive concentration of antral mucosal gastrin and the increased number of antral G cells observed here may contribute to it. Somatostatin is thought to inhibit gastrin release in the antrum and acid secretion in the fundic mucosa by both hormonal and paracrine pathways[2,12,13]. Significant reduction in antral somatostatin concentration and significant decrease in the number of antral D cells may be another pathogenesis for inducing hypergastrinemia. Several studies have demonstrated an inverse relationship between gastrin and somatostatin release in animals[17-19]. However, in the present study there was a significant correlation between mucosal concentration of antral gastrin and somatostatin in normal control subjects, suggesting that regularity in distribution of both peptides and interaction may exist between G and D cells int he antrum and fur-

thermore the existence of positive feedback mechanism between gastrin (G cells) and somatostatin (D cells). On the contrary, such correlation could not be seen in patients with duodenal ulcer, mainly hyperchlorhydric, suggesting that such a regularity and interaction were not present in duodenal ulcer.

Somatostatin release in stimulated by perfusion of the stomach with acid, and injection of somatostatin antiserum stimulates gastrin release from the isolated perfused stomach[18,19]. This suggests that somatostatin D cells exert an inhibitory effect on G cells.

It can be therefore said that somatostatin mediates acid inhibition of gastrin release. In other words, somatostatin may control gastrin release from G cells through acid. A significant reduction of mucosal concentration of antral somatostatin as well as D cells in the antrum indicates a defect in the control mechanism of patients with hyperchlorhydric duodenal ulcer, although data are controversial about concentration and the number of antral D cells in duodenal ulcer patients[1,4,6,16,23]. A functional alteration in release of gastrin in patients with hyperchlorhydric duodenal ulcer may be due to decrease in the number of D cells in the antrum.

As for gastric acid secretion and somatostatin, a significant correlation was observed between MA but not MAO and mucosal concentration of somatostatin in normal control subjects, suggesting that hydrogen ion concentration may control the function of somatostatin D cells in the antrum. On the contrary, no correlation was seen between MA and somatostatin in patients with hyperchlorhydric duodenal ulcer, suggesting that hydrogen ion concentration can not control D cells and, therefore, the resulting somatostatin mediated by acid can not inhibit gastrin release in hyperchlorhydric cases with duodenal ulcer. This may be another pathogenesis of basal hypergastrinemia in patients with hyperchlorhydric duodenal ulcer.

Lastly, it remains to be elucidated in the present study what factors can initiate antral G cell hyperplasia observed in patients with hyperchlorhydric duodenal ulcer. Diathesis to duodenal ulcer may be one factor and genesis may be another factor. There are, however, no evidences for both factors. For elucidation of the pathogenesis of G cell hyperplasia it is necessary to examine more cases with hyperchlorhydric duodenal ulcer.

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