Marked Increase in Fundic Mucosal Histidine Decarboxylase Activity in a Patient with H⁺,K⁺-ATPase Antibody-Positive Autoimmune Gastritis

Yoshiji MIYAZAKI, Yasuhisa SHINOMURA, Yoko MURAYAMA, Ikuo IMAMURA*,
Hiroyuki FUKUI*, Masatomo MAEDA**, Masamitsu FUTAI** and Yuji MATSUZAWA

A 63-year-old woman was diagnosed as autoimmune gastritis by the presence of serum antibody against α -subunit of gastric H⁺,K⁺-ATPase. The patient did not have pernicious anemia, but showed achlorhydria, marked hypergastrinemia, enterochromaffin-like cell hyperplasia and an extremely high histidine decarboxylase activity in the gastric fundic mucosa. Intragastric acidification by infusion of hydrochloric acid via a nasogastric tube induced a transient reduction of serum gastrin level and fundic mucosal histidine decarboxylase activity. A marked increase in fundic mucosal histidine decarboxylase activity as well as hypergastrinemia appears to be the pathophysiologic response to achlorhydria caused by autoimmunity against gastric H⁺,K⁺-ATPase. (Internal Medicine 32: 602–606, 1993)

Key words: histamine, gastrin, atrophic gastritis, enterochromaffin-like cell, achlorhydria

Introduction

It has been well established that achlorhydria induced by H⁺,K⁺-ATPase inhibitors, such as omeprazole, exerts a great influence on histamine metabolism in the fundic mucosa of rats. Long-term treatment with high doses of omeprazole causes in the rat fundic mucosa hyperplasia of so-called enterochromaffin-like (ECL) cells, the major histamine producing endocrine cells, and increase in histamine content and histamine-producing enzyme, histidine decarboxylase (HDC) activity (1-3). In addition, investigations have indicated that hypergastrinemia plays a major role in the over-production of histamine and proliferation of ECL cells in the fundic mucosa of rats treated with omeprazole (1-3).

In the case of humans, achlorhydria, hypergastrinemia and fundic mucosal ECL cell hyperplasia have often been reported in patients with Type A or autoimmune gastritis, a type of gastritis characteristic of diffuse corporal atrophy with an intact antral mucosa (4-7). However, the major histamine containing cells in the human gastric mucosa have been considered to be mast cells and not ECL cells (8-10), and the pathophysiologic changes in histamine metabolism in the gastric mucosa associ-

ated with achlorhydria in humans are still not well understood. Herein, we report a case of autoimmune gastritis who was positive for serum anti H⁺,K⁺-ATPase antibody and showed achlorhydria, hypergastrinemia, ECL cell hyperplasia and a marked increase in fundic mucosal HDC activity.

Case Report

A 63-year-old woman was diagnosed with multiple gastric polyps at Osaka University Medical School Hospital in July 1989. She had a past history of Basedow's disease which was treated with isotope therapy in 1973. Upper gastrointestinal series showed the presence of multiple polyps distributed in the corpus area but she had no symptoms associated with gastric polyps. Laboratory examinations revealed an extremely high concentration of serum gastrin. Fasting serum gastrin level was 3,492 pg/ml (normal range: 30–180 pg/ml). Basal acid output was 0 mEq/hr which was not changed after pentagastrin injection (6 μ g/kg, s.c.). These data indicate that the hypergastrinemia was due to achlorhydria.

Serological tests revealed the existence of several auto-antibodies. Serum anti-nuclear antibody was positive at a titer

From the Second Department of Internal Medicine and *the Department of Pharmacology II, Osaka University Medical School, **the Department of Organic Chemistry and Biochemistry, Institute of Scientific and Industrial Research, Osaka University, Suita

Received for publication January 20, 1993; Accepted for publication July 13, 1993

Reprint requests should be addressed to Dr. Yoshiji Miyazaki, the Second Department of Internal Medicine, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565, Japan

of 1:160. Antimicrosomal antibody was present at a titer of $1:2^{100} \times 100$ but anti-thyroglobulin antibody was negative. Serum parietal cell autoantibodies (PCA) as measured by immunofluorescence were present at a titer of 1:320. Serum antibody against intrinsic factor was negative and serum vitamin B₁₂ level was within normal range (905 pg/ml, normal range: 200–1,000 pg/ml). The patient showed no abnormalities in the peripheral blood examinations. The presence of antibody against H⁺,K⁺-ATPase was checked. Isolated porcine tubulovesicular membranes were solubilized by boiling at 55°C for 15 minutes (11). The solubilized proteins were separated by 10% SDS-PAGE and electrophoretically transferred to nitrocellulose membranes (12). The membranes were first incubated with sera (diluted 1:50 in phosphate-buffered saline) for 60 minutes at room temperature, and then with iodinated Protein A for 60 minutes. Bound Protein A was detected by autoradiography. The positive band at 94 kD indicates the presence of antibody against H⁺,K⁺-ATPase α -subunit (Fig. 1). The epitopes recognized were also determined by ELISA assays using fusion proteins containing segments of the α -subunit (data not shown) (13). Antibody against β -subunit could not be detected.

Endoscopy was first performed on August 12, 1990. Biopsy specimens were obtained from polyps and background mucosa to exclude the presence of carcinoid since the high prevalence of gastric carcinoid and/or diffuse ECL cell hyperplasia has often been discussed in patients with hypergastrinemia in the literature (4-6). Informed written consent was obtained from the patient before endoscopy. Endoscopic biopsies from polyps, stained with hematoxylin and eosin, showed hyperplasia

of foveolar cells (Fig. 2A). Several biopsies from apparently normal fundic mucosa, stained with Grimelius' silver impregnation, showed loss of normal fundic glands and severe hyperplasia of ECL cells (Fig. 2B). Hyperplasia of gastrin cells was observed in the antral mucosa (data not shown).

It is well recognized that ECL cell population, histidine decarboxylase (HDC) activity and histamine content are dramatically increased in the fundic mucosa of rats by drug-induced achlorhydria (1-3). Then, gastric mucosal HDC activity and histamine content were determined in endoscopic biopsy specimens of this patient. Histamine content and HDC activity were determined by the method described elsewhere (14, 15). Biopsy specimens were homogenized in 0.1 M potassium-phosphate buffer (pH 7.0) containing 0.01 mM amino-guanidine, 1.0% polyethyleneglycol (relative molecular weight 300), 0.01 mM pyridoxal-5'-phosphate and 20 μ g/ml phenyl-methylsulfonyl fluoride (PMSF). Each 50 μ l of homogenate was deproteinized by adding 450 μ l of 3% perchloric acid and centrifuged at 15,000 rpm for 10 minutes. Histamine in the supernatant was determined by the high performance liquid chromatography (HPLC) method developed in our laboratory

Fig. 1. Western blot analysis showing the existence of antibody to H⁺,K⁺-ATPase. Lane C: rabbit anti IgG against porcine H⁺,K⁺-ATPase α -subunit. Lanes N1 and N2: normal human sera. Lane P: patient's serum. The band at 94 kD corresponds to H⁺,K⁺-ATPase α -subunit.

Fig. 2. Polyp tissue stained with hematoxylin and eosin (A) The polyp tissue is composed mostly of foveolar cells ($\times 100$). Fundic mucosa stained by the Grimelius method (B) Loss of normal fundic glands and severe hyperplasia of enterochromaffin-like (ECL) cells are seen ($\times 200$).

(16). HDC was assayed by the determination of histamine formed from L-histidine in the extract. Each remaining extract was centrifuged at 15,000 rpm for 10 minutes at 4°C. The supernatant was dialyzed twice against the extraction buffer at 4°C. The reaction was started by adding L-histidine (0.25 mM final concentration) to the dialysate and stopped after 3 hours of incubation at 37°C. Histamine formed during the reaction was determined by the HPLC method. Blanks with α -fluoromethyl-histidine (α -FMH), a potent and specific inhibitor of HDC, were used throughout the study.

The results of gastric mucosal HDC activity and histamine content in the patient were compared with those in control subjects described previously (15). Fundic mucosal HDC activity in the patient was 1,527 f mol/min/mg protein, which was 13-fold that of control subjects (Table 1). The levels of HDC activity in the polyp tissue and in the antral mucosa were 87 and 42 fmol/min/mg protein, respectively. Fundic mucosal histamine content in the patient was about 2-fold that of controls (Table 1).

An attempt was made to determine if the extremely high HDC activity in the fundic mucosa as well as hypergastrinemia was a physiological response to achlorhydria and not due to hyperfunction of tumorous cells. It was reported that intragastric acidification can differentiate hypergastrinemia due to achlorhydria from that due to gastrinoma (17). The following examinations were performed with written informed consent. Serum gastrin concentration and HDC activity were determined after intragastric infusion of hydrochloric acid on October 8, 1990. Three hundred milliliters of 0.1N hydrochloric acid was infused into the stomach via a nasogastric tube for 10 minutes. Blood samples were collected every 15 minutes until 120 minutes after intragastric acidification, when endoscopic biopsy was performed. Intragastric acidification caused a dramatical decrease in the serum gastrin level, indicating that hypergastrinemia was due to achlorhydria (Fig. 3). In addition, endoscopic biopsy specimens obtained 120 minutes after intragastric acidification showed a marked decrease in the fundic mucosal HDC activity (599 fmol/min/mg protein) (Table 2). The levels of serum gastrin and HDC activity, however, were still higher than those in controls after intragastric acidification. Hypergastrinemia and increased HDC activity in the fundic mucosa

Table 1. Serum Gastrin, Gastric Mucosal Histamine Content and HDC Activity

	Serum gastrin (pg/ml)	Histamine content (nmol/g tissue)		HDC Activity (fmol/min/mg protein)	
		Corpus	Antrum	Corpus	Antrum
Patient	4360	239	65	1527	42
		153(P)		87(P)	
Controls	78±9	143±10	120±14	120±13	39±5

Data of control subjects, expressed as mean±S.E. (n=19), are quoted from our previous publication (15). P: Tissue specimen obtained from one of the gastric polyps in the corpus area, HDC: histidine decarboxylase.

Fig. 3. Serum gastrin response to acidification of the stomach by infusion of 300 ml of 0.1 N hydrochloric acid via a nasogastric tube. Endoscopic biopsy was performed 120 minutes after intragastric acidification.

Table 2. Serum Gastrin, Fundic Mucosal Histamine Content and HDC Activity of the Patient during 2-year Observation Period

Date	Serum gastrin (pg/ml)	Histamine content (nmol/g tissue)	HDC Activity (fmol/min/mg protein)
8/12/90	4360	239	1527
10/1/90	3539	236	1480
10/8/90*	1711	243	599
6/17/91	4485	295	1695
4/22/92	3550	193	1375

*Endoscopy after intragastric HCl infusion was performed on October 8, 1990. HDC: histidine decarboxylase.

of the patient persisted until the latest observation in April 1992 (Table 2).

Discussion

The present case was considered to be Type A or autoimmune gastritis, a clinical entity first proposed by Strickland and Mackay (18, 19), characterized by the presence of serum parietal cell autoantibody (PCA), achlorhydria and hypergastrinemia. Two major circulating gastric autoantibodies were proposed as PCA of Type A gastritis, previously. De Aizpurua et al proposed the primary importance of a circulating autoantibody to the gastrin receptor in patients with pernicious anemia which evolves almost exclusively from type A gastritis (20). More recently, Burman et al showed the presence of antibody against H^+,K^+ -ATPase in the sera of patients with pernicious anemia which inhibits H^+,K^+ -ATPase activity *in vitro* (21-23). The present case had no pernicious anemia but showed the presence of serum autoantibody against α -subunit of H^+,K^+ -ATPase that might play an important role in sustained achlorhydria.

The following two pathophysiologic features have often been described in Type A gastritis. First, hypergastrinemia caused by hyperplasia and hyperfunction of antral G-cells. Second, diffuse ECL cell hyperplasia and/or development of multiple ECL cell carcinoidosis (ECLoma) in the fundic mucosa (4-7). The present case also showed hypergastrinemia and fundic mucosal ECL cell hyperplasia. Recent investigations revealed that sustained achlorhydria induced by inhibition of H^+,K^+ -ATPase in rats causes hypergastrinemia and fundic mucosal ECL cell hyperplasia. There is considerable evidence which suggests that the proliferation rate of ECL cells in the rat fundic mucosa is regulated by serum gastrin concentration (1-3). In addition, Hirschowitz et al reported that ECL gastric carcinoids in patients with pernicious anemia disappeared after antrectomy (7). This suggests that the proliferation rate of ECL cells in humans as well as in rats is under the control of serum gastrin concentration. Thus, fundic mucosal ECL cell hyperplasia in the present case is suspected to be promoted by persistent hypergastrinemia due to sustained achlorhydria.

It has been well recognized that ECL cells in the rat gastric fundic mucosa contain large amounts of histamine and histamine producing enzyme, HDC (10, 24, 25). Therefore, proliferation of ECL cells results in a great increase in histamine content and HDC activity in the rat fundic mucosa. However, there are few reports about pathophysiologic changes in histamine metabolism in the human gastric mucosa associated with achlorhydria. Only Cattani et al have recently described that fundic mucosal HDC activity and histamine content are correlated with ECL cell density in atrophic gastritis with hypergastrinemia (26). Previous histochemical investigations have demonstrated that mast cells are rather numerous compared to ECL cells in the stomach of such species like humans and dogs (8-10). In addition, the presence of histamine has been demonstrated mostly in mast cells but not in ECL cells in human and canine gastric mucosa (8-10). Biochemical investigations showed that human gastric mucosa has an extremely low or no HDC activity, despite the high content of histamine (27, 28). The low level of HDC activity in the gastric mucosa where histamine-containing mast cells are rich may be explained by the slow rate of histamine turn-over in mast cells.

The present case showed some pathophysiologic changes in histamine metabolism in the gastric mucosa. First, the patient showed an extremely high HDC activity (1.5 pmol/min/mg protein) compared to controls in the fundic mucosa where ECL cells exist abundantly. The fundic mucosal HDC activity in the patient was as high as that in fasted rats determined by our own method (2 pmol/min/mg protein) (29). The levels of HDC activity in the polyp tissue and antral mucosa where ECL cells scarcely exist, however, were lower compared to that of control fundic mucosa. Thus, HDC activity appears to reflect the population of ECL cells in the gastric mucosa. Second, histamine content was also increased in the patient's fundic mucosa where ECL cells were rich. However, increase in histamine content was not so prominent as in HDC activity, that is to say, histamine turn-over is rather rapid in ECL cells compared to mast cells. ECL cells may produce and secrete histamine but

Fig. 4. Hypothesis for the pathophysiologic changes in the present case.

may not store histamine for a long time. Third, intragastric acidification induced simultaneous reduction of serum gastrin concentration and fundic mucosal HDC activity, indicating that the increase in fundic mucosal HDC activity as well as hypergastrinemia is a partially reversible change and that gastrin may control histamine formation in ECL cells located in the fundic mucosa. Fundic mucosal HDC activity in the patient appears to reflect not only the ECL cell population but also activation of each ECL cell by gastrin.

Figure 4 shows a hypothesis for the pathophysiologic changes in the present case. Achlorhydria caused by autoimmunity against gastric H^+,K^+ -ATPase appears to induce hypergastrinemia, resulting in ECL cell hyperplasia and a great increase in HDC activity in the fundic mucosa. The present case suggests that sustained achlorhydria in human subjects induced by inhibition of gastric H^+,K^+ -ATPase may increase fundic mucosal histamine formation as well as the serum gastrin concentration.

References

- 1) Larsson H, Carlsson E, Mattson H, et al. Plasma gastrin and gastric enterochromaffin-like cell activation and proliferation. Studies with omeprazole and ranitidine in intact and antrectomized rats. *Gastroenterology* **90**: 39, 1986.
- 2) Tieleman Y, Håkanson R, Sundler F, Willems G. Proliferation of enterochromaffin-like cells in omeprazole-treated hypergastrinemic rats. *Gastroenterology* **96**: 723, 1989.
- 3) Tieleman Y, Håkanson R, Sundler R, Willems G. Proliferation of enterochromaffin-like cells in omeprazole treated hypergastrinemic rats. *Gastroenterology* **96**: 723, 1989.
- 4) Borch K, Renvall H, Liedberg G. Gastric endocrine cell hyperplasia and carcinoid tumors in pernicious anemia. *Gastroenterology* **88**: 638, 1985.
- 5) Borch K. Atrophic gastritis and gastric carcinoid tumors. *Ann Med* **21**: 291, 1989.
- 6) Roucayrol AM, Cattani D. Evolution of argyrophil cell hyperplasia in nonantral atrophic gastritis. *Gastroenterology* **99**: 1307, 1990.
- 7) Hirschowitz BI, Griffith J, Pellegrin D, Cummings OW. Rapid regression of enterochromaffin-like cell gastric carcinoids in pernicious anemia after

- antrectomy. *Gastroenterology* **102**: 1409, 1992.
- 8) Håkanson R, Lijia B, Owman CH. Cellular localization of histamine and monoamines in the gastric mucosa of man. *Histochemie* **18**: 74, 1969.
 - 9) Mohri K, Reimann HJ, Lorenz W, Troidl H, Weber D. Histamine content and mast cells in human gastric and duodenal mucosa. *Agents Actions* **8**: 327, 1978.
 - 10) Håkanson R, Bottcher G, Ekblad E, et al. Histamine in endocrine cells in the stomach. A survey of several species using a panel of histamine antibodies. *Histochemistry* **86**: 5, 1986.
 - 11) Maeda M, Tagaya M, Futai M. Modification of gastric (H^+K^+)-ATPase with pyridoxal 5'-phosphate. *J Biol Chem* **263**: 3652, 1988.
 - 12) Towbin HS, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc Natl Acad Sci USA* **76**: 4350, 1979.
 - 13) Wang X-H, Miyazaki Y, Shinomura Y, et al. Characterization of human autoantibodies reactive to gastric parietal cells. *Biochem Biophys Res Commun* **190**: 207, 1993.
 - 14) Taguchi Y, Watanabe T, Kubota H, Hayashi H, Wada H. Purification of histidine decarboxylase from the liver of fetal rats and its immunochemical and immunohistochemical characterization. *J Biol Chem* **259**: 5214, 1984.
 - 15) Miyazaki Y, Imamura I, Shinomura Y, Wada H, Tarui S. Marked increase in gastric histidine decarboxylase activity in patients with hypergastrinemia. *Life Sci* **48**: 51, 1991.
 - 16) Yamatodani A, Fukuda H, Wada H, Iwaeda T, Watanabe T. High-performance liquid chromatographic determination of plasma and brain histamine without previous purification of biological samples: Cation-exchange chromatography coupled with post-column determination fluorometry. *J Chromatograph* **344**: 115, 1985.
 - 17) Griffith JL, Cummings OW, Hirschowitz BI. Development of sustained achlorhydria in a patient with the Zollinger-Ellison syndrome treated with omeprazole. *Gastroenterology* **101**: 242, 1991.
 - 18) Strickland RG, Mackay IR. A reappraisal of the nature and significance of chronic atrophic gastritis. *Am J Dig Dis* **18**: 426, 1973.
 - 19) Mackay IR. Autoimmune serological studies in chronic gastritis and pernicious anemia. *Gut* **5**: 23, 1964.
 - 20) De Aizpurua HJ, Ungar B, Toh BH. Autoantibody to the gastrin receptor in pernicious anemia. *N Engl J Med* **313**: 479, 1985.
 - 21) Burman P, Mardh S, Norberg L, Karlsson FA. Parietal cell antibodies in pernicious anemia inhibit H^+K^+ adenosine triphosphatase, the proton pump of the stomach. *Gastroenterology* **96**: 1434, 1989.
 - 22) Kahlsson FA, Burman P, Loof L, Mardh S. The major parietal antigen in autoimmune gastritis with pernicious anemia is the acid-producing H^+K^+ -ATPase of the stomach. *J Clin Invest* **81**: 475, 1988.
 - 23) Toh BH, Gleeson PA, Simpson RJ, et al. The 60- to 90-kDa parietal cell autoantigen associated with autoimmune gastritis is a β -subunit of the gastric H^+K^+ -ATPase (proton pump). *Proc Natl Acad Sci USA* **87**: 6418, 1990.
 - 24) Soll AH, Levin KJ, Beaven MA. Isolation of histamine containing cells from rat gastric mucosa: Biochemical and morphologic differences from mast cells. *Gastroenterology* **80**: 717, 1981.
 - 25) Kubota H, Taguchi Y, Tohyama M, et al. Electron microscopic identification of histidine decarboxylase-containing endocrine cells of the rat gastric mucosa. *Gastroenterology* **87**: 496, 1984.
 - 26) Cattan D, Roucayrol AM, Launay JM, et al. Circulating gastrin, endocrine cells, histamine content, and histidine decarboxylase activity in atrophic gastritis. *Gastroenterology* **97**: 586, 1989.
 - 27) Aures D, Davidson WD, Håkanson R. Histidine decarboxylase in gastric mucosa of various mammals. *Eur J Pharmacol* **8**: 100, 1969.
 - 28) Dencker H, Kahlson G, Kockum I, Norrby C, Rosengren E. Histamine metabolism in human gastric mucosa. *Clin Sci Mol Med* **45**: 225, 1973.
 - 29) Kawabata S, Kanayama S, Shinomura Y, et al. Effect of cholecystokinin receptor antagonists, MK-329 and L-365,260, on cholecystokinin-induced acid secretion and histidine decarboxylase activity in the rat. *Regul Pept* **35**: 1, 1991.