Histamine Synthesis after Administration of Gastrin and Blockade of Acid Secretion in the Rat Stomach

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Histidine decarboxylase (HDC) activity in the oxyntic gland and gastric volume were measured in rats treated with tetragastrin, cimetidine or omeprazole. HDC activity was dose dependently activated by not only tetragastrin but also cimetidine and omeprazole treatment. Histamine concentration in the oxyntic gland was reduced, but the amount of histamine in the gastric contents was increased by tetragastrin treatment. In rats premedicated with cimetidine or omeprazole, histamine concentration in the oxyntic gland and the amount of histamine in the gastric contents were not changed by administration of tetragastrin.

It was concluded that tetragastrin activated HDC which increased histamine release into the gastric contents. Cimetidine and omeprazole induced the secretion of endogeneous gastrin, leading to the activation of HDC.

(Key Word: Gastric histamine, histidine decarboxylase activity, gastric secretion, histamine output, tetragastrin stimulation)

INTRODUCTION

Peptic ulcers are one of the most common diseases affecting adults and stress is known to be one of the causes. In the formation of ulcers, it has been postulated that stress activates gastrin production, which subsequently activates histidine decarboxylase in the oxyntic glands (6, 9). Gastric mucosal histamine is then released into the gastric contents or plasma, stimulating gastric acid production in the stomach (7). Histamine is thus a key modulator not only in the onset but also in the prognosis of peptic ulcer disease. Recently, the histamine H2-receptor blocker cimetidine has been used in patients with gastric ulcers. In spite of daily administration of a H2 blocker, some patients still have recurrent or intractable ulcers. This appears to be related to changes in gastric histamine and HDC activity. We investigated the effects of cimetidine or omeprazole premedication on HDC activity, the histamine concentration in the oxyntic gland and gastric juice in tetragastrin stimulated rats, and also examined the changes in histamine synthesis in the rat stomach after administration of gastrin and acid secretion blockers.

MATERIALS AND METHODS

Male rats of the JCL-Wistar strain, weighing about 250g, were used. Before each experiment, the animals were placed in wire mesh bottom cages and were deprived of food but not water for 24 hours. For measurement of gastric histamine concentration and HDC activity, the rats were randomly assigned to five groups. Group A was administered tetragastrin (10, 50, 100, 200μg/kg) intraperitoneally. Group B was administered cimetidine (5, 10, 30, 100, 200mg/kg) intraperitoneally. Group
C was administered omeprazole (5, 10, 50, 100, 200μmol/kg) intraperitoneally. Group D was premedicated with cimetidine (100mg/kg) intraperitoneally 30 minutes before tetragastrin (10, 50, 100, 200μg/kg, ip) administration. Group E was premedicated with omeprazole (100μmol/kg) intraperitoneally 30 minutes before tetragastrin (10, 50, 100, 200μg/kg, ip) administration. The abdomen and thorax were opened under diethyl ether anesthesia 2 hours after administration of tetragastrin (Group A, D, E), cimetidine (Group B) or omeprazole (Group C). Blood samples were taken from the left ventricle for measurement of endogeneous gastrin. The stomach was removed, cut open along the major curvature and washed with ice-cold NaCl solution. Samples were collected from the total gastric layer of the fundus. For measurement of gastric juice volume and the amount of histamine in the gastric juice, the rats were assigned to three groups. One group was administered tetragastrin (200μg/kg, ip) alone. The other two groups were premedicated with cimetidine (100mg/kg, ip) or omeprazole (100μmol/kg, ip) 30 minutes before tetragastrin administration. Gastric juice samples were taken by the pylorus-ligation method 2 hours after tetragastrin administration.

1) Histamine concentration
We used a modification of the method of Shore (10). Briefly, each sample was weighed, homogenized in a glass homogenizer at a dilution of 10:1 in 0.25N perchionic acid and centrifuged at 6,000g (4°C, 15 min) to remove tissue debris. After centrifugation, 40μl of the supernatant was mixed with 0.25N HCl. Then 200μl of 2N NaOH and 100μl of 1% Orthophthalaldehyde-methanol solution were successively added to the sample to develop fluorescence. After mixing for exactly 5 min in darkness, 200μl of 1M H2SO4 was added to stop the fluorescence reaction. Then a 20μl volume of the mixture was injected for HPLC-fluorescence analysis.

The liquid-chromatographic system consisted of a Shimadzu LC-5A, a Shimazu RF-530 spectrometer and a Shimadzu Chromatopac CR3A. A stainless-steel ODS-inertsil column was used. The mobile phase was a mixture of 0.1M acetic acid buffer adjusted to pH 3.8 with 2N NaOH using a pH meter and acetonitrile (30:10).

2) Histidine decarboxylase activity
We used a modification of the method of Kobayashi (5). Histidine decarboxylase was prepared from rat tissue as follows. The tissue was excised, and washed quickly with physiological saline. The full thickness of the fundus and antrum was scraped off and homogenized in 0.1M phosphate buffer, pH 6.8 (final tissue concentration: 100 mg wet weight per ml). Then the sample was centrifuged at approximately 6,000 g for 15 minutes at 4°C and the cell free solution was decanted. Enzyme activity was measured as 14CO2 produced from (1-14C)-L-Histidine. The reaction mixture contained, in a final volume of 2.2 ml. 10 μg of pyridoxal phosphate carboxyl 14C-L-histidine (0.1μCi containing 5 μg of histidine and 0.1M phosphate buffer, pH6.8. The reaction was carried out in a microtube which was incubated at 37°C for 2h, after which the reaction was stopped by the addition of 200 μl of 1M citric acid. Sample was immersed in scintillation fluid for determination of radioactivity after further incubation for 1h. All assays were performed in duplicate and the enzyme activities were expressed as pmole CO2 formed per mg of tissue per hour. The results were corrected using a boiled tissue blank.

3) Endogeneous gastrin concentration
Blood samples were taken by cardiac puncture 2 hours after injection of tetragastrin (Groups A D and E) and cimetidine (Group B) and omeprazole (Group C). After centrifugation, plasma was stored in a freezer until analysis. Serum gastrin was measured by the gastrin RIA-PEG method (Special Reference Laboratories).

RESULTS

1) Serum gastrin concentration
Fig. 1 shows the changes in serum gastrin concentration in Group B (administration of cimetidine) and Group C (administration of omeprazole). Serum gastrin concentration was significantly higher than the basal control value in both groups.

2) HDC activity of the fundic gland
Fig. 2 shows changes in HDC activity in the
Fig. 1 Effects of cimetidine (Group B) and omeprazole (Group C) on serum gastrin concentration.

Fig. 2 Changes of HDC activity in the fundic gland after administration of tetragastrin (Group A), cimetidine (Group B), omeprazole (Group C) and premedication with cimetidine (Group D) and omeprazole (Group E).
fundic gland. HDC activity remarkably increased in Group A (administration of tetragastrin). Group B and Group C. Similar changes were observed in Group D (premedication with cimetidine) and Group E (premedication with omeprazole). However, Groups D and E showed high activities from the first due to cimetidine (100 mg/kg) and omeprazole (100 μmol/kg) premeditation.

3) Concentration of tissue histamine in the fundic gland

Groups B and C did not show any changes in histamine concentration in the fundic gland. However, Group A showed significantly reduced histamine concentration. In contrast, Groups D and E did not show any changes (Fig. 3).

4) Gastric juice secretion and the amount of histamine in the gastric juice

In Group A, gastric juice secretion and the amount of histamine in the gastric juice were significantly increased in comparison with the basal control. In Groups D and E, not only gastric juice secretion but also the amount of histamine in the gastric juice was significantly decreased (Fig. 4).

DISCUSSION

In this study, the amount of histamine in the oxyntic gland area and gastric contents, HDC activity in the oxyntic gland and gastric juice secretion were investigated in tetragastrin stimulated rats and cimetidine or omeprazole premedicated rats. HDC activity was dose dependently activated not only by tetragastrin but also by cimetidine or omeprazole treatment. Histamine concentration of the oxyntic gland was reduced, but histamine release into gastric contents was increased by tetragastrin treatment. In cimetidine or omeprazole premedicated rats, histamine concentration and histamine output did not change due to administration of tetragastrin. The results of HDC activity and histamine concentration in rats treated with tetragastrin can be explained by the hypothesis of Kahlson et al. (4) and Hakansson et al. (3) that acid secretion of gastrin is related to gastric mucosal histamine. The administration of tetragastrin induced activation of HDC, which led to the secretion of histamine in the oxyntic gland. Histamine increased gastric acid production and part of the histamine was immediately released into the gastric contents.

Interesting findings were obtained in the rats treated with cimetidine and omeprazole. Our results showed that both drugs activated HDC, and activation of HDC should increase the concentration of histamine in the oxyntic gland. However, our results were the opposite. Since the amount of histamine in the gastric contents did not change due to cimetidine or omeprazole treatment, it appears that histamine produced by HDC is not released into the gastric contents. Therefore, two explanation are possible: (1) the release of histamine into the blood circulation and (2) the degradation of histamine. Although increased concentration in the plasma has been reported, we could not detect such an increase. Histamine methyltransferase (HMT) is an enzyme which degrades histamine. In a previous paper (1) the administration of H2 antagonist was reported to increase HMT activity in the gastric mucosa. It has been suggested that activating HMT suppresses the increase of the mucosal histamine concentration by administration of cimetidine or omeprazole.

If acid secretion blockers were administered for a long period, histamine concentration increased in the rat stomach (11). It appeared that gastric mucosal histamine was increased by hypergastrinemia and acceleration of HDC activity. Histamine containing cells, such as mast cells and enterochromaffin-like cells, differ among various species of animals (2). However, in a previous study (8), long-term treatment of cimetidine caused an increase in gastric histamine concentration in peptic ulcer patients. Therefore, the increase in HDC activity after cimetidine and omeprazole treatment might lead to an interesting assumption as to why some patients suffer from intractable ulcers or recurrent ulcers. From these results, it was evident that serum gastrin concentration is increased and HDC is activated by the administration of H2 antagonists or proton pump inhibitors. Therefore, it is probable that HDC activity in the oxyntic gland of patients taking such drugs will be increased. Since these drugs inhibit the secretion of gastric juice, histamine should be dealt with accordingly. Some patients may not be able to cope with high con-
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**Fig. 3** Changes of gastric histamine concentration in the fundic gland by administration of tetragastrin, cimetidine or omeprazole alone and premedication with cimetidine or omeprazole before administration of tetragastrin.

**Fig. 4** Changes of gastric volume and the amount of histamine in the gastric juice in the controls, and after administration of tetragastrin (200 μg/kg, ip) alone and premedication with cimetidine (100 mg/kg, ip) or omeprazole (100 μmol/kg, ip).
centrations of histamine, and this may cause the above-mentioned ulcers.

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REFERENCES