Activity and Distribution of Histidine Decarboxylase and Histamine Concentration in Rat Stomach

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The involvement of histamine in mediating gastric function under normal and pathological conditions has been largely established. Histidine decarboxylase (HDC) is the only synthetic enzyme of histamine in the mast cell or enterochromaffin like (ECL) cell. In this study, we examined, the activity of HDC by a method based on the modification of Kobayashi's method, the histological distribution of HDC containing cells was determined by an immunohistochemical method and the histamine concentration by a HPLC method in rat stomach. Histamine concentration in the total gastric layer of the fundus is overwhelming large compared to that in the antrum. On the other hand, histamine concentration of the mucosal layer of the fundus is 72.1 ± 9.4 μg/g (mean ± S.D.) and that in both the muscular and serosal layers is 7.2 ± 0.4 μg/g. HDC activity of the total gastric layer of the fundus was about ten times as high as that in the antrum. Histological distribution of HDC-containing endocrine cells was observed in the basal part of the gastric mucosa of the fundus, but could not be observed in the muscular and serosal layer. These results suggest that HDC-containing endocrine cells, in only the basal part of the mucosal layer of the fundus, were similar to ECL cells and have important parts for biosynthesis of internal gastric histamine. Therefore, using the total gastric layer of the stomach was thought to be easier than using only the mucosal layer for measuring gastric histamine and HDC-activity in rats.

(Key Words: Histamine, Histidine decarboxylase (HDC), HDC containing cell)

INTRODUCTION

HDC plays a very important part in the formation of histamine. Histamine is involved in several physiological processes including gastric secretion, peripheral circulation, allergic and similar hypersensitivity reaction. A key role histamine in the physiological control of gastric acid secretion was established with the advent of the histamine H2 Receptor antagonists which have been shown in vivo to inhibit, virtually all forms of basal and stimulated secretion. In the past little enzymological research on HDC was performed. However, since the advent of histamine H2 receptor antagonist, there has been a tendency to focus were attention on HDC than before.

We have investigated the relationship of the activity and distribution of HDC and histamine concentration of the stomach in rats, furthermore we examined the method using total gastric layer of the fundus on the measurement of gastric histamine and HDC-activity.

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 24h. The abdomen was opened immediately under diethyl ether anesthesia. After removing the stomach, it was cut open along the major curvature and washed with ice-cold NaCl solution. The total gastric layer of the fundus and the antrum were collected for the following experiments. Furthermore, the mucosal...
layer, the muscular and serosal layer were separated from the fundic region.

1) Histamine concentration

Our study used a method based on a modification of the method described by Shore (9). Briefly, each sample was weighed, homogenized in a glass homogenizer with 10:1 in 0.25N perchloric acid and centrifuged at 6000g (4 °C, 15 min) to remove tissue debris. After centrifugation, 40 μl of the supernatant was mixed with 0.25N HCl. Then 200 μl of 2N NaOH and 100 μl of a 1% ortho-phthalaldehyde-methanol solution were successively added to the sample to develop fluorescence. After mixing for exactly 5 min, in the dark, 200 μl of 1M H2SO4 was added to stop the fluorescence reaction. Then a 20 μl volume to the mixture was injected for HPLC-fluorescence analysis.

The liquid-chromatographic system consisted of a Shimadzu LC-5A, Shimadzu RF-550 Spectrometer and a Shimadzu chromatopac C-R3A. A stainless-steel column was used ODS-inertsil column. The mobile phase was a mixture of 0.1M acetic acid buffer adjusted to pH 3.8 with 2N NaOH, by measurement with a pH meter and acetonitrile (30:10).

2) Histidine decarboxylase activity

Our study used a method based on a modification of Kobayashi's method (4). The preparation of histidine decarboxylase from rat tissue was as follows. The tissue was excised, washed quickly with physiological saline, and scraped off the full thickness of the fundus and the antrum 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 6000g for 15 minutes at 4°C and the cell free solution decanted. Enzyme activity was measured as 14CO2 produced from (1,14C)-L-Histidine. Reaction mixture contained, in a final volume of 2.2 ml: 10 μg pyridoxal phosphate, carbamyl 14C-L-histidine (0.1 μCi containing 5 μg histidine), 0.1M phosphate buffer (pH 6.8).

The reaction was carried out in a micro tube, incubated at 37°C for 2h, after which the reaction was stopped by the addition of 200 μl of 1M citric acid. A vessel of sample immersed in scintillation fluid for determination of radioactivity, after further incubated for 1h. All assays were made in duplicate and the enzyme activities were expressed as moles 14CO2 formed per mg and hour. The results were corrected by using, boiled tissue blank.

3) Histological examination of HDC containing cells

The specimens of the stomach (the total gastric layer of the fundus and antrum) were immediately immersed in cold Zamboni's fixative for 24h at 4°C and then rinsed for 24h at 4 °C in Zamboni's fluid containing sucrose. Serial frozen sections were cut on a cryostat at a thickness of 6 μm. Antiserum was used rabbit antiserum against histidine decarboxylase which was kindly provided by Prof. Hiroshi Wada, Department of Pharmacology, Osaka University School of Medicine. The Secondary antibody used was goat antirabbit IgG Fab-HRP which was made in the Department of Pathology, Tokai University School of Medicine. Each antibody was reacted for 24h, 4°C, in moist chamber. Then the peroxidase activity was detected by periodic acid dihydrate. After reaction of diaminobenzidine (DAB), the sections were stained with a 1% methyl green solution.

RESULTS

1) Gastric histamine concentration

Fig. 1 shows the separation, by HPLC gradient-elution, of histamine in a standard solution and the total gastric layer of the fundus. On our method, the separation and the quantity of histamine have been satisfactorily.

Histamine concentration in the total gastric layer of the fundus (N = 5): mean ± S.D. was 51.9 ± 7.5 μg/g (means ± S.D.), in the total gastric layer of the antrum (N = 5) was 7.2 ± 0.4 μg/g. The histamine concentration in the fundus was higher than in the antrum (Fig. 2). On the other hand, histamine concentration in the mucosal layer of the fundus (N = 5) was 72.1 ± 6.2 μg/g, in the muscular and serosal layer of the fundus (N = 5) it was 5.8 ± 0.5 μg/g (Fig. 3).

2) Histidine decarboxylase activity

Fig. 4 shows HDC activity of the total gastric layer of the fundus and the antrum. HDC activity of the fundic region, which was 0.98 ± 0.06 pmol 14CO2/hr/mg protein, was nearly ten times as high as that in the antral.
region (0.10 ± 0.03 pmol $^{14}$CO$_2$/hr/mg protein).

3) Localization of HDC containing cells (Fig. 5, 6)

A number of HDC containing cells were located in the basal portion of the fundic mucosal region. The HDC containing cells were observed to be of various shape and size. Certain cells had a meandering projection at the cell margin. Except for the basal portion of the fundic mucosal region, HDC containing cells could not be detected in any other region.

DISCUSSION

Hakanson (2) and Thrunberg (10) reported that gastric histamine containing cells of rat were few in the pyloric gland area but numerous in the entire oxyntic gland area. We examined histamine concentration in the total gastric layer of each regions by biochemical study. Our result agreed with past reports in which gastric histamine existed almost entirely in the fundic region.

HDC, present in several mammalian tissue, is more important in the formation of histamine and is the only biosynthetic enzyme of histamine (3, 6).

The activity of HDC was found to be high in the fundic region, however, it was low in the antral region. Since HDC activity and histamine concentration of the fundic region were higher than the antral region, it is suggested that the fundic region is the central area of biosynthesis of histamine in the rat stomach.

In several recent papers (1, 7, 8) the presence of histamine store in the gastric mucosa has clearly been established as being predominantly the enterochromaffin-like cell in the rat. The cellular localization of histamine has been es-

![Fig. 1 Chromatogram of the standard and the total gastric layer of the fundic region in rat on inertsil ODS, 5 μm particle size. Injection volume 20 μl. The mobile phase consisted of 0.1M acetic acid buffer and acetonitrile (50:20, v/v) and 0.15×10$^{-3}$M 1-octanesulfonic acid, sodium salt. Flow rate: 1.0 ml/min; emission wavelength 450nm, excitation wavelength 350 nm.]}
Fig. 2 Histamine concentration of the total gastric layer of the fundic and pyloric gland area. Histamine concentration in the total gastric layer of the fundus overwhelmingly involved by comparison with the total gastric layer of the antrum.

Fig. 3 For the most part gastric histamine concentration exists in the mucosal layer, gastric histamine concentration in the muscular and serosal layer is low.

Fig. 4 HDC activity of the fundic region was nearly ten times as high as the antral region.
Fig. 5  Localization of HDC containing cells. A number of HDC containing cells were located in the basal portion of the fundic mucosal region. No other region was found to contain HDC cells.

Fig. 6  HDC containing cells with various shapes and sizes. Certain cells had meandering projections.
established by a histochemical technique, in which freeze-dried tissue sections are exposed to ortho-phthaldialdehyde (OPT) vapor, which converts histamine into a fluorophore, which can be detected by fluorescence microscopy. However, the specificity of the OPT-method has not been definitely established, because OPT can stain histamine as well as polyamine. In the electron microscopic radioautographic studies, Rubin et al. (8) reported that the ECL is the histamine synthesizing endocrine cell of the rat stomach. On the other hand, recently, an antiserum to HDC was produced from the liver of the fetal rat. Using this antiserum, we determined the localization of HDC containing cell in the endocrine cells of the rat stomach. HDC containing cells has a characteristic distribution in the basal portion of the mucosa in the oxyntic gland area and they are not related to the parietal cells. This result agreed with those of Kubota et al. (5) and it was thought that these cells were the same as ECL-cell. It is concluded that HDC containing cells, have high enzymatic activity, localized in almost the basal portion of the fundic mucosal region and this portion is the central area of histamine biosynthesis in the stomach of rat. Gastric histamine and HDC-activity were veinely present in the mucosal layer of the fundus and HDC containing cell exist basal portion of the fundic mucosal region. In the many reports, measurement of gastric histamine and HDC-activity have been made use of separated mucosal mcosa. However, except mucosal region, histamine and HDC-activity of the other parts was few, hence, we thought that the method of using the total gastric layer of the fundic region was useful and easier than using separated mucosal layer on measurement of gastric histamine and HDC-activity in rats.

REFERENCES


