Histidine Decarboxylase Expression and Histamine Metabolism in Gastric Oxyntic Mucosa during Hypergastrinemia and Carcinoid Tumor Formation*

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ABSTRACT
Histamine is an important stimulator of gastric acid secretion. In experimental animals, inhibition of acid secretion by long term histamine receptor blockade causes hypergastrinemia, proliferation of enterochromaffin-like (ECL) cells, and formation of histamine-producing gastric carcinoids. The aim of this study was to examine the role of gastrin in histamine synthesis and metabolism of the oxyntic mucosa of normal, hyperplastic, and carcinoid bearing Mastomys natalensis. Administration of exogenous gastrin to normal animals increased histidine decarboxylase (HDC) messenger RNA (mRNA) expression in the oxyntic mucosa within 30 min, indicating that gastrin stimulates histamine synthesis by regulating HDC mRNA abundance. Endogenous hypergastrinemia, induced by short term histamine receptor blockade (loxotidine) for 3-29 days, did not induce tumors, but enhanced the expression of HDC mRNA (2- to 4-fold elevated) and histamine contents (2-fold elevated) in the oxyntic mucosa. Long term histamine receptor blockade (7-21 months) resulted in sustained hypergastrinemia and ECL tumor formation. Tumor-bearing animals had a 4-fold increase in HDC mRNA expression and histamine contents of the oxyntic mucosa. Urinary excretion of the histamine metabolite methyl-imidazole-acetic acid was 2-fold elevated. Tumor-bearing animals recovering from histamine receptor blockade were normogastrinemic and had normal levels of HDC mRNA and histamine in the oxyntic mucosa as well as normal excretion of methyl-imidazole-acetic acid. The results indicate that ECL cell carcinoids developing during hypergastrinemia are well differentiated tumors that respond to high gastrin levels with increased histamine synthesis and secretion. (Endocrinology 137: 4435-4442, 1996)

GASTRIC ACID secretion is stimulated by several hormones and neurotransmitters, e.g. histamine, gastrin, and acetylcholine. Histamine has emerged as a fundamental regulator of gastric acid secretion, as blockade of peripheral histamine receptor almost completely inhibits acid secretion. In the gastric mucosa, histamine is mainly localized to mast cells and endocrine cells, so-called enterochromaffin-like (ECL) cells. ECL cells are on of the major sources of gastric histamine and play a central role in the regulation of acid secretion. Histamine is synthesized by decarboxylation of the amino acid L-histidine using L-histidine decarboxylase (HDC). After synthesis, histamine is stored in specific secretory granules. When released, histamine is efficiently degraded in the tissues before excretion in the urine. The main metabolite of histamine is methyl-imidazole-acetic acid (MelMAA), and the amount excreted in the urine is a measure of the total histamine turnover. Urinary secretion of MelMAA is clinically used as a marker of histamine-producing tumors or mastocytosis (1). The tissue histamine content, on the other hand, reflects the functional mass of histamine-producing cells and their histamine storage capacity.

Pharmacological inhibition of gastric acid secretion has become a common treatment of gastric and duodenal ulcer disease. However, in experimental animals, long term inhibition of acid secretion, e.g. by histamine receptor blockade or proton pump inhibition, has been shown to cause ECL cell proliferation and development of ECL cell carcinoids. Hypergastrinemia, induced by acid inhibition, has been proposed as the agent responsible for the ECL cell proliferation and tumor formation (the gastrin hypothesis) (2). This hypothesis is supported by the fact that continuous infusion of gastrin in intact animals causes ECL cell proliferation, and administration of gastrin to isolated ECL cells stimulates DNA synthesis (3, 4). In man, gastric carcinoids are rare tumors, but an increased incidence has been observed in conditions associated with hypergastrinemia, i.e. chronic atrophic gastritis type A and the Zollinger-Ellison/Multiple Endocrine Neoplasia-1 syndrome (5-8). Therapeutic inhibition of acid secretion and subsequent hypergastrinemia of relatively limited duration have not been shown to induce gastric carcinoids in man. Additional factors, such as chronic inflammation or genetic susceptibility, may be required for...
tumor development. The African rodent *Praomys* (*Mastomys*) *natalensis* represents a unique model for studying gastric carcinoid formation. In this species, spontaneous gastric carcinoids develop at 2 yr of age, but carcinoid tumors may be rapidly induced by 4–6 months of histamine, receptor blockade, with a similar frequency in both sexes (9, 10).

Histamine synthesis and HDC messenger RNA (mRNA) expression in the oxyntic mucosa have previously only been studied in normal rats (11–13). The aim of the present study was to investigate the role of elevated plasma gastrin levels in HDC mRNA expression and histamine content of normal, hyperplastic, and tumor-bearing gastric mucosa of *Praomys* (*Mastomys*) *natalensis*. Urinary secretion of MelMAA was studied in tumor-bearing animals to evaluate its usefulness as a marker for ECL cell proliferation.

**Materials and Methods**

**Animals**

A colony of *Praomys* (*Mastomys*) *natalensis* was established at the University of Goteborg in 1988 from 10 breeding pairs kindly provided by I. M. Modlin, Yale University (West Haven, CT). Animals were housed in sawdust-floor macrolon cages, with free access to standard food pellets and drinking water. Room temperature was kept at 20 C, and illumination was provided on a 12-h light, 12-h dark cycle. A total of 175 adult *Mastomys*, weighing 50–100 g, were used in the study. Both male (n = 64) and female (n = 111) animals were studied, because both sexes develop gastric carcinoids (9, 14). The age of the animals was 5–14 months in the group subjected to acute gastrin stimulation, 6–8 months in the group receiving short term loxtidine treatment, and 12–26 months in the group given long term loxtidine treatment. At the end of each experiment, animals were killed by decapitation, and tissues and plasma were collected as described below. Experiments were approved by the ethical committee for experimental animals, University of Goteborg.

**Drugs**

Loxtidine hemisuccinate, an insurmountable histamine, receptor antagonist, was a generous gift from Glaxo (Greenford, UK) (15, 16). Rat gastrin I was purchased from Sigma (catalog no. G1276, St. Louis, MO).

**Experimental design**

Animals were divided into the following experimental groups.

*Exogenous gastrin stimulation.* Unfasted animals were injected sc with a bolus dose of rat gastrin I (500 µg/kg) and killed 0.5–24 h later. Fasted animals (12 h fasting overnight with free access to water) were injected with rat gastrin I (250 µg/kg) and killed 2 h later. These doses result in supramaximal gastrin levels for this species, but are in the same range as the gastrin levels seen in omeprazole-treated rats (17) or humans with a similar frequency in both sexes (9, 10).

*Short term loxtidine treatment.* Animals were given loxtidine in the drinking water at a concentration of 10 g/liter for 3–29 days. This treatment induces ECL cell hyperplasia, but no ECL cell tumors (9). Age- and sex-matched control animals received tap water.

*Long term loxtidine treatment.* One group of animals (TL) received loxtidine at a concentration of 2 g/liter in the drinking water for 7–21 months. This treatment induces ECL cell hyperplasia, dysplasia, and neoplasia (9). Only animals harboring gross tumors were studied. Loxtidine treatment was given continuously until death. A second group of animals (TW) received loxtidine at the same dose and for the same period of time, but the treatment was ended 2 weeks before death. A third group of control animals (C) was given tap water only. At the end of experiments, animals were killed by decapitation, and blood was collected for assay of gastrin. The stomachs were opened along the lesser curvature and briefly rinsed in tap water. The corpus was dissected, weighed, and snap-frozen in liquid nitrogen. Samples of liver, kidney, brain, antrum, and colon were also frozen in liquid nitrogen and stored at −80 C until assay.

**Determination of plasma gastrin**

Blood from decapitated animals was collected in 1.5-ml Eppendorf tubes on ice. Each tube contained 100 IU heparin and 200 IU aprotinin. Blood samples were centrifuged at 5000 g for 20 min, and plasma was withdrawn and stored at −80 C until assay. Plasma gastrin levels were determined using a competitive RIA based on antisera 4562 (a generous gift from J. Rehfeld), using human gastrin-17 as the standard. This rabbit antiserum is directed toward the C-terminal portion of gastrin and shows 5% cross-reactivity with cholecystokinin (CCK). The cross-reactivity for gastrin-34 was 64% and with sulfated gastrins considerably lower than that with nonsulfated gastrins (19). The tracer used was [125I]gastrin-17, which was prepared by the chloramine-T method and purified by HPLC before use. Intra- and interassay coefficients of variation were 6% and 8%, respectively, at 50 pmol/liter.

**Determination of urinary MelMAA**

To allow collection of urine, animals were kept overnight in clean cages with a wire bottom and a plastic container underneath. The container was moistened with 2 ml 0.1 M HCl before collection of urine and washed with an additional 2 ml 0.1 M HCl after collection. Urine samples were stored in plastic tubes at −20 C until assay. Animals were fasted during the collection period, but were allowed free access to water.

**Sample preparation.** Tele-MelMAA was added to a 0.5-ml aliquot as an internal standard. The urine was evaporated under a stream of air at 95–100 C until complete dryness. Acids were converted to isopropyl esters by the addition of 2 ml isopropanol and 0.20 M acetylchloride, followed by heating at 80 C. The esters were separated by extraction with dichloromethane at pH 6.0 after an initial extraction at an acidic pH. For the final separation, a small cellulose column (id, 4 cm x 8 mm; Econo-Column, Bio-Rad Laboratories, Hemel Hempstead, UK) and cellulose phosphate (cation exchanger, medium mesh; Sigma, St. Louis, MO) were used; the column was washed twice with 2 ml water-saturated ether, and elution was carried out with 8 ml dichloromethane and water-saturated ether at a volume ratio of 4:1.

**HPLC.** Samples were analyzed on a HPLC system under isocratic conditions. The column system was Waters Z-module equipped with a Novapec C18 Radial-Pak cartridge (id, 10 cm x 0 mm, 4 µm packing), and the mobile phase consisted of 15 g SDS/liter water (sodium lauryl sulfate puriss, Fluka, Buchs, Switzerland), 0.8 ml/liter triethylamine, and phosphoric acid to obtain pH 3.5. The filtered aqueous phase was mixed with acetonitrile (38%). The flow rate was 3.0 ml/min, and UV detection was performed at 214 nm. This system also allowed a direct determination of the creatinine concentration in urine samples diluted 1:250. The results are given as millimoles of MelMAA per mol creatinine.

**Determination of oxyntic mucosal histamine**

**Tissue extraction.** Biopsies of gastric corpus were thawed, and the mucosa was carefully scraped off and weighed. To each sample of oxyntic mucosa (25 mg), 3-methylhistamine was added as an internal standard. Samples were sonicated in 400 µl 0.6 M perchloric acid (PCA). Homogenates were placed on ice for 2–3 h and centrifuged at 10,000 x g for 2 min at 4 C. Histamine was extracted from homogenates using cationic cartridge columns (Bond Elute SCX, Varian, Harbor City, CA). Columns were equilibrated with 3 ml methanol and 2 ml 0.6 M PCA. Samples (400 µl) were applied to the columns, followed by 1 ml 0.6 M PCA, and repeated washings were performed with 1 ml 0.15 M phosphate buffer (pH 8.0). Histamine was eluted from the column with 1.5 ml 1.5% triethylamine.

**HPLC.** Histamine was analyzed by HPLC under isocratic conditions. The HPLC system consisted of a BAS PM 60 pump, a CMA 200 refrigerated autosampler (CMA Microdialysis, Stockholm, Sweden), and a Kratos Spectroflow 980 detector (Applied Biosystems, Foster City, CA) with filters for excitation at 330 nm and emission at 418 nm. The signal was recorded by a Data Jet integrator (Spectra-Physics, San Jose, CA). A Kromasil C8 column (250 x 4.6 mm; 5 µm; Hichrom, Berkshire, UK) was used.
HDC mRNA abundance was analyzed by Pitman's and Pearson's tests. Values are given as the mean ± SEM.

### Results

#### Localization of histamine in gastric corpus

Scattered histamine-immunoreactive cells were observed in the corpus mucosa of untreated animals. Labeled cells were almost exclusively located in the glandular epithelium and were identified as ECL cells by their morphology and chromogranin content. A few histamine-storing mast cells were observed in the mucosa and submucosa, as shown by long toluidin blue staining (23, 24). In loxtidine-treated animals, histamine-storing ECL cells increased in number (Fig. 1), in agreement with previous observations (9, 14).

### Histidine decarboxylase mRNA

Expression of HDC mRNA could be detected in brain, gastric antrum, and gastric corpus of untreated animals. No expression could be detected in the large bowel, liver, or kidney. The highest expression of HDC mRNA was found in the gastric corpus (Fig. 2). A single 2.7-kb RNA species was detected; this is in agreement with the situation in the mouse where a single 2.7-kb transcript is expressed (21), but differs from the situation in the rat where alternative RNA splicing generates two transcripts, 2.7 and 3.5 kb in size (12, 25).

#### Effect of exogenous gastrin stimulation

Five groups of freely fed animals were studied 0.5, 2, 6, 12, and 24 h after the injection of rat gastrin I (500 μg/kg) and compared with untreated animals. The plasma gastrin levels were maximally elevated 0.5 and 2 h postinjection (3.924 ± 1.716 and 2.602 ± 1.216 pmol/liter, respectively) compared to the control value (53.1 ± 8.9 pmol/liter), but returned to normal at 6 h. The expression of HDC mRNA in the oxyntic mucosa was also elevated 0.5 and 2 h postinjection, although it did not reach significance (Fig. 3A). Fasted animals were studied 2 h after the injection of rat gastrin I (250 μg/kg) and compared with untreated fasted controls. Plasma gastrin levels were markedly elevated 2 h postinjection (951 ± 278 pmol/liter) compared to the control value (29.5 ± 0.8 pmol/liter). HDC mRNA expression was 3-fold elevated, which was statistically significant (P < 0.01; Fig. 3B).

#### Effect of short term loxtidine treatment

Four groups of animals were treated with loxtidine for 3, 7, 14, and 29 days, respectively, and compared with untreated animals. Plasma gastrin levels were significantly elevated (5-10 times) in all loxtidine-treated animals compared with control levels (Fig. 4A). The expression of oxyntic mucosal HDC mRNA was also significantly elevated (2-4 times) in loxtidine-treated animals compared with that in controls at the same time intervals (Fig. 4, A and B). A significant positive correlation between plasma gastrin levels and oxyntic mucosal HDC mRNA expression was found (P = 0.0014; r = 0.466; n = 48).

In separate groups of animals, oxyntic mucosal histamine concentrations were determined after loxtidine treatment for similar periods of time. A slight, but gradual, increase in...
FIG. 1. Immunohistochemical demonstration of histamine in the oxyntic mucosa of *Mastomys natalensis*. Histamine immunoreactivity is localized to endocrine cells (ECL cells) of the oxyntic mucosa. A. Scattered cells are seen in normal mucosa. B. Numerous ECL cells are seen in the hyperplastic mucosa of loxtidine-treated animals.

FIG. 2. Northern analysis of HDC mRNA expression in various normal tissues of *Mastomys natalensis* and in loxtidine-induced gastric carcinoid. A single 2.7-kb HDC mRNA transcript was detected in normal gastric mucosa, with highest expression in the gastric corpus. High expression of HDC mRNA was also detected in the loxtidine-induced gastric carcinoid, whereas no expression could be detected in the colon, brain, kidney, or liver.

Oxynic mucosal histamine concentrations was observed during treatment, with maximal elevation (2-fold the control level) after 29 days (Fig. 4A).

**Effect of long term loxtidine treatment**

Animals were subjected to long-term treatment with loxtidine until ECL cell tumors developed. One group of tumor-bearing animals was kept on loxtidine (TL) until death, and another group of tumor-bearing animals was given drinking water (TW) 2 weeks before death. Both groups were compared with untreated control animals (C). None of the C animals, but all animals in the TL and TW groups, had gross tumors in the oxyntic mucosa at death. The weights of the gastric corpus in the C, TL, and TW groups were 234 ± 15, 536 ± 83, and 672 ± 84 mg, respectively. Plasma gastrin levels were 2-fold elevated in the TL group compared to those in the TW or C group. When HDC mRNA was analyzed in these animals, a significant elevation was seen in the TL group, but not in the TW group (Fig. 5). In separate groups of tumor-bearing animals (TL and TW), the levels of mucosal histamine and the urinary excretion of MelmAa were studied. These parameters followed a pattern similar to that observed for plasma gastrin and HDC mRNA expression, with significant elevations in the TL group compared to the TW and C groups (Fig. 5, A and B). All groups (C, TL, and TW) consisted of both male and female animals, with no detectable differences between the sexes in HDC mRNA expression, histamine content, or MelmAa excretion in response to hypergastrinemia.

**Discussion**

The regulation of gastric acid secretion from the parietal cell is a complex function influenced by neural and hormonal stimuli. The ECL cell system seems to be an important regulator of gastric acid secretion and can be influenced via peptide receptors, e.g. CCK-B and somatostatin receptors as well as via histamine autoreceptors (4, 26–28). The relative importance of histamine stimulation of parietal cells has been clinically proven by the efficacy of H2 receptor antagonists. The main cellular source of histamine in the gastric mucosa is the ECL cell population, but mast cells also contain histamine. In mice, ECL cells are by far the most common histamine-producing cells (22), an observation confirmed in the present study. The biosynthesis of histamine is initiated by uptake of the amino acid histidine into the amine-handling cells. Histidine is subsequently decarboxylated within...
the cell by HDC, which is the rate-limiting enzyme. It has previously been shown that the HDC enzymatic activity of the gastric mucosa will increase in response to hypergastrinemia in man, rats, and Mastomys (9, 11, 13).

In the present study the influence of acute (exogenous) and short term (3–29 days; endogenous) hypergastrinemia on the ECL cell system was investigated. Gastric acid inhibition was achieved by administration of the irreversible H₂ receptor blocker loxtidine in the drinking water. This treatment was accompanied by increased plasma gastrin levels and activation of the ECL cell system, reflected by an elevated expression of HDC mRNA and an increased content of histamine in the oxyntic mucosa. The increase in HDC mRNA expression preceded and was much higher than the increase in histamine content, which may be due in part to the various effects of gastrin in ECL cells, i.e. stimulation of histamine secretion as well as stimulation of HDC mRNA expression (4). When hypergastrinemia was induced acutely by the injection of gastrin, activation of the ECL cell system was evident, as judged by HDC mRNA expression, by 30 min postinjection. The response was similar in freely fed and fasted animals, although the relative increase in HDC mRNA expression was greater in the fasted animals and reached statistical significance. This is most likely due to a lower expression of HDC mRNA in the fasted control animals compared to freely fed animals (12). Short term (3–29 days) hypergastrinemia induced by loxtidine treatment gave a similar increase in HDC mRNA expression as acute supramaximal stimulation with exogenous gastrin. These findings suggest that gastrin regulates histamine synthesis in ECL cells by controlling HDC mRNA abundance in the oxyntic mucosa. Gastrin may exert its actions on histamine synthesis via a direct activation of ECL cells, but may also influence intermediary regulatory systems. The fact that gastrin levels were not closely correlated to HDC mRNA levels in the different experimental situations, i.e. 1 HDC mRNA was higher in the loxtidine-treated animals than in gastrin-injected animals, despite lower plasma gastrin levels suggests that such regulatory systems may, in fact, be operating in animals subjected to short term treatment (no hyperplasia). One possible explanation is that sustained hypergastrinemia may up-regulate CCK-B receptors on ECL cells as well as increase their numbers (29). An alternative explanation is that sustained hypergastrinemia may ablate inhibitory systems such as the somatostatin producing D cells.

In Mastomys subjected to long term loxtidine treatment (6 months), the entire sequence of carcinoid formation can be followed: initially linear and nodular hyperplasia, followed by dysplasia of ECL cells, and finally micro- and macrocarcinoids (9). The histopathological changes up to dysplasia are slowly reversible after cessation of loxtidine treatment. However, after malignant transformation, no reversibility is evident (14). In the present series only animals with gross tumors (macrocarcinoids) were studied after long term loxtidine treatment (7–21 months). To examine the effects of elevated levels of endogenous gastrin on ECL cell tumors and their expression of HDC mRNA, the following experimental groups were studied: 1) one group receiving loxtidine treatment that was continuously hypergastrinemic until death (T1); and 2) one
group in which loxtidine treatment was ended 2 weeks before death (TW), resulting in normogastrinemia. In the TL group, plasma gastrin levels and HDC mRNA expression were elevated, resulting in a marked increase in tissue histamine. In the clinical diagnosis and follow-up of patients with histamine-producing carcinoids, urinary excretion of MelMAM has proven to be a useful marker of tumor burden (1). The urinary excretion of MelMAM was, therefore, studied and shown to be elevated in these animals. In the group with discontinuation of loxtidine treatment 2 weeks before death (TW), gastrin levels, HDC mRNA expression, and urinary excretion of MelMAM were all normal. One explanation for this unexpected finding is that the synthesis and secretion of histamine in ECL cell tumors are closely regulated by endogenous levels of gastrin. However, it cannot entirely be excluded that the ECL cell tumors produce relatively minor amounts of histamine compared with the surrounding hyperplastic mucosa. However, this explanation is contradicted by the fact that these tumors are composed of cells with ultrastructural features of ECL cells and immunohistochemically proven contents of histamine-immunoreactive material. Our interpretation of these results is that the ECL cell tumors developing during hypergastrinemia are well differentiated tumors that respond to hypergastrinemia with histamine synthesis and secretion. It has even been suggested that tumor-produced histamine may act as an intermediate growth factor, participating in an autocrine
stimulatory loop, during tumor development (30). With this interpretation, withdrawal of the gastrin stimulus will result in decreased histamine synthesis as well as arrest of tumor growth.

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