Differentiation of the Gastric Mucosa

I. Role of histamine in control of function and integrity of oxyntic mucosa: understanding gastric physiology through disruption of targeted genes

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Chen, Duan, Takeshi Aihara, Chun-Mei Zhao, Rolf Hákanson, and Susumu Okabe. Differentiation of the Gastric Mucosa. I. Role of histamine in control of function and integrity of oxyntic mucosa: understanding gastric physiology through disruption of targeted genes. Am J Physiol Gastrointest Liver Physiol 291: G539–G544, 2006; doi:10.1152/ajpgi.00178.2006.—Many physiological functions of the stomach depend on an intact mucosal integrity; function reflects structure and vice versa. Histamine in the stomach is synthesized by histidine decarboxylase (HDC), stored in enterochromaffin-like (ECL) cells, and released in response to gastrin, acting on CCK2 receptors on the ECL cells. Mobilized ECL cell histamine stimulates histamine H2 receptors on the parietal cells, resulting in acid secretion. The parietal cells express H2, M3, and CCK2 receptors and somatostatin sst2 receptors. This review discusses the consequences of disrupting genes that are important for ECL cell histamine release and synthesis (HDC, gastrin, and CCK2 receptor genes) and genes that are important for “cross-talk” between H2 receptors and other receptors on the parietal cell (CCK2, M3, and sst2 receptors). Such analysis may provide insight into the functional significance of gastric histamine.

knockout mice; gastric acid secretion; oxyntic mucosal proliferation and differentiation; gastrin; histamine; gastrin receptor

THE VERTEBRATE STOMACH performs a variety of functions including serving as a reservoir for food, exposing ingested food to acid (secreted by the parietal cells) and pepsin (secreted by the chief cells), and providing a barrier that prevents microorganisms from entering the intestines. In addition, the stomach is exceedingly rich in the following peptide hormone-producing cells: enterochromaffin-like (ECL) cells (histamine), D cells (somatostatin), A-like cells (ghrelin and obestatin), D1/P cells (unknown products), EC cells (serotonin), and G cells (gastrin) (Table 1). The diverse physiological functions of the stomach depend on an intact gastric mucosal integrity. The stomach wall consists of a mucous layer, mucosa, submucosa, muscularis, and serosa. The mucous layer protects the mucosal surface from harmful components in the lumen (e.g., HCl and pepsin) capable of damaging the epithelial barrier. In this review, we will deal with the disruption of specific genes and the consequences for gastric function with respect to the role of histamine in the control of acid secretion and mucosal integrity.

Histamine is produced by decarboxylation of L-histidine. Most histamine in the body is stored in mast cells and basophils, although some is found also in eosinophils and platelets. In the gastric mucosa, histamine occurs mainly in ECL cells and mast cells (12). The role of mast cell histamine probably reflects the pathophysiological role of mast cells in immune reactions. The ECL cells, which produce a peptide hormone of unknown identity, are located mainly in the basal part of the oxyntic mucosa without contact with the gastric lumen. They are rich in histidine decarboxylase (HDC) and are actively producing and releasing histamine (see Refs. 6 and 13).

Targeted gene disruption has been used to study the role of histamine in the control of acid secretion and gastric mucosal integrity. Functional analysis of a series of knockout mouse models, including HDC knockouts, histamine H2-receptor knockouts, CCK2-receptor knockouts, gastrin knockouts and gastrin and CCK double knockouts, muscarinic M3-receptor knockouts, and somatostatin sst2-receptor knockouts, may provide insight into the functions of the stomach in general and of gastric histamine in particular.

THE ROLE OF HISTAMINE IN REGULATION OF ACID SECRETION

Gastric acid secretion is stimulated or inhibited by endocrine, paracrine, and neuroendocrine signals via at least three messenger pathways; gastrin-histamine, CCK-somatostatin, and acetylcholine.

The Gastrin-Histamine Pathway

Circulating gastrin acts on the CCK2 receptors of the ECL cells, resulting in increased HDC mRNA expression and accelerated release and synthesis of histamine, which, in turn, stimulates acid secretion by activating the histamine H2 receptors of the parietal cells. As expected, acid secretion was impaired in gastrin knockout mice and even more so in CCK2-receptor knockout mice (6, 7). The greater impairment of acid secretion in the CCK2-receptor knockout mice could be due to the loss of typical ECL cells and their replacement by histamine-free endocrine-like cells, displaying an ultrastructure distinct from that of the ECL cells (ECL cell replacements) (6). HDC knockout mice had little or no de novo histamine synthesis in the gastric mucosa, resulting in severely impaired acid secretion and a failure to respond to gastrin (10, 22). H2-receptor knockout mice showed a complete lack of acid response to both histamine and gastrin (15). Thus targeted gene disruption of gastrin, CCK2 receptor, HDC, and H2 receptor showed that histamine has a key role in the gastrin-triggered pathway that controls acid secretion (Fig. 1).
CCK<sub>1</sub> receptors recognize CCK preferentially; in fact, sulfated CCK-8 binds to the receptor with 500 times greater affinity than gastrin-17. CCK mobilizes somatostatin from D cells by acting on CCK<sub>1</sub> receptors in both the antral and oxyntic mucosa, thereby inhibiting the gastrin-histamine pathway (G cells and ECL cells) and the activity of the parietal cells by an effect of somatostatin on sst<sub>2</sub> receptors (4). However, acid secretion in CCK1-receptor knockout mice was not much different from that in wild-type mice (Zhao CM, Kopin AS, and Chen D, unpublished observation). The CCK-somatostatin pathway has been uncovered by generating gastrin and CCK double-knockout mice, which were found to have normally functioning parietal cells despite inactive ECL cells (7). In gastrin and CCK double-knockout mice, little or no histamine was mobilized from the ECL cells to stimulate the H<sub>2</sub> receptors. At the same time, there was no circulating CCK to stimulate gastric mucosal D cells to release somatostatin. Despite the lack of gastrin (and CCK), the parietal cells were still capable of producing gastric acid in response to vagal stimulation (pylorus ligation) and a single injection of histamine (but not gastrin) (7). The low acid secretion in the gastrin knockout mice and the relatively high acid output in the gastrin and CCK double-knockout mice can be explained by assuming that CCK somehow counteracts the acid-stimulating effect of gastrin. Indeed, administration of sulfated CCK-8 to gastrin and CCK double-knockout mice effectively inhibited acid secretion (a single injection) and increased fundic somatostatin mRNA expression more than twofold (2-day infusion) (7). Thus the net acid output may be determined by the balance between stimulating signals from the gastrin-histamine pathway on one hand and inhibiting signals from the CCK-somatostatin pathway on the other (Fig. 1). In fact, the studies of sst<sub>2</sub>-receptor knockout mice indicated that endogenous somatostatin acts on the sst<sub>2</sub> receptor to suppress gastric acid secretion by an inhibitory action directly on the parietal cell and by inhibition (probably together with galanin) of the action of gastrin on the ECL cells (17, 26).

**Neural Pathway (Acetylcholine and Neuropeptides)**

Central mechanisms control the sympathetic and parasympathetic inputs to myenteric and submucosal ganglia in the stomach wall; command neurons in these ganglia control nerve signaling to the parietal cells. Thus parietal cell function is regulated not only by circulating hormones (e.g., gastrin and CCK) and paracrine messengers (e.g., histamine and somatostatin) but also by neurotransmitters from enteric neurons (e.g., acetylcholine, catecholamines, and neuropeptides such as pituitary adenylate cyclase-activating peptide (PACAP), VIP, and galanin (for review, see Ref. 13). Acetylcholine acts on muscarinic receptors, which are of five subtypes (M<sub>1</sub>–M<sub>5</sub>). All M<sub>1</sub>–M<sub>5</sub> receptors seem to be expressed in the stomach wall, and M<sub>1</sub>–M<sub>4</sub> are expressed in the oxyntic mucosa as revealed by RT-PCR analysis (3). Identification of the precise cellular localization of these different receptor subtypes has turned out to be difficult. However, it has been suggested that M<sub>1</sub> receptors are expressed by chief cells and surface mucous cells, M<sub>2</sub> and M<sub>4</sub> receptors occur on D cells, M<sub>3</sub> on parietal cells and G cells, and M<sub>3</sub> on postganglionic enteric nerve fibers (see Ref. 3). Acetylcholine is known to stimulate acid secretion. In fact, M<sub>1</sub>-receptor knockout mice had an impaired parietal cell function as evidenced by elevated intragastric pH, reduced acid output in response to pylorus ligation, reduced proportion of secreting parietal cells, and elevated serum gastrin concentration in the fasted state (1). It may be noted that pylorus ligation induces acid secretion through vago-vagal reflexes independent of gastrin and ECL cells (25). Although the ECL cells seem to lack muscarinic receptors, they mobilize histamine in response to adrenaline/nonadrenaline (acting on β<sub>2</sub>-receptors) and to certain neuropeptides that occur in enteric neurons, such as PACAP (acting on PAC<sub>1</sub> receptor) and VIP (acting on VPAC<sub>2</sub> receptor). Galanin inhibited gastrin-induced mobilization of ECL cell histamine in vitro as well as in vivo by acting on Gal<sub>1</sub> receptors. Carbachol (a stable acetylcholine analog) stimulated acid secretion in wild-type mice but reduced acid secretion (pylorus ligation model) in gastrin and CCK double-knockout mice (7). Conceivably, carbachol mobilizes soma-
tostatin from the D cells (by acting on M₂ and/or M₄ receptors), which leads to inhibition of the parietal cells (by acting on sst₂ receptors), resulting in low acid secretion (Fig. 1). In fact, the somatostatin concentration in the oxyntic mucosa was almost doubled, and secretory granules were notably numerous in the D cells of the oxyntic mucosa of gastrin and CCK double-knockout mice (7).

“Cross-talk” Between the H₂, CCK₂, M₃, and sst₂ Receptors of the Parietal Cell

Parietal cells harbor at least three types of acid-stimulating receptors (H₂, M₃, and CCK₂) and one type that inhibits acid secretion (sst₂). The targeted gene disruption of any of the three stimulating receptors will result in impaired acid secretion. Activation of the H₂ receptor (by histamine) appears to play a crucial role in acid secretion. Thus, when histamine was missing from the ECL cells, as is the case in HDC knockout mice (10) and CCK₂-receptor knockout mice (6), gastrin, carbachol, or pylorus ligation induced little or no acid secretion. When the H₂ receptor was missing (as in H₂-receptor knockout mice), there was no acid response to gastrin, and the acid response to carbachol was impaired at 3–4 mo of age (9) and lost at 6 and 14 mo of age (19). Moreover, when exogenous histamine was provided, carbachol was found to stimulate acid secretion in HDC knockout mice, an effect that could be prevented by H₂-receptor blockade (famotidine) (10). On the other hand, M₃-receptor knockout mice not only failed to respond with stimulated acid secretion to 2-deoxy-D-glucose (a vagal stimulant) but responded poorly also to both gastrin and histamine, suggesting that the M₃ receptor on the parietal cell is needed to ensure full secretory capacity (1). In sst₂-receptor knockout mice, the acid response to gastrin was enhanced greatly but not to histamine (26). It is becoming increasingly evident that cross-talk exists between these receptors on the parietal cells, probably via overlapping intracellular second messenger systems. Studies of isolated parietal cells or isolated oxyntic glands from pig, rat, guinea pig, or rabbit have shown that the CCK₂ and M₃ receptors are coupled to G₄ trimeric protein, which upon stimulation activates phospholipase C to induce a rise in inositol trisphosphate, causing a release of intracellular calcium. The H₂ receptor is coupled to both G₉ and G₆ transduction pathways. The G₉ pathway activates adenylate cyclase and increases intracellular cAMP. Moreover, the sst₂ receptor has been suggested to be coupled to the G₁ trimeric protein that inhibits the PAC₁ receptor-coupled G₆ pathway in the ECL cells and perhaps also the CCK₂ receptor-coupled G₄ pathway in ECL cells and parietal cells. It has been suggested that an increase in both cAMP and intracellular calcium is needed to stimulate acid secretion (5).

THE ROLE OF HISTAMINE IN GASTRIC MUCOSAL DIFFERENTIATION AND PROLIFERATION

All gastric mucosal epithelial cells are thought to originate from a common progenitor cell type (stem cells) in the isthmus and neck regions of the gland. Some of these cells migrate upwards and become mucus-secreting surface epithelial cells, whereas others migrate downwards, differentiating into parietal cells, chief cells, or endocrine cells with a lifetime of ~80 days in parietal cells, ~200 days in chief cells, and ~60 days in ECL cells (14, 23). In addition, the ECL cells are capable of self-replication under the influence of gastrin (23). Increased mucosal proliferation is manifested as increased mucosal weight and thickness, DNA and protein contents, mitotic index, and number of parietal cells and ECL cells. Targeted gene disruption that results in lack of histamine in the ECL cells or lack of the H₂ receptor on the parietal cells was associated with abolished acid secretion, secondary hypergastrinemia, and oxyntic mucosal hyperplasia (Table 2). It has been convincingly demonstrated that hypergastrinemia stimulates proliferation and differentiation in the oxyntic mucosa (for review, see Ref. 16). Gastrin and CCK double-knockout mice had a normal-appearing oxyntic mucosa (7), whereas CCK₂-receptor knockout mice displayed mucosal atrophy (6). Conceivably, the CCK₂ receptor (but neither gastrin nor CCK) is responsible for the normal maintenance (through differentiation and proliferation) of the oxyntic mucosa, although the ligands (gastrin and CCK) are eminently capable of inducing growth when present in excess. To explore the putative role of histamine in maintaining the mucosal architecture it is imperative to distinguish between the effects of histamine and those of gastrin. As illustrated in Table II and Fig. 2, the HDC and H₂-receptor knockout mice displayed oxyntic mucosal thickening, whereas the H₂ and CCK₂ receptors double-knockout mice displayed mucosal atrophy. Hence, it may be concluded that histamine and the H₂ receptor do not exert a trophic effect and that they do not mediate the trophic action of gastrin. The finding that the ECL cells and parietal cells were more numerous in HDC knockout mice than in omeprazole-treated wild-type mice probably reflects the longer duration of the hypergastrinemia in the HDC knockout mice (>3 mo) than in the omeprazole-treated mice (2 mo) (18). Interestingly, M₃-receptor knockout mice were hypergastrinemic, but gastrin-induced trophic effects did not manifest themselves, suggesting that the CCK₂ and M₃ receptors (but not the H₂ receptor) are necessary for gastrin’s action (1).

Differentiation is a normal maturation process by which cells become progressively more specialized; in the gastric mucosa this results in the appearance of many different cell populations and in the development of the functional activity typical of each one of these cell populations. Among the endocrine cells, only the ECL cells are capable of producing histamine, which is essential for maintaining the structure and function of these particular cells (6). The ECL cells are rich in secretory organelles, electron-lucent vesicles (numerous) and

Table 2. Serum gastrin levels, oxyntic mucosal thickness, and number of ECL and parietal cells in various knockout mouse strains (compared with wild-type mice)

<table>
<thead>
<tr>
<th>Gene Knockout</th>
<th>Serum Gastrin</th>
<th>Mucosal Thickness</th>
<th>ECL Cell Number</th>
<th>Parietal Cell Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDC</td>
<td>increased</td>
<td>increased</td>
<td>increased</td>
<td>increased</td>
</tr>
<tr>
<td>H₂ receptor</td>
<td>increased</td>
<td>increased</td>
<td>increased</td>
<td>increased</td>
</tr>
<tr>
<td>CCK₂ receptor</td>
<td>increased</td>
<td>decreased</td>
<td>replaced</td>
<td>decreased</td>
</tr>
<tr>
<td>M₃ receptors</td>
<td>increased</td>
<td>decreased</td>
<td>7** decreased</td>
<td>decreased</td>
</tr>
<tr>
<td>M₃ receptor</td>
<td>increased</td>
<td>unchanged</td>
<td>unchanged</td>
<td>unchanged</td>
</tr>
</tbody>
</table>

*Typical ECL cells (detectable, e.g., by histamine or HDC immunostaining) were missing, but the ECL cell replacements were as numerous as the ECL cells of wild-type mice. **Information on ultrastructure is missing, and very little immunocytochemical information is available (8). Hence, data available at present do not permit a correct assessment of the cell number.
granules (less numerous). These secretory organelles develop from microvesicles that bud off from the trans-Golgi network, loaded with cargo (e.g., proteolytic enzymes, chromogranin A, and prohormone). As a result of protein condensation, the electron-lucent microvesicles are promptly transformed into dense-cored granules. When the granules embark upon their journey towards the periphery of the cell, they start to take up histamine from the cytosol by means of the vesicle monoamine transporter type 2 (VMAT2), which is located in the granule/vesicle membrane. The continued accumulation of histamine is associated with transformation of the granule into a large electron-lucent secretory vesicle. As a consequence of stimulation of the cell (by e.g., gastrin), the secretory vesicles will fuse with the cell membrane ultimately to release their contents by exocytosis (Fig. 3). In response to sustained gastrin stimulation, the secretory vesicles will fuse not only with the cell membrane but also with each other to form vacuoles, a process that is accompanied by the appearance of lipofuscin bodies. The time-dependent accumulation of vacuoles and lipofuscin bodies is associated with a progressive impairment of the ECL cell function (24). In HDC knockout mice, an altered ECL cell ultrastructure was observed; the secretory vesicles were greatly reduced in number, whereas the granules became more numerous; nonetheless, the cells could still be recognized as ECL cells (Zhao CM, Falus A, and Chen D, unpublished observation). However, in CCK2-receptor knockout mice, the ECL cells had no HDC and contained little or no histamine; consequently, typical ECL cells could no longer be demonstrated by histamine or HDC immunostaining. Instead, numerous novel endocrine-like cells (probably ECL-cell replacements) could be visualized by immunostaining for chromogranin A or VMAT2, or by electron microscopy (6). Thus lack of the HDC gene resulted in profound ultrastructural changes but not in altered differentiation, whereas lack of the CCK2-receptor gene altered the differentiation of the ECL cells (ECL cells being replaced by another population of endocrine-like cells with an ultrastructure different from that of the ECL cells). In H2- as well as M3-receptor knockout mice, ECL cells were still recognizable as ECL cells, but, not surprisingly, they had the appearance of ECL cells that had been exposed to hypergastrinemia for a long time; i.e., the cytoplasm contained conspicuous vacuoles and lipofuscin bodies (1).

Despite the altered ECL cell ultrastructure in HDC, H2-, CCK2-, and M3-receptor knockout mice, the parietal cells in

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**Fig. 2.** Photomicrographs of the hematoxylin and eosin-stained oxyntic mucosa in wild-type (A), CCK2-receptor knockout (B), H2-receptor knockout (C), and CCK2 and H2 double-knockout (D) mice at 9 mo of age. Note the mucosal surface (top) and mucosal thickness (decreased in B and D, increased in C). Bar = 150 μm.

**Fig. 3.** Schematic drawing illustrating the synthesis, storage, and release of histamine from the ECL cells. The ECL cell expresses mRNAs for the histamine-forming enzyme histidine decarboxylase (HDC) and the vesicle monoamine transporter 2 (VMAT2). There are at least 3 HDC isoforms; 74, 63, and 54 kDa. During the transformation from granule to secretary vesicle, the granule/secretry vesicles, which contains chromogranin A (CGA), accumulates histamine via VMAT2. Upon stimulation, the vesicle membrane fuses with the plasma membrane, in preparation for exocytosis.
these mutant mice, including sst2-receptor knockouts, seemed unaffected with a fairly normal display of tubulovesicles and secretory canaliculi. It was observed, however, that vacuoles appeared in the parietal cells of HDC, H2-, and M3-receptor knockout mice, conceivably because of the hypergastrinemia (acting on the CCK2 receptors of the parietal cells). Hence, neither histamine nor the H2 and M3 receptors seem to play a role in the differentiation of the parietal cell. It has been suggested that the H2 receptor might be responsible for chief cell maturation (8), but modified/altered chief cells could not be observed in the knockout strains that we have analyzed so far.

THE ROLE OF HISTAMINE IN GASTRIC DISEASES

The discovery of H2-receptor antagonists and the use of such drugs in the treatment of acid-related disorders (e.g., peptic ulcer and gastro-esophageal reflux) represent a milestone in medical history (for review, see Ref. 2).

Menetrier’s disease is said to develop after long-term treatment of patients with histamine H2-receptor antagonists (11). This disease is characterized by enlargement of the gastric folds with foveolar hyperplasia and cystic dilation of the glands, primarily in the oxyntic glandular area, and by hypochlorhydria, hypoalbuminemia, and enhanced mucus secretion. Hypergastrinemia and oxyntic mucosal hyperplasia were observed in both HDC and H2-receptor knockouts, but the hypoalbuminemia typical of Menetrier’s disease was seen in H2-receptor knockout mice only (20). Phenotyping of the H2-receptor knockout mice from birth to 17 mo of age revealed that the oxyntic mucosa started to show signs of Menetrier’s disease, suggesting that malfunctioning H2 receptors might be involved in its pathogenesis. Patients with this disease have been treated with anticholinergics (propantheline and pirenzepine) and antisecretagogues (cimetidine and omeprazole) and without success. In the H2-receptor knockout mouse model, the use of a CCK2-receptor antagonist (YM022) showed therapeutic promise (20).

It has been shown also that the combination of CCK2-receptor and H2-receptor antagonists slows down the development of gastric atrophy and cancer in Helicobacter-carrying mice with hypergastrinemia (gastrin transgenic mice) (21).

CONCLUDING REMARKS

Disruption of specific genes may help us understand the physiological role played by these genes in vivo. Lack of histamine (HDC knockouts) or lack of histamine action (H2-receptor knockouts) was associated with impaired parietal cell function (low acid secretion). Lack of the CCK2 receptor eliminated the typical ECL cells and replaced them with a novel endocrine-like cell population, resulting in abolished histamine synthesis and release and little or no acid secretion. The gastrin-histamine and CCK-somatostatin pathways seem to interact in regulating acid secretion. In the parietal cells, the H2 receptor communicates with the M3 receptor, whereas the sst2 receptor interacts with the CCK2 receptor. The CCK2 receptors were found to be responsible for the trophic effects of gastrin. Mice deficient in HDC or H2 receptors displayed oxyntic mucosal hyperplasia, most likely because of the hypergastrinemia observed in the two mouse strains. The M3 but not the H2 or the sst2 receptors was found to be essential for the ability of gastrin (acting on CCK2 receptors) to generate a trophic response in the oxyntic mucosa, emphasizing the integration between endocrine and neurocrine signaling.

GRANTS

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G543

HISTAMINE AND OXYNTIC MUCOSA

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