

# Crucial Role of Histamine for Regulation of Gastric Acid Secretion Ascertained by Histidine Decarboxylase-Knockout Mice

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Received March 27, 2003; accepted June 11, 2003

## ABSTRACT

Histidine decarboxylase (HDC) represents the sole enzyme that produces histamine in the body. The present work investigated the role of endogenous histamine in carbachol- and gastrin-induced gastric acid secretion with HDC-knockout (HDC<sup>-/-</sup>) mice. Acid secretion was measured in either mice subjected to acute fistula production under urethane anesthesia or conscious mice that had previously undergone pylorus ligation. In wild-type mice, carbachol and gastrin significantly stimulated acid secretion, increasing gastric mucosal histamine. In contrast, in HDC<sup>-/-</sup> mice, carbachol and gastrin had little impact when either delivered alone or together. Nonetheless, the two agents achieved a synergistic effect when delivered together with exogenous histamine, stimulating acid secretion in HDC<sup>-/-</sup> mice. Such synergism was abolished by the histamine

H<sub>2</sub>-receptor antagonist famotidine. cAMP involvement in acid secretion was also examined with theophylline, a phosphodiesterase inhibitor, and forskolin, an adenylate cyclase activator. In wild-type mice, theophylline significantly increased acid secretion, enhancing carbachol- and gastrin-stimulated acid secretion. In contrast, in HDC<sup>-/-</sup> mice, theophylline failed to exert an effect on basal acid secretion, as well as carbachol- and gastrin-stimulated acid secretion. Although forskolin interacted with carbachol, allowing acid secretion in HDC<sup>-/-</sup> mice, similar results were not achieved with gastrin. Such results suggest that 1) histamine is essential for carbachol- and gastrin-stimulated gastric acid secretion in mice; and 2) histamine-induced cAMP production contributes to the *in vivo* response to carbachol or gastrin.

The regulation of gastric acid secretion involves a complex network of mediators that both stimulate secretion in response to meal ingestion and maintain gastric mucosal homeostasis (Hersey and Sachs, 1995; Hirschowitz et al., 1995; Aihara et al., 2003). Because histamine represents a potent stimulant for gastric acid secretion, H<sub>2</sub>-receptor antagonists are widely used for the treatment of acid-related peptic disease. Histamine is synthesized from histidine by histidine decarboxylase (HDC) and stored in mast cells, enterochromaffin-like (ECL) cells, and enteric nerve fibers in the stomach (Hakanson et al., 1986; Prinz et al., 2003). Acetylcholine and gastrin also represent major mediators of gastric acid secretion (Wilkes et al., 1991). It has been suggested that ECL cells are stimulated by carbachol (an acetylcholine analog) and gastrin, resulting in histamine release from cytoplasmic granules, which in turn activates parietal cells to

secrete acid (Lindstrom et al., 2001; Prinz et al., 2003). Because H<sub>2</sub>-receptor antagonists inhibit vagus nerve- and gastrin-stimulated gastric secretion, histamine is thought to represent the final mediator of acid secretion (Grossman and Konturek, 1974). In contrast, *in vitro* studies have demonstrated that both carbachol and gastrin directly stimulate isolated parietal cells to result in acid secretion (Soll, 1980; Pfeiffer et al., 1990; Li et al., 1995). In addition, histamine amplifies parietal cell stimulation by carbachol and gastrin. Although the above-mentioned studies summarize the involvement of histamine release in carbachol- and gastrin-stimulated gastric acid secretion, until the present work, the role played by endogenous histamine for *in vivo* carbachol- and gastrin-induced gastric acid secretion remained poorly understood.

Previous reports have demonstrated that treatment with  $\alpha$ -fluoromethylhistidine ( $\alpha$ -FMH), an irreversible inhibitor of HDC, resulted in an 80% reduction in gastric mucosal histamine levels (Andersson et al., 1996).  $\alpha$ -FMH is thought to act

Article, publication date, and citation information can be found at <http://jpet.aspetjournals.org>.  
DOI: 10.1124/jpet.103.052019.

**ABBREVIATIONS:** HDC, histidine decarboxylase; HDC<sup>-/-</sup>, histidine decarboxylase-knockout; ECL, enterochromaffin-like;  $\alpha$ -FMH,  $\alpha$ -fluoromethylhistidine; CCK<sub>2</sub> receptor, cholecystokinin subtype-2 receptor; H<sub>2</sub>R<sup>-/-</sup>, histamine H<sub>2</sub>-receptor-knockout.

by depleting histamine stores in ECL cells, leaving mucosal mast cells apparently unaffected (Andersson et al., 1996). Although  $\alpha$ -FMH treatment was found to inhibit basal acid secretion by >60%, ranitidine inhibited basal acid secretion by approximately 85%. In the same experiments, vagal stimulation was found to increase acid secretion in  $\alpha$ -FMH-treated animals, although ranitidine prevented such a rise. These findings suggest that  $\alpha$ -FMH treatment is not sufficient to eliminate histamine's influence on gastric secretion.

HDC<sup>-/-</sup> mice were recently generated by gene-targeting methods (Ohtsu et al., 2001, 2002; Tanaka et al., 2002). Such mice exhibited trace histamine levels and no de novo gastric mucosal histamine synthesis. Accordingly, HDC<sup>-/-</sup> mice represent an ideal model to assess the direct effects of carbachol and gastrin on parietal cells.

The present work examined the effects of carbachol, gastrin, and histamine, alone and in combination, on gastric acid secretion in HDC<sup>-/-</sup> mice. In addition, the effects of theophylline, a nonselective phosphodiesterase inhibitor, and forskolin, an adenylate cyclase activator, on carbachol- and gastrin-stimulated acid secretion were studied to elucidate the role played by cAMP in parietal cells.

## Materials and Methods

**Animals.** Male and female HDC<sup>-/-</sup> mice, as well as control wild-type littermate mice, were generated on a mixed genetic 129/Sv × ICR background (Ohtsu et al., 2001; Tanaka et al., 2002) and raised until 10 to 12 weeks old. The animals were housed at 23°C with 13 h of light (7:00 AM–8:00 PM). Wild-type mice were fed ad libitum standard diet. HDC<sup>-/-</sup> mice were fed a low histamine diet (Nosan Co., Kanagawa, Japan). Before the experiments, both wild-type and knockout mice were deprived of food for 21 h and water for 2 h. Animal maintenance and experimental procedures were carried out in accordance with the guidelines of the Ethics Committee of Kyoto Pharmaceutical University.

**Drugs.** Drugs used were histamine 2HCl (0.1–10 mg/kg, histamine; Sigma-Aldrich, St. Louis, MO), carbamylcholine chloride (0.05 mg/kg, carbachol; Sigma-Aldrich), human gastrin-17 (1 mg/kg, gastrin; Sigma-Aldrich), theophylline (100 mg/kg; Nakarai Chemicals, Ltd., Kyoto, Japan), forskolin (5 mg/kg; Wako Pure Chemicals, Osaka, Japan), famotidine (10 mg/kg; Yamanouchi Pharmaceutical Co., Tokyo, Japan), and urethane (1.25 g/kg; Kyoto Kasei, Kyoto, Japan). Both theophylline and famotidine were suspended in 0.5% carboxymethylcellulose. Forskolin was first dissolved in dimethyl sulfoxide and then diluted with saline to the desired concentration. All other drugs were dissolved in saline. All agents and vehicle were prepared before administration and subcutaneously injected at a volume of 0.05 ml/10 g of body weight.

**Measurement of Intra-gastric pH.** Under ether anesthesia, each abdomen was opened and each stomach was removed and incised along the greater curvature. Intra-gastric pH was immediately measured with a pH meter (M-11; Horiba Co., Kyoto, Japan) that was directly placed on the fundic mucosa.

**Measurement of Gastric Mucosal Histamine.** Gastric mucosal histamine contents in wild-type and HDC<sup>-/-</sup> mice were measured according to a previously reported method (Kobayashi et al., 2000). In a limited number of mice, mucosal histamine levels were determined 1 h after carbachol or gastrin administration. In brief, each stomach was removed, rinsed with phosphate-buffered saline, weighed, and then homogenized in 0.01 M sodium phosphate buffer. The buffers contained 10<sup>-6</sup> M semicarbazide HCl to prevent histamine degradation. Each homogenate was diluted 1:10 with sodium phosphate buffer and heated in boiling water for 10 min so as to release bound histamine. The homogenates were then centrifuged at 500g for 20 min, and the supernatants were used as samples. Quan-

titative determination of sample histamine levels was performed with the histamine enzyme immunoassay kit (SPI-bio, Massy Cedex, France).

**Determination of Gastric Acid Secretion in Mice with a Gastric Fistula.** Each mouse was anesthetized with an intraperitoneal injection of urethane. After tracheotomy, a polyethylene 60 tube was inserted into the trachea to ensure a patent airway. Acid secretion was measured in the mice according to a previously reported method (Tanaka et al., 2002). In brief, the abdomen was incised and both the stomach and duodenum were exposed. An acute catheter (inside diameter of 2 mm), created with a polyethylene tube, was inserted into the stomach from a small incision made in the duodenum and secured with a ligature around the pylorus. At the beginning of each experiment, each stomach was rinsed several times with physiological saline and filled with 0.4 ml of saline through the catheter. Physiological saline (0.4 ml) was injected through the fistula and collected every 15 min. The acidity of the collected gastric fluid was determined by titration against 0.01 mol/l NaOH to a pH of 7.0 using an automatic titrator (Comtite 550; Hiranuma, Tokyo, Japan). Gastric acid output (volume × acidity) was expressed as microequivalents per 15 min. The cumulative acid output represents the sum of the acid output for 2 or 5 h after administration of each stimulant. In all experiments, basal gastric acid secretion was determined for at least 45 min before drug administration.

**Determination of Gastric Acid Secretion in Mice Subjected to Pylorus Ligation.** The effects on gastric secretion of subcutaneous administration of a single dose of histamine, carbachol, and gastrin were examined. In certain experiments, carbachol or gastrin was subcutaneously injected either immediately after or 45 min after histamine administration to HDC<sup>-/-</sup> mice. A histamine H<sub>2</sub>-receptor antagonist, famotidine, was subcutaneously delivered 30 min before administration of histamine alone, histamine and carbachol, histamine and gastrin, or theophylline. To examine the role played by cAMP, theophylline or forskolin was subcutaneously injected 30 min before administration of the other agents. The theophylline dose was based on a previous report (Cheng et al., 1997). Control animals received vehicle alone. To compare results obtained with anesthetized mice to those obtained with conscious mice, the acid secretion-stimulating effects of histamine, carbachol, and gastrin were examined without urethane treatment. Each mouse was lightly anesthetized with ether for epigastric laparotomy and pylorus ligation. After recovery from anesthesia, histamine, carbachol, or gastrin was subcutaneously injected. Subsequently, 1.5 h after pylorus ligation, animals were lightly anesthetized with ether again and each stomach was removed to collect gastric fluid. Gastric fluid was analyzed with regards to volume and acidity. Control animals received vehicle alone.

**Data Analysis.** Data are presented as means ± S.E.M. Comparisons between control means and single treatment means were made with a two-tailed, paired, or unpaired Student's *t* test, as deemed appropriate. Comparisons of control means with multiple treatment means were made by one-way variance analysis followed by Dunnett's test. *P* values of <0.05 were regarded as significant.

## Results

**Intra-gastric pH in Wild-Type (WT) and HDC<sup>-/-</sup> Mice.** To determine whether the lack of histamine affected basal gastric secretion, intra-gastric pH was determined. Intra-gastric pH in wild-type mice and HDC<sup>-/-</sup> mice was found to be 2.1 ± 0.2 and 4.7 ± 0.2, respectively; the difference was noted to be significant.

**Effects of Carbachol and Gastrin on Wild-Type and HDC<sup>-/-</sup> Mice.** In anesthetized wild-type mice subjected to acute fistula production, carbachol (0.05 mg/kg) and gastrin (1 mg/kg) significantly increased gastric acid secretion (Fig.

1, a and b). Combined treatment with carbachol and gastrin was found to synergistically stimulate acid secretion (Fig. 2, a and c). Cumulative analysis of acid output in such mice revealed that cotreatment with carbachol and gastrin significantly increased acid secretion compared with vehicle; wild-type mice treated with carbachol or gastrin alone exhibited similar effects (Fig. 2c). Pretreatment with famotidine (10 mg/kg) resulted in complete inhibition of acid secretion induced by both carbachol and gastrin (Fig. 2, a and c). In contrast, carbachol, gastrin, or carbachol simultaneously administered with gastrin did not stimulate gastric acid secretion in  $HDC^{-/-}$  mice (Figs. 1, a and b, 2b). Based upon the above results, an effect of famotidine on gastric acid secretion was not observed in response to each secretagogue in  $HDC^{-/-}$  mice (Fig. 2c).

**Effects of Histamine on Carbachol- and Gastrin-Stimulated Gastric Acid Secretion in Wild-Type and  $HDC^{-/-}$  Mice.** In both wild-type and  $HDC^{-/-}$  mice, gastric acid secretion was stimulated by exogenous histamine in a dose-related manner (Fig. 3). Nonetheless,  $HDC^{-/-}$  mice exhibited hypersensitive gastric acid secretion in response to histamine; the increased secretion was significant at  $>3$  mg/kg. Because histamine, at a dose of 0.3 mg/kg, led to a significant increase in acid secretion in  $HDC^{-/-}$  mice, this dose was used in the following study.

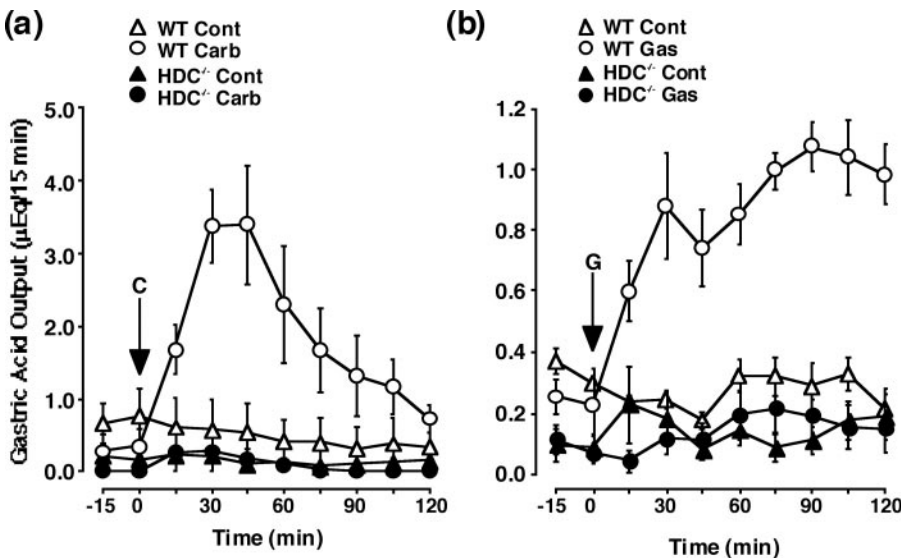
To investigate the potentiative effect of histamine for carbachol and gastrin, the effects of low doses of histamine on the response to carbachol and gastrin were examined in  $HDC^{-/-}$  mice. Combined treatment with carbachol (0.05 mg/kg) and histamine (0.3 mg/kg) clearly resulted in a synergistic increase in gastric acid secretion in  $HDC^{-/-}$  mice (Fig. 4a). For 2-h cumulative acid secretion, the synergistic response was significantly greater than the response observed for controls and mice treated with histamine or carbachol alone (Fig. 4b). Famotidine (10 mg/kg) completely inhibited secretion after combined carbachol and histamine administration (Fig. 4b). Gastrin alone (1 mg/kg) had no effect on acid secretion in  $HDC^{-/-}$  mice (Fig. 5a); however, gastrin (1 mg/kg) significantly augmented acid secretion 45 min after histamine treatment (0.3 mg/kg) (Fig. 5a). Famotidine (10 mg/kg) treatment completely inhibited enhanced acid secretion, resulting from combined gastrin and histamine (Fig. 5b).

The present study confirmed with conscious wild-type mice subjected to pylorus ligation that histamine significantly potentiated acid secretion in response to gastrin. In contrast, histamine only exhibited a tendency for potentiated secretion with carbachol stimulation (Fig. 6a). Nonetheless, histamine significantly potentiated acid secretion in response to both carbachol and gastrin in  $HDC^{-/-}$  mice (Fig. 6b).

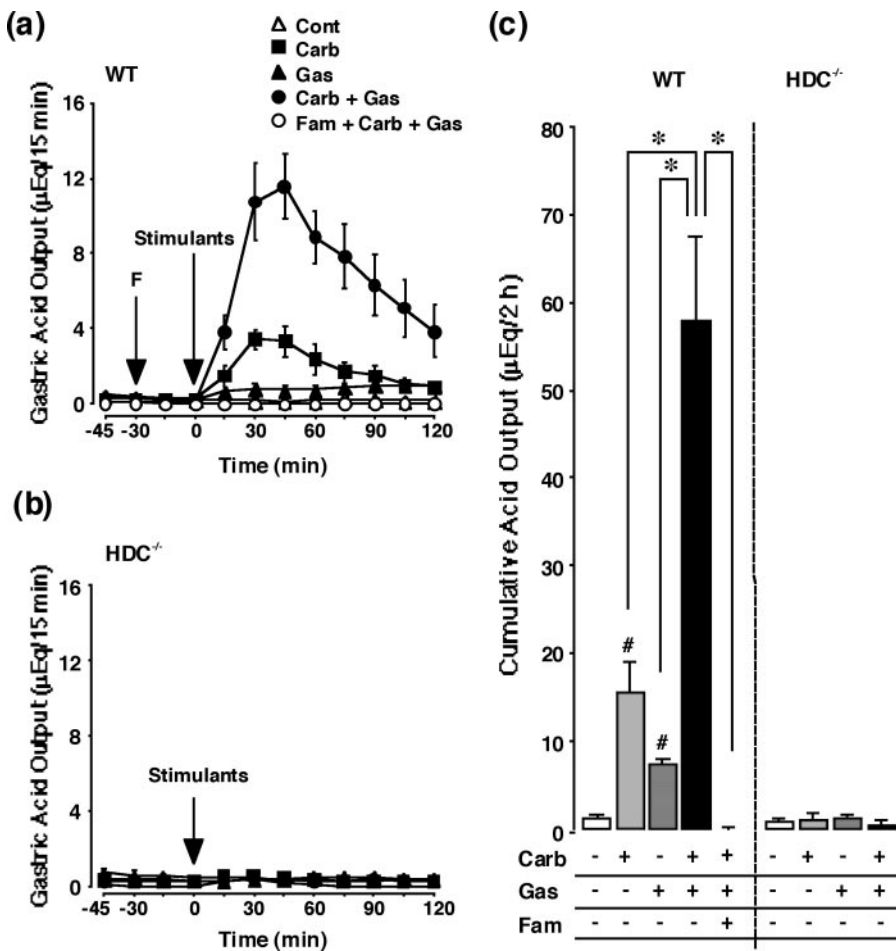
**Effects of Carbachol and Gastrin on Gastric Mucosal Histamine Levels in Wild-Type Mice.** Gastric mucosal histamine increased to  $26.1 \pm 1.5$  and  $22.0 \pm 1.7$   $\mu\text{g/g}$  of wet tissue after treatment with carbachol and gastrin, respectively. Such levels were statistically greater than levels measured in control mice ( $15.6 \pm 1.8$   $\mu\text{g/g}$  of wet tissue; Fig. 7).

**Effects of Theophylline and Forskolin on Gastric Acid Secretion in Wild-Type and  $HDC^{-/-}$  Mice.** It has been suggested that theophylline augments the secretory response to histamine via inhibition of phosphodiesterase, because  $H_2$ -receptors are  $G_s$  protein-coupled receptors that are known to increase cAMP. To further investigate the role of cAMP in histamine-mediated acid secretion, the effects of theophylline and gastric secretagogues on basal and stimulated acid secretion were examined in anesthetized wild-type and  $HDC^{-/-}$  mice. Theophylline (100 mg/kg) significantly stimulated 5-h cumulative gastric acid secretion in wild-type mice but failed to achieve a similar effect in  $HDC^{-/-}$  mice (Fig. 8, a–c). Famotidine completely prevented theophylline-stimulated acid secretion in wild-type mice (Fig. 8, a and c). In addition, theophylline tended to increase mucosal histamine levels in wild-type mice stomachs (Fig. 8d). Moreover, theophylline enhanced gastric acid secretion induced by carbachol, gastrin, and histamine in wild-type mice (Fig. 9a). In contrast, theophylline administered together with either carbachol or gastrin failed to affect acid secretion in  $HDC^{-/-}$  mice. Nonetheless, upon concurrent administration of theophylline and histamine (0.3 mg/kg), significantly enhanced secretion was observed (Fig. 9b). Theophylline delivered with 10 mg/kg histamine, however, did not result in further enhanced acid secretion; thus, it seems that maximal stimulation was achieved with the lower dose (Fig. 9b).

The effect of forskolin, an adenylate cyclase activator, was examined to further study the role played by cAMP for histamine-mediated acid secretion. Although forskolin (5 mg/kg)



**Fig. 1.** Gastric acid secretion induced by carbachol (a) or gastrin (b) in WT and  $HDC^{-/-}$  mice. Gastric acid secretion was measured with the gastric fistula method under urethane anesthesia. Carbachol (Carb, C) and gastrin (Gas, G) were subcutaneously injected at doses of 0.05 and 1 mg/kg, respectively. Control mice (Cont) were administered vehicle alone. The acid output profiles are shown as means  $\pm$  S.E.M. for five to eight mice.

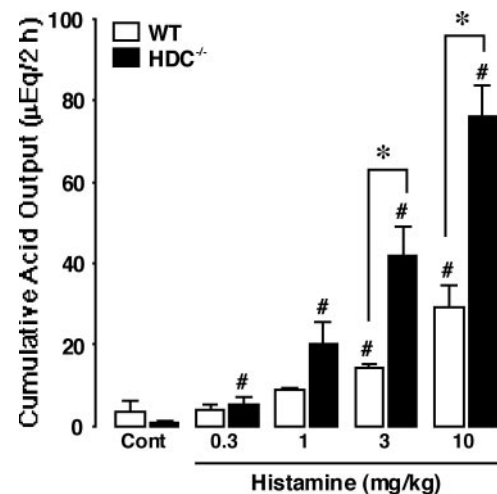


**Fig. 2.** Gastric acid secretion induced by carbachol combined with gastrin in WT and HDC<sup>-/-</sup> mice. Gastric acid secretion was measured with the gastric fistula method under urethane anesthesia. Carbachol (Carb), gastrin (Gas), or famotidine (Fam, F) was subcutaneously injected at doses of 0.5, 1, and 10 mg/kg, respectively. Control mice (Cont) were administered vehicle alone. Acid-output profiles for wild-type (a) and HDC<sup>-/-</sup> (b) mice are shown as means  $\pm$  S.E.M. for four to six mice. The cumulative acid outputs after stimulation are as shown (c). #,  $P < 0.05$ , significant difference from vehicle-treated control mice (Dunnett's test). \*,  $P < 0.05$ , significant difference between the indicated groups (Student's  $t$  test).

significantly stimulated acid secretion in wild-type mice, it failed to stimulate gastric acid secretion in HDC<sup>-/-</sup> mice (Fig. 10, a and b). Combined treatment with forskolin and theophylline tended to stimulate gastric acid secretion, yet the increase was not significant compared with forskolin alone. In HDC<sup>-/-</sup> mice, both forskolin and combined forskolin and theophylline did not significantly stimulate gastric acid secretion (Fig. 10b). Nonetheless, carbachol (0.05 mg/kg), but not gastrin (1 mg/kg), significantly enhanced acid secretion in HDC<sup>-/-</sup> mice treated with combined forskolin and theophylline (Fig. 10b).

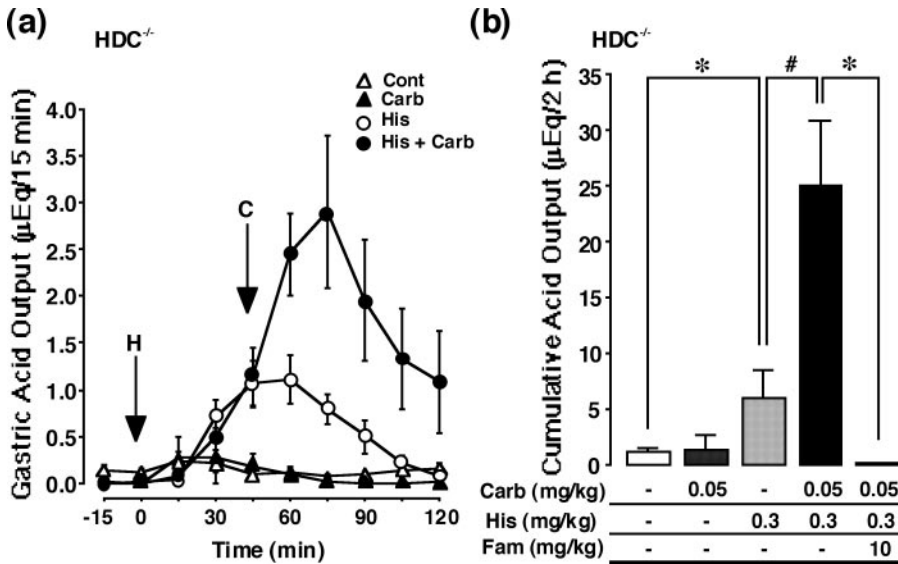
## Discussion

The above-mentioned results demonstrated that, although carbachol and gastrin individually exerted little influence on secretion in HDC<sup>-/-</sup> mice, the two secretagogues synergistically combined with exogenous histamine to stimulate acid secretion. The present study also revealed that although HDC<sup>-/-</sup> mice lack gastric mucosal histamine, such mice exhibit increased parietal cell sensitivity to exogenous histamine, which results in enhanced acid secretion. Although Tanaka et al. (2002) demonstrated slightly reduced basal acid secretion in HDC<sup>-/-</sup> mice (Tanaka et al., 2002), the present study found that intragastric pH was significantly higher in HDC<sup>-/-</sup> mice compared with wild-type mice. In addition, serum gastrin levels in HDC<sup>-/-</sup> mice were also significantly higher than those of wild-type mice, a result of the increased pH (data not shown).

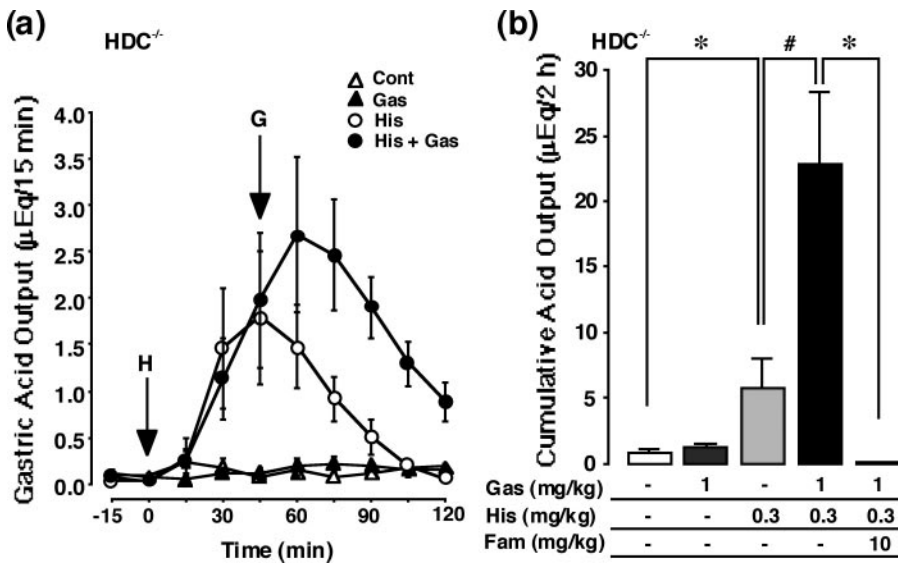


**Fig. 3.** Gastric acid secretion induced by histamine in WT and HDC<sup>-/-</sup> mice. Gastric acid secretion was measured with the gastric fistula method under urethane anesthesia. Histamine was subcutaneously injected at the indicated doses. Control mice (Cont) were administered vehicle alone. Cumulative acid output after stimulation for wild-type and HDC<sup>-/-</sup> mice are shown as means  $\pm$  S.E.M. for five to six mice. # and \*,  $P < 0.05$ , significant difference from vehicle-treated mice (Dunnett's test) and wild-type mice (Student's  $t$  test) treated with identical histamine doses, respectively.

Some disparities exist between the present study and a previous study. Tanaka et al. (2002) reported that carbachol stimulated weak and transient acid secretion in HDC<sup>-/-</sup>



**Fig. 4.** Gastric acid secretion stimulated by histamine combined with carbachol in  $HDC^{-/-}$  mice. Gastric acid secretion was measured with the gastric fistula method under urethane anesthesia. In dual agonist studies in  $HDC^{-/-}$  mice, carbachol (Carb, C) was subcutaneously injected at a dose of 0.05 mg/kg 45 min after histamine (His, H) treatment with a dose of 0.3 mg/kg (a). Famotidine (Fam; 10 mg/kg) was subcutaneously injected 30 min before histamine treatment with a dose of 0.3 mg/kg. Control mice (Cont) were administered vehicle alone. The acid output profiles are shown as means  $\pm$  S.E.M. for five to seven mice. The cumulative acid outputs after stimulation of  $HDC^{-/-}$  mice are as shown (b). \*,  $P < 0.05$ , significant difference from mice treated with either vehicle or carbachol (0.05 mg/kg) combined with histamine (0.3 mg/kg) (Student's *t* test). #,  $P < 0.05$ , significant difference from mice treated with histamine (0.3 mg/kg) (Dunnett's test).



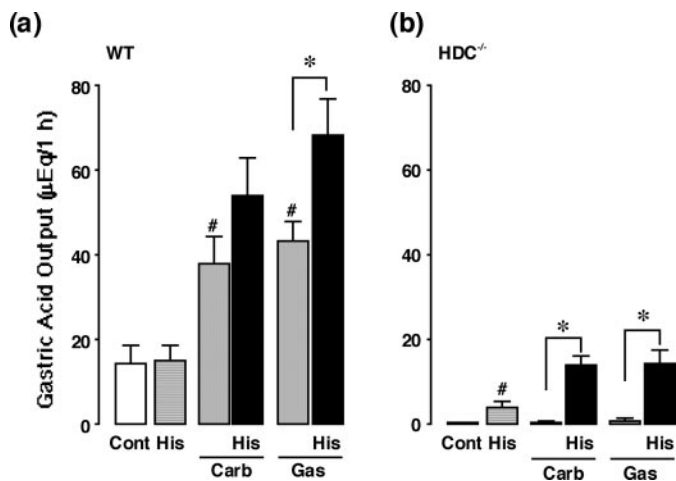
**Fig. 5.** Gastric acid secretion stimulated by histamine combined with gastrin in wild-type and  $HDC^{-/-}$  mice. Gastric acid secretion was measured with the gastric fistula method under urethane anesthesia. In dual agonist studies in  $HDC^{-/-}$  mice, gastrin (Gas, G) was subcutaneously injected at a dose of 1 mg/kg 45 min after histamine (His, H) treatment with a dose of 0.3 mg/kg (a). Famotidine (Fam; 10 mg/kg) was subcutaneously injected 30 min before histamine treatment with a dose of 0.3 mg/kg. Control mice (Cont) were administered vehicle alone. The acid-output profiles are shown as means  $\pm$  S.E.M. for five to seven mice. The cumulative acid outputs after stimulation of  $HDC^{-/-}$  mice are as shown (b). \*,  $P < 0.05$ , significant difference from mice treated with either vehicle or gastrin (1 mg/kg) combined with histamine (0.3 mg/kg) (Student's *t* test). #,  $P < 0.05$ , significant difference from mice treated with histamine (0.3 mg/kg) (Dunnett's test).

mice. Nonetheless, in the present study, carbachol did not stimulate acid secretion in  $HDC^{-/-}$  mice. Recently, existence of a histamine-uptake mechanism for mast cells was reported (Ohtsu et al., 2002). Given the possibility that  $HDC^{-/-}$  mice might uptake and store exogenous histamine obtained from food into mast cells, potentially influencing acid secretion, the difference observed in the results might stem from the fact that a diet low in histamine was used for the present work.

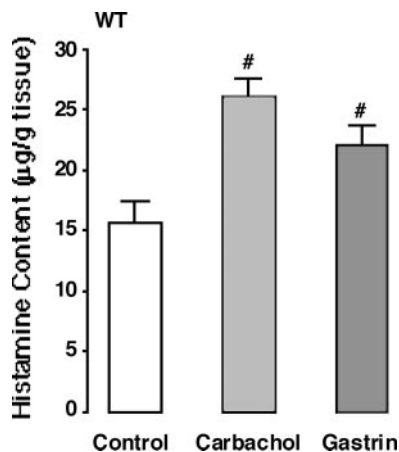
It has been suggested that carbachol and gastrin stimulate histamine release from ECL cells, inducing acid secretion. In the present study, the finding that carbachol and gastrin synergistically stimulated acid secretion in the presence of exogenous histamine, but not in the absence of exogenous histamine in  $HDC^{-/-}$  mice, should be stressed. In addition, such stimulated acid secretion was completely blocked by pretreatment with famotidine. Considering that histamine release from ECL cells is absent in  $HDC^{-/-}$  mice, such results indicate that carbachol and gastrin can directly act on parietal cells, but require  $H_2$ -receptor activation to initiate acid secretion. We postulate that both sufficient intracellular

cAMP, an effect achieved with histamine, and  $Ca^{2+}$ , an effect achieved with carbachol and gastrin, in parietal cells is required to initiate acid secretion. Accordingly, in  $HDC^{-/-}$  mice fistula experiments, if histamine, i.e., cAMP, is present, carbachol and gastrin are able to potentiate acid secretion more than with histamine stimulation alone.

It is important to note that, in wild-type mice, gastrin significantly stimulated acid secretion, maintaining an elevated level 2 h after administration. In contrast, gastrin-stimulated acid secretion in histamine-pretreated  $HDC^{-/-}$  mice gradually declined to baseline after peaking at a maximal level. Accordingly, gastrin seems to stimulate acid secretion in wild-type mice by causing a continuous release of histamine from ECL cells. Such results agree with previous reports (Gerber and Payne, 1992; Prinz et al., 1993). It has been reported that, although gastrin was found to induce histamine release from ECL cells via interaction with  $CCK_2$  receptors, carbachol did not stimulate such histamine release (Norlen et al., 2001). Interestingly, the neurotransmitter pituitary adenylate cyclase-activating polypeptide was noted to exert a strong stimulatory effect on ECL cell histamine mo-



**Fig. 6.** The effects of histamine on responses to carbachol and gastrin in wild-type and  $\text{HDC}^{-/-}$  mice. Gastric acid secretion was measured using the pylorus-ligation method (1.5 h). Histamine (His), carbachol (Carb), or gastrin (Gas) was subcutaneously injected at doses of 0.3, 0.05, and 1 mg/kg, respectively. Control mice (Cont) were administered vehicle alone. Data are expressed as means  $\pm$  S.E.M. for six to nine mice. #,  $P < 0.05$ , significant difference from vehicle-treated control mice (Dunnett's test). \*,  $P < 0.05$ , significant difference between the indicated groups (Student's  $t$  test).

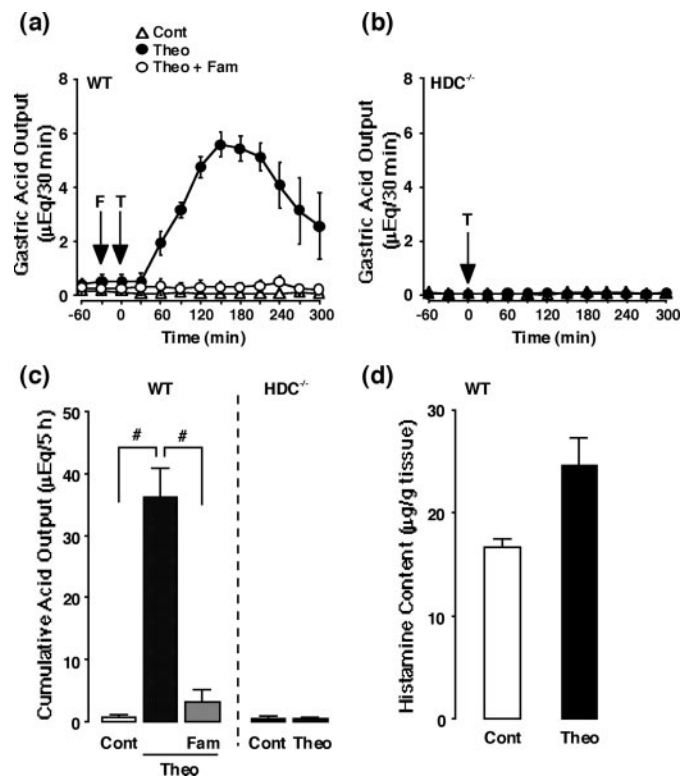


**Fig. 7.** Effects of carbachol and gastrin on gastric mucosal histamine levels in WT mice. Either carbachol or gastrin was subcutaneously injected at doses of 0.05 or 1 mg/kg, respectively. Control mice (Cont) were administered vehicle alone. Data are expressed as means  $\pm$  S.E.M. for five mice. #,  $P < 0.05$ , significant difference from vehicle-treated control mice (Dunnett's test).

bilization (Norlen et al., 2001; Sandvik et al., 2001). Consequently, the mechanism underlying carbachol-induced acid secretion might be partially mediated by pituitary adenylate cyclase-activating polypeptide.

It is of interest that theophylline treatment resulted in markedly different acid secretory responses in wild-type and  $\text{HDC}^{-/-}$  mice. Theophylline significantly stimulated acid secretion in wild-type mice, but not  $\text{HDC}^{-/-}$  mice. Moreover, famotidine significantly inhibited acid secretion induced by theophylline in wild-type mice, despite the fact that theophylline only tended to increase histamine levels. Such results suggest that endogenous histamine remains essential for theophylline-stimulated acid secretion. Because increases in intracellular cAMP have been suggested to result in histamine release (Miyazaki et al., 1992; Prinz et al., 1993), theophylline might induce histamine release from either ECL or mast cells.

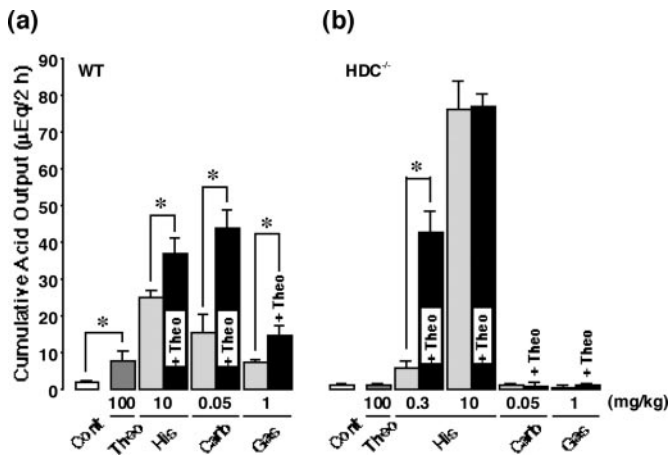
Theophylline treatment in wild-type mice extensively in-



**Fig. 8.** Effects of theophylline on gastric acid secretion and mucosal histamine levels in WT and  $\text{HDC}^{-/-}$  mice. Gastric acid secretion was measured with the gastric fistula method under urethane anesthesia. The acid output profiles for wild-type (a) and  $\text{HDC}^{-/-}$  (b) mice are shown as means  $\pm$  S.E.M. for three to four mice. Theophylline (Theo, T) or famotidine (Fam, F) was subcutaneously injected at doses of 100 or 10 mg/kg, respectively. Control mice (Cont) were administered vehicle alone. The cumulative acid outputs after stimulation are as shown (c). Data are expressed as means  $\pm$  S.E.M. for three to four mice. Gastric mucosal histamine levels 1 h after treatment with theophylline (100 mg/kg) were measured (d). #,  $P < 0.05$ , significant difference from theophylline-treated mice (Dunnett's test).

creased gastric acid secretion in response to histamine, carbachol, and gastrin. Nonetheless, theophylline treatment in  $\text{HDC}^{-/-}$  mice only significantly increased acid secretion in response to histamine; no increase in secretion was observed with concurrent administration with carbachol or gastrin. Such results imply that, in wild-type mice, carbachol, gastrin, and histamine stimulate acid secretion by increasing cAMP levels in parietal cells. In theophylline-treated  $\text{HDC}^{-/-}$  mice, however, carbachol and gastrin could not stimulate acid secretion, because endogenous histamine was not present. The results strongly suggest that histamine is required for cAMP-mediated acid secretion by carbachol and gastrin.

It is of interest that pretreatment with forskolin and theophylline resulted in different acid secretory responses in wild-type and  $\text{HDC}^{-/-}$  mice. Namely, forskolin, delivered either with or without theophylline, significantly stimulated acid secretion in wild-type mice, but not  $\text{HDC}^{-/-}$  mice. It remains likely that such distinct responses represent the product of whether or not endogenous histamine is present in the gastric mucosa. Previous studies have demonstrated that histamine induced an increase in intracellular  $\text{Ca}^{2+}$ , as well as intracellular cAMP, via interaction with  $\text{H}_2$ -receptors (Delvalle et al., 1992a,b). Accordingly, it seems that histamine stimulates acid secretion by facilitating an increase in both intracellular cAMP and  $\text{Ca}^{2+}$ . In contrast, forskolin,



**Fig. 9.** Effects of theophylline on histamine-, carbachol-, and gastrin-stimulated gastric acid secretion in WT (a) and HDC<sup>-/-</sup> (b) mice. Gastric acid secretion was measured with the gastric fistula method under urethane anesthesia. The cumulative acid outputs after stimulation are as shown. Theophylline (Theo), histamine (His), carbachol (Carb), or gastrin (Gas) was subcutaneously injected at doses of 100, 0.3, 0.05, and 1 mg/kg, respectively. Control mice (Cont) were administered vehicle alone. Data are expressed as means  $\pm$  S.E.M. for four to five mice. \*,  $P < 0.05$ , significant difference between the indicated groups (Student's *t* test).

combined with theophylline, failed to increase acid secretion in HDC<sup>-/-</sup> mice. Such findings suggest that an increase in cAMP alone is insufficient to stimulate acid secretion. The mechanism by which carbachol potentiates acid secretion to a small degree in HDC<sup>-/-</sup> mice treated with combined forskolin and theophylline might stem from increased second messenger levels, i.e., cAMP and Ca<sup>2+</sup>, in parietal cells. The reason underlying the inability of gastrin to increase acid secretion in HDC<sup>-/-</sup> mice most likely results from the inability of gastrin to significantly induce elevation of second messenger levels compared with carbachol.

Current genetic technology has allowed generation of knockout mice lacking genes for such receptors as CCK<sub>2</sub> and H<sub>2</sub>, as well as such mediators as gastrin. The functional and morphological changes in the gastric mucosa of such knockout mice has been reported previously (Nagata et al., 1996; Langhans et al., 1997; Friis-Hansen et al., 1998; Kobayashi

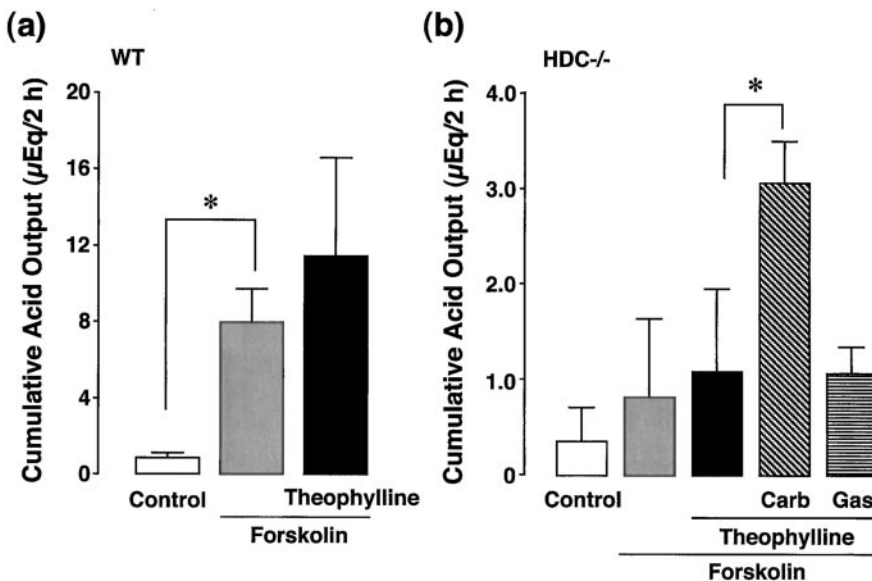
et al., 2000; Ogawa et al., 2003). A study of gastrin-knockout mice demonstrated that histamine or carbachol administration failed to stimulate acid secretion (Friis-Hansen et al., 1998). Nonetheless, such a study demonstrated that gastrin administration for 6 days reversed the effects of gastrin deficiency, leading to an increase in the number of mature H<sup>+</sup>, K<sup>+</sup>-ATPase-positive parietal cells and a partial restoration of acid secretion. Such results strongly suggest that deficiency of mature parietal cells contributes to impaired acid secretion in gastrin-knockout mice. Decreased acid secretion in CCK<sub>2</sub> receptor knockout mice is also thought to result from a deficiency of mature parietal cells (Nagata et al., 1996; Langhans et al., 1997). In contrast, because the histological makeup and appearance of the mucosal surface of HDC<sup>-/-</sup> mice closely resembles that of wild-type mice, HDC<sup>-/-</sup> mice can be used to investigate the mechanisms underlying acid secretion.

H<sub>2</sub>-receptor knockout (H<sub>2</sub>R<sup>-/-</sup>) mice were generated and analyzed in terms of gastric functional and morphological changes (Kobayashi et al., 2000; Ogawa et al., 2003). The basal intragastric pH of H<sub>2</sub>R<sup>-/-</sup> mice was normal (<3.0). It should also be noted that carbachol, but not gastrin, clearly stimulated acid secretion in such mice. Accordingly, Kobayashi hypothesized that in H<sub>2</sub>R<sup>-/-</sup> mice, a cholinergic pathway might allow maintenance of basal acid secretion in the absence of a histamine-mediated pathway. As stated above, the present study has advanced the understanding of the mechanisms underlying acid secretion by demonstrating that carbachol stimulation of acid secretion is dependent on increased levels of intracellular cAMP induced by histamine.

In conclusion, studies with HDC<sup>-/-</sup> mice demonstrated that carbachol and gastrin directly stimulate parietal cells to secrete gastric acid only in the presence of gastric mucosal histamine. In addition, it was also found that high intracellular cAMP levels are requisite for carbachol, gastrin, and histamine to stimulate gastric acid secretion.

#### Acknowledgments

We thank C. J. Hurt (John Hopkins University School of Medicine) for a critical reading of the manuscript and T. Kataoka, Y. Nakamura, K. Mizukoshi, and M. Shiomi for technical assistance.



**Fig. 10.** Effects of forskolin on carbachol- and gastrin-stimulated gastric acid secretion in WT (a) and HDC<sup>-/-</sup> (b) mice. Gastric acid secretion was measured with the gastric fistula method under urethane anesthesia. The cumulative acid outputs after stimulation are as shown. Forskolin, theophylline, carbachol (Carb), or gastrin (Gas) was subcutaneously injected at doses of 5, 100, 0.05, and 1 mg/kg, respectively. Control mice were administered vehicle alone. Data are expressed as means  $\pm$  S.E.M. for four to six mice. \*,  $P < 0.05$ , significant difference between the indicated groups (Student's *t* test).

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