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Unique roles of G protein-coupled histamine H_2 and gastrin receptors in growth and differentiation of gastric mucosa

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Abstract

Disruption of histamine H_2 receptor and gastrin receptor had different effects growth of gastric mucosa: hypertrophy and atrophy, respectively. To clarify the roles of gastrin and histamine H_2 receptors in gastric mucosa, mice deficient in both (double-null mice) were generated and analyzed. Double-null mice exhibited atrophy of gastric mucosae, marked hypergastrinemia and higher gastric pH than gastrin receptor-null mice, which were unresponsive even to carbachol. Comparison of gastric mucosae from 10-week-old wild-type, histamine H_2 receptor-null, gastrin receptor-null and double-null mice revealed unique roles of these receptors in gastric mucosal homeostasis. While small parietal cells and increases in the number and mucin contents of mucous neck cells were secondary to impaired acid production, the histamine H_2 receptor was responsible for chief cell maturation in terms of pepsinogen expression and type III mucin. In double-null and gastrin receptor-null mice, despite gastric mucosal atrophy, surface mucous cells were significantly increased, in contrast to gastrin-null mice. Thus, it is conceivable that gastrin-gene product(s) other than gastrin-17, in the stimulated state, may exert proliferative actions on surface mucous cells independently of the histamine H_2 receptor. These findings provide evidence that different G-protein coupled-receptors affect differentiation into different cell lineages derived from common stem cells in gastric mucosa.

Keywords: G protein; Histamine H_2; Double-null, mouse

1. Introduction

Recently, gene-targeting techniques have made it possible to generate mice deficient in a number of genes involved in gastric acid secretion (Friis-Hansen et al., 1998; Fukushima et al., 2003; Kobayashi et al., 2000; Koh et al., 1997; Langhans et al., 1997; Lloyd et al., 1997; Matsui et al., 2000; Nagata et al., 1996; Tanaka et al., 2002). Of these gene products, histamine H_2, gastrin, and muscarine M_3 receptors are direct targets of secretagogues and are involved in acid production in parietal cells. Targeted disruption of the histamine H_2 receptor caused hypertrophy of gastric mucosa due to marked hyperplasia of parietal, mucous neck and enterochromaffin-like (ECL) cells (Fukushima et al., 2003). Despite prominent hypergastrinemia, surface mucous cells were not as increased in number as downward migrating cells in histamine H_2 receptor-null mice (Fukushima et al., 2003). In contrast, gastrin receptor-null mice exhibited remarkable

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gastric mucosae atrophy accompanied by decreases in parietal and ECL cell numbers (Nagata et al., 1996). Although differences in pH values between wild-type mice and histamine H_2 receptor-null mice were minimal (Fukushima et al., 2003; Kobayashi et al., 2000), gastrin-dependent acid production was impaired in histamine H_2 receptor-null mice. In gastrin receptor-null mice, basal acid productions were lower than those in wild-type mice (Langhans et al., 1997; Nagata et al., 1996). In this study, to further clarify the distinct roles of histamine H_2 receptor and gastrin receptor in gastric mucosa, mice deficient in both the histamine H_2 and the gastrin receptors (double-null mice) were generated. We also analyzed gastric mucosa from aged histamine H_2 receptor-null mice and aged double-null mice. Herein, we present evidence that these different G-protein coupled-receptors mediate differentiation into different cell lineages derived from common stem cells in gastric mucosa.

2. Materials and methods

2.1. Mice

All animal experimental procedures were reviewed and approved by the Institutional Animal Care and Research Advisory Committee of the University of Tokyo. Mice deficient in histamine H_2 receptors were generated as described previously (Fukushima et al., 2003; Shindo et al., 2002).

2.2. Generation of mice deficient in both the histamine H_2 receptor and the gastrin receptor (double-null mice)

Histamine H_2 receptor-null mice and gastrin receptor-null mice with the genetic background of the 129/Sv×C57BL/6 hybrid were used (Fukushima et al., 2003; Nagata et al., 1996). Offspring obtained by crossing histamine H_2 receptor-null and gastrin receptor-null mice were confirmed to be heterozygous for both the histamine H_2 receptor and the gastrin receptor. These mice were then crossed and the offspring thus obtained were genotyped with PCR and/or Southern blot analysis using genomic DNA prepared from tail biopsies. Of these offspring, wild-type, histamine H_2 receptor-null, gastrin receptor-null and double-null mice were used for the following studies. Double-null mice appeared normal, were healthy into adulthood and both sexes were fertile.

2.3. Generation of polyclonal antibody against murine pepsinogen C

Polyclonal antibody against murine pepsinogen C was generated by a previously described method (Fukushima et al., 1993). The 100 carboxyl-terminal amino acids of murine pepsinogen C were fused to Glutathione *S*-transferase, which was used to immunize female New Zealand white rabbits. Serum collected from the immunized rabbits was passed through Affigel-10 beads, which had been cross-linked to Glutathione *S*-transferase. The flow-through was collected and passed through Afvfigel-10 beads, which had been cross-linked to the fusion protein. Antibody adsorbed to the beads was collected. This polyclonal antibody specifically recognizes chief cells in mouse oxyntic mucosa.

2.4. Histological analysis

Gastric specimens were fixed in 3% phosphate-buffered paraformaldehyde (pH 7.4), embedded in paraffin, and cut into 3 μm sections. The sections were stained with periodic acid-Schiff (PAS), hematoxylin and eosin, and examined under a light microscope. Paraffin-embedded gastric tissue sections were dewaxed and rehydrated with graded concentrations of ethanol. After treatment with 2% H_2O_2/phosphate buffered saline for 10 min, tissue sections were incubated with anti-pepsinogen C antibody, anti-histidine decarboxylase (HDC) polyclonal antibody, anti-$H^{(+)}/K^{(+)}$-ATPase monoclonal antibody (Fukushima et al., 1999), anti-type III mucin monoclonal antibody HIK1087 (Kanto-Kagaku, Japan) or normal rabbit or mouse immunoglobulin G (IgG) overnight at 4 °C. The sections were rinsed and then incubated for 30 min with biotinylated anti-rabbit or mouse IgG (1:400 dilution). The tissue sections were then rinsed and incubated for 30 min with peroxidase-labeled streptavidin (1:70 dilution). The slides were rinsed again in phosphate buffered saline and reacted with diaminobenzidine for 5 min at room temperature. Finally, the sections were rinsed and counterstained with hematoxylin.

2.5. Incorporation of the thymidine analog bromodeoxyuridine (BrdU)

BrdU (80 mg/kg BW(body weight)) was injected intraperitoneally into mice 2 h before sacrifice. Gastric tissues were removed and fixed in 3% phosphate-buffered paraformaldehyde. Immunohistochemistry with anti-BrdU monoclonal antibody was performed using paraffin-embedded sections from these samples.

2.6. Measurement of gastric pH

Wild-type and histamine H_2 receptor-null mice were fasted overnight with free access to water. At 1.5 h after subcutaneous injection of vehicle (0.5% methylcellulose), 10 mg/kg BW of famotidine, 10 mg/kg BW of pirenzepine dihydrochloride (a muscarine M_1 receptor antagonist) or 10 mg/kg BW of (*R*)-1-[2,3-dihydro-1-(2′ -methylphenacyl)-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl]-3-(3-methylphenyl)urea (YM022), a gastrin receptor antagonist, the mice were sacrificed and their stomachs were immediately excised. Gastric pH was measured using an ultra-thin pH monitor (Horiba, Japan).

2.7. Measurement of secretagogue induced acid secretion

Mice were maintained on anesthesia in chambers infused with oxygen gas saturated with diethylether. The stomach and duodenum were exposed via an epigastric midline incision. A tube inserted from the duodenum was placed in the gastric lumen. Stomachs were washed with 1 ml of prewarmed physiologic saline three times. After extraction of the tube and ligation of the pylorus, physiologic saline or secretagogue solution was administered peritoneally. A total of 10 mg/kg BW of histamine dihydrochloride, 0.05 mg/kg BW of carbachol or 0.1 mg/kg BW of gastrin-17 were administered, i.e. 2.5 ml/kg BW of physiologic saline as a control, histamine dihydrochloride solution (4 mg/ml), carbachol solution (0.02 mg/ml) or gastrin-17 solution (0.04 mg/ml). Thirty minutes after administration, the mice were sacrificed and their stomachs were excised. Gastric juice was collected with 1.5 ml of physiologic saline. Secreted gastric acid was measured by titrating the collected gastric juice to pH 7.0.

2.8. Statistical analysis

Quantitative values were expressed as means±S.E. Statistical significance was tested using the unpaired *t*-test (two tailed). A value of $P<0.05$ was considered significant.

3. Results

3.1. Comparison of gastric mucosae and serum gastrin levels of 10-week-old histamine H_2 receptor-null, gastrin receptor-null, double-null and wild-type mice

Stomachs from 10-week-old double-null mice weighed significantly less than those of 10-week-old wild-type mice (double-null 60.0±0.6 g/kg BW, wild-type 79.0±1.0 g/kg BW, $P<0.0001$). Macroscopically, oxyntic mucosae from double-null mice were more atrophic than those from wild-type mice (Fig. 1). Serum gastrin levels in double-null mice were significantly higher than those in wild-type mice, while being comparable to and lower than those in gastrin receptor-null mice and histamine H_2 receptor-null mice, respectively (Fig. 2). In addition, except in wild-type mice serum gastrin levels were not elevated by feeding (Fig. 2).

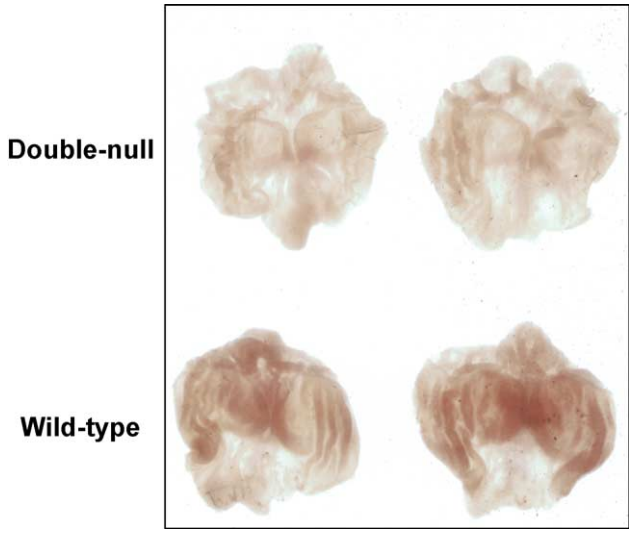

Fig. 1. Macroscopic views of stomachs from 10-week-old wild-type and double-null mice. The excised stomachs were opened along the greater curvature.

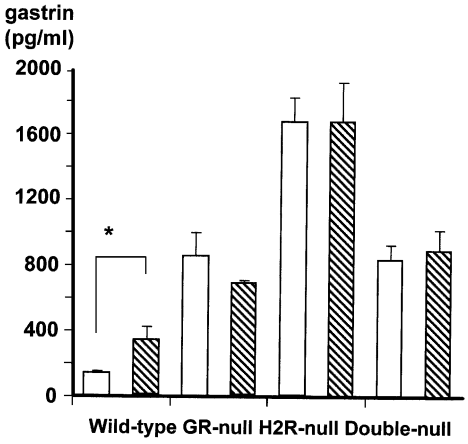

Fig. 2. Serum gastrin levels in wild-type, histamine H_2 receptor-null, gastrin receptor-null and double-null mice. Serum gastrin levels were measured in fasting (open bars) and fed states (hatched bars) in 10- to 12-week-old wild-type, histamine H_2 receptor-null (H2R-null), gastrin receptor-null (GR-null) and double-null mice. Data are presented as means±S.E. (n=15). *$P<0.0001$ between fasting and fed states.

To explore the effects of disrupting gastrin receptor and histamine H_2 receptor genes, we examined oxyntic mucosae from the four types of mice at 10 weeks of age, PAS staining of gastric mucosa from 10-week-old double-null mice showed no hypertrophy of oxyntic mucosae in double-null mice (Fig. 3D).

In histamine H_2 receptor-null mice, oxyntic mucosal hypertrophy was attributable to hyperplasia of ECL, parietal and mucous neck cells, and parietal cells were small (Table 1). In some portions of oxyntic mucosae from histamine H_2 receptor-null mice, peculiar mucous neck cells full of mucin protruded into the gastric gland lumen. Despite marked hypergastrinemia surface mucous cells were not as increased in number as the downward migrating cells, resulting in a decreased percentage of surface mucous cells per gland in histamine H_2 receptor-null mice. These findings confirm our previous report on histamine H_2 receptor-null mice (Table 1, Fig. 3B) (Fukushima et al., 2003). However, on closer examination, we found the number of surface mucous cells to be significantly increased as compared to wild-type mice (Table 1).

In gastrin receptor-null mice, numbers of downward migrating cells were decreased as previously reported ($P<0.001$, vs. wild-type mice) (Table 1) (Nagata et al., 1996). Interestingly, surface mucous cell cells were increased in number as compared with wild-type mice (26.7±1.6

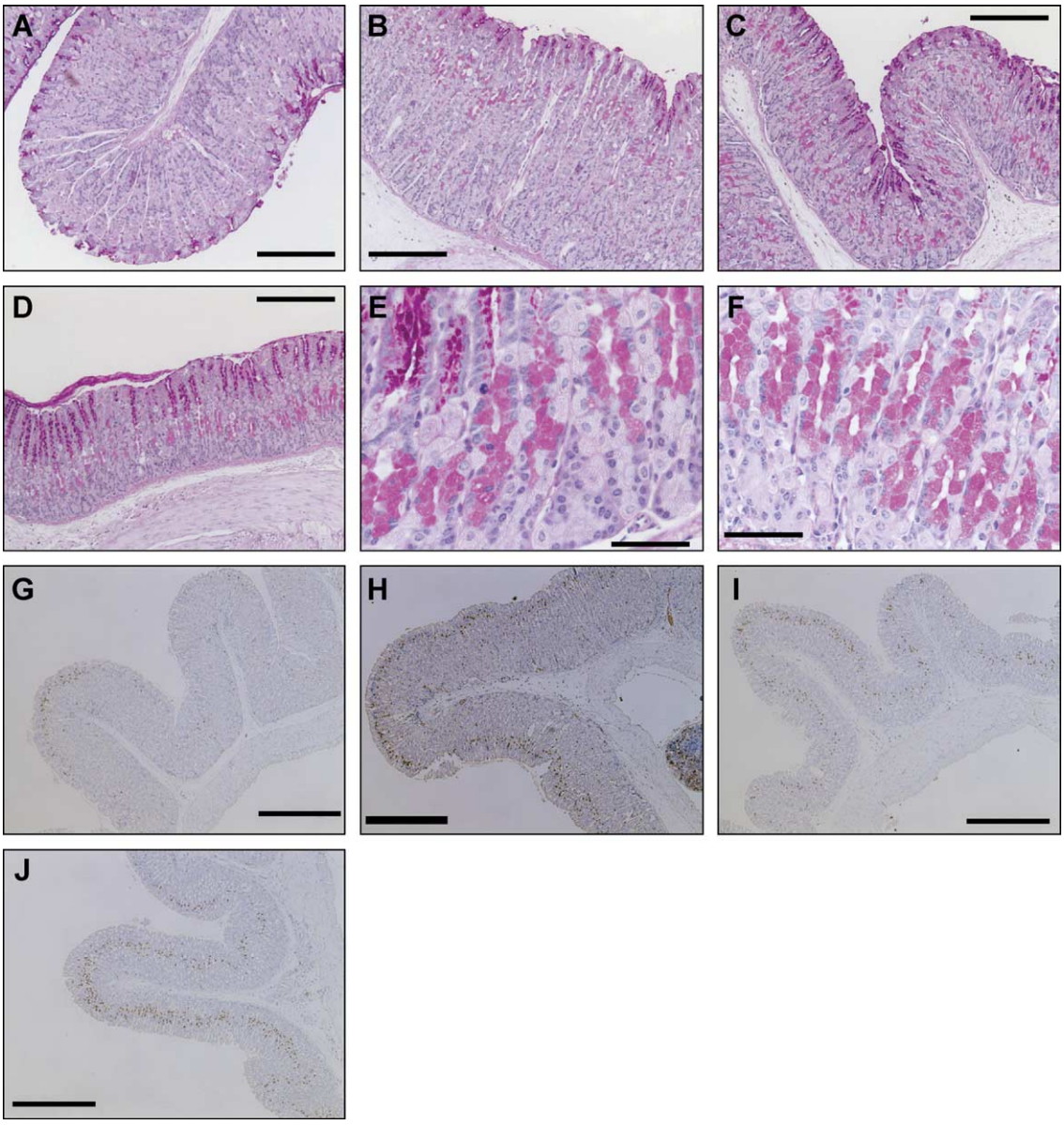

Fig. 3. Oxyntic mucosa from 10-week-old wild-type, histamine H_2 receptor-null, gastrin receptor-null and double-null mice. Sections of oxyntic mucosa from wild-type (A, G), histamine H_2 receptor-null (B, H), gastrin receptor-null (C, E, I) and double-null (D, F, J) mice were subjected to PAS staining (A, B, C, D, E, F) or BrdU labeling (G, H, I, J). Scale bars, 200 μm (A, B, C, D), 50 μm (E, F), 500 μm (G, H, I, J).

arbitrary units per gland vs. 20.3±0.5 arbitrary units per gland, $P<0.001$) (Table 1, Fig. 3C). Thus, although numbers of downward migrating cells were decreased, the total number of cells per gland did not differ significantly between gastrin receptor-null and wild-type mice (Table 1). In addition, an increase in the number of BrdU positive cells per gland was observed in gastrin receptor-null mice (gastrin receptor-null, 2.76±0.14 arbitrary units per gland, wild-type, 0.95±0.09 arbitrary units per gland, $P<0.001$) (Table 1, Fig. 3I). Just as in histamine H_2 receptor-null mice, some portions of the oxyntic mucosa, especially at the greater curvature and near the antrum, contained mucous neck cells full of mucins (Fig. 3E). Small parietal cells were observed in gastrin receptor-null mice as well (gastrin receptor-null mice, 5.37±0.10 arbitrary units per cell, wild-type mice, 8.86±0.17 arbitrary units per cell, $P<0.001$) (Table 1). In double-null mice, numbers of ECL cells, and parietal cells as well as the total number of downward migrating cells, were decreased (Table 1). As in gastrin receptor-null mice, the number of surface mucous cells was increased as compared with those from wild-type mice (25.3±0.8 arbitrary units per gland vs. 20.3±0.5 arbitrary units per gland, $P<0.001$) (Table 1, Fig. 3D). BrdU positive cells per gland were increased in number in double-null mice (double-null, 1.75±0.13 arbitrary units per gland, wild-type, 0.95±0.09 arbitrary units per gland, $P<0.001$) (Table 1, Fig. 3J). Total number of cells per gland did not differ significantly between wild-type and double-null mice (Table 1). Mucous neck cells with

Table 1
Quantitative analyses of gastric glands from 10-week-old wild-type, histamine H_2 receptor-null, gastrin receptor-null and double-null mice

	Total cell number	Surface mucous cell number	Gland cell number	Parietal cell		ECL cell number	BrdU positive cell number
				Number	Size		
Wild-type	63.7±0.7	20.3±0.5	43.4±0.7	20.3±0.4	8.86±0.17	1.44±0.13	0.95±0.09
Histamine H_2 receptor-null	114.2±3.6[a]	26.7±1.6[a]	87.5±2.9[a]	38.6±1.3[a]	4.79±0.11[a]	7.61±0.32[a]	2.01±0.11[a]
Gastrin receptor-null	61.5±1.2	26.7±0.7[a]	34.8±0.9[a]	13.8±0.2[a]	5.37±0.10[a]	0.53±0.08[a]	2.76±0.14[a]
Double-null	61.1±1.1	25.3±0.8[a]	35.8±0.8[a]	14.2±0.4[a]	5.01±0.09[a]	0.81± 0.07[a]	1.75±0.13[a]

Numbers of cells were counted in gastric glands sectioned centrally and in a manner parallel to their longitudinal axes, then expressed as arbitrary units per gland. Parietal cell size was determined by measuring the longitudinal cross sectional area of parietal cells from these gastric glands and expressed as arbitrary units per cell. One hundred glands from 10 mice (10 glands per mouse) were used for each type of mouse. Data are expressed as arbitrary units per gland or parietal cell since the data obtained are proportional but not equivalent to the actual cell numbers or parietal cell mass.

[a] $P<0.0001$ vs. wild-type mice.

characteristics similar to those in histamine H_2 receptor-null mice and gastrin receptor-null mice were seen in similar portions of the gastric mucosa (Fig. 3F). Small parietal cells were also observed in double-null mice (double-null mice, 5.01±0.09 arbitrary units per cell, wild-type mice, 8.86±0.17 arbitrary units per cell, $P<0.001$) (Table 1).

3.2. Comparison of chief cell lineage in gastric mucosae from 10-week-old wild-type, histamine H_2 receptor-null, gastrin receptor-null and double-null mice

Next, to explore the effects of histamine H_2 receptor and gastrin receptors on maturation of the chief cell lineage, expressions of pepsinogen and type III mucin were examined in gastric glands in each type of mouse. Fig. 4 is a schematic representation of a gastric gland. Fig. 5 shows that type III mucin positive cells were increased in number in histamine H_2 receptor-null, gastrin receptor-null and double-null mice as compared with wild-type mice. In addition, type III mucin positive cells, although present in the base regions of gastric glands from histamine H_2 receptor and double-null mice (Fig. 5J,L), were very scarce at the bases of gastric glands from wild-type and gastrin receptor-null mice (Fig. 5I,K). In wild-type mice, numbers of pepsinogen positive cells in gastric glands gradually increased from the isthmus to the base and pepsinogen expression per cell had already peaked in the neck region (Fig. 5A). In gastrin receptor-null mice, pepsinogen expression in gastric glands was maximal only at the base (Fig. 5C). It is noteworthy that mature chief cells, without type III mucin and with abundant pepsinogen, were present at the base region of gastric glands from gastrin receptor-null mice (Fig. 5C,G). In contrast, gland cells with abundant pepsinogen expression and without type III mucin were not present in histamine H_2 receptor-null mice and double-null mice (Fig. 5B,D,F,H). In addition to the low pepsinogen expression, pepsinogen levels per cell did not increase from the isthmus to the base in histamine H_2 receptor-null and double-null mice (Fig. 5B,D).

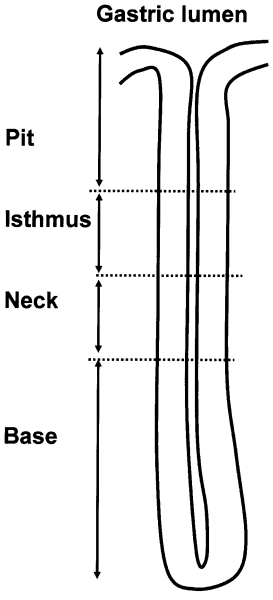

Fig. 4. Schematic drawing of a gastric gland.

3.3. Gastric pH and gastric acid productions in 10-week-old wild-type, histamine H_2 receptor-null, gastrin receptor-null and double-null mice

First, in vivo acid productions in response to secretagogues were measured. Histamine H_2 receptor-null mice were responsive to carbachol, but not to histamine or gastrin-17 (Fukushima et al., 2003). Secretagogue-induced acid secretion (10 mg/kg BW of histamine, 0.05 mg/kg BW of carbachol) was not observed in either gastrin receptor-null nor double-null mice (data not shown). Gastric pH values in double-null mice were the highest among the four types of mice (Fig. 6). Those in gastrin receptor-null mice were higher than those in wild-type or histamine H_2 receptor-null mice and lower than those in double-null mice. Treatment of gastrin receptor-null mice with famotidine (10 mg/kg BW) or pirenzepine (10 mg/kg BW) raised gastric pH values, indicating that histaminergic and muscarine pathways, although severely impaired, are functional in gastrin receptor-null mice. Because fasting gastric pH values in double-null mice were too high to assess the inhibitory effects of pirenzepine, the effect of

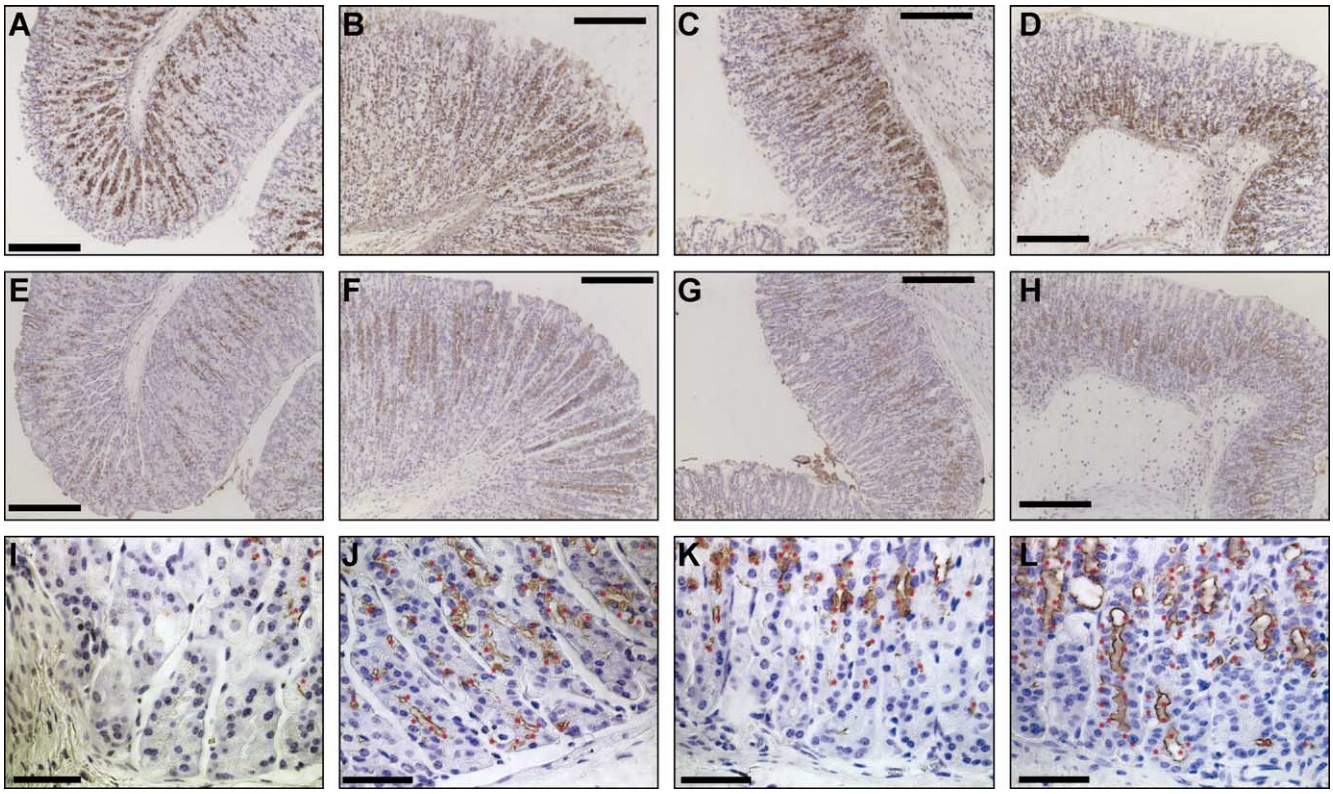

Fig. 5. Expressions of pepsinogen and type III mucin in oxyntic mucosa from 10-week-old wild-type, histamine H_2 receptor-null, gastrin receptor-null and double-null mice. Sections of oxyntic mucosa from wild-type (A, E, I), histamine H_2 receptor-null (B, F, J), gastrin receptor-null (C, G, K) and double-null (D, H, L) mice were stained with anti-pepsinogen antibody (A, B, C, D) and anti-type III mucin antibody (E, F, G, H, I, J, K, L). In I, J, K, L, type III mucin-positive cells are marked with asterisks. Scale bars, 200 μm (A, B, C, D, E, F, G, H), 50 μm (I, J, K, L).

carbachol at 1 mg/kg BW, a dose which is too high to be tolerated in measuring in vivo acid production, was examined in double-null mice. Fig. 6 shows that while gastrin receptor-null mice were responsive to both histamine and carbachol, double-null mice were unresponsive to both.

3.4. Long term follow-up of histamine H_2 receptor-null mice and double-null mice

At 6 months, while there were no changes in gastric mucosa from wild-type mice, further elongation of gastric glands was observed in histamine H_2 receptor-null mice

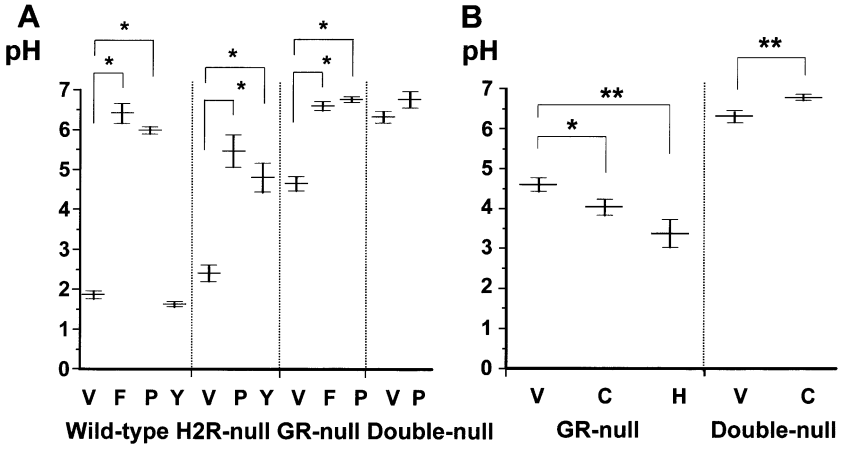

Fig. 6. Gastric pH in wild-type, histamine H2 receptor-null, gastrin receptor-null and double-null mice. Wild-type, histamine H_2 receptor-null (H2R-null), gastrin receptor-null (GR-null) and double-null (10 to 12 weeks old) mice were fasted overnight with free access to water. (A) At 1.5 h after subcutaneous injection of 0.5% methylcellulose as a vehicle (V) (n=20), 10 mg/kg BW of famotidine (F) (n=20), 10 mg/kg BW of pirenzepine (P) (n=20) or 10 mg/kg BW of YM022 (Y) (n=20), the mice were killed and their stomachs immediately excised. Gastric pH was measured using an ultra-thin pH monitor. (B) At 15 min after subcutaneous injection of vehicle (V) (n=20), 10 mg/kg BW of histamine (H) (n=20) or 1 mg/kg BW of carbachol (C) (n=20), the mice were killed and their stomachs immediately excised. Gastric pH was measured using an ultra-thin pH monitor. Data are presented as means±S.E. *P<0.001 vs. respective values.

Table 2
Stomach weight and gastric pH in aged wild-type and aged histamine H_2 receptor-null mice

	Stomach weight (g)	Fasting gastric pH
Wild-type	0.16±0.02	1.61±0.13
Histamine H_2 receptor-null	0.38±0.02[a]	2.14±0.13[b]

Stomach weight and fasting gastric pH were measured in 12-month-old wild-type and histamine H_2 receptor-null mice. Data are expressed as means±S.E. (n=10, each group).
[a] P<0.0001 vs. wild-type mice.
[b] P=0.0134 vs. wild-type mice.

(data not shown). However, the structure of gastric oxyntic mucosa from 6-month-old histamine H_2 receptor-null mice was very similar to that of mucosa from 10-week-old histamine H_2 receptor-null mice, except for the presence of cysts near the basal region. In 12-month-old histamine H_2 receptor-null mice, in addition to the marked increase in stomach weight (Table 2), oxyntic mucosal structures appeared to differ strikingly from those of wild-type and younger histamine H_2 receptor-null mice (Fig. 7B). Oxyntic mucosa from aged histamine H_2 receptor-null mice was full of cystic structures (Fig. 7B). Most gastric glands were dilated and, in addition, interstitial tissues between cysts were markedly increased (Fig. 7D), which is in sharp contrast to the findings in gastric mucosa from aged wild-type mice (Fig. 7C). Some cells lining the cysts were positive for $H^{(+)}/K^{(+)}$-ATPase, pepsinogen and HDC (Fig. 7E,F,G), indicating that the cysts were derived from dilated gastric glands. However, small portions of oxyntic mucosa remained mostly unaltered (Fig. 7H), suggesting that the program for formation of normal gastric glands is preserved in gastric mucosal stem cells. Gastric pH values in aged histamine H_2 receptor-null mice were essentially preserved (Table 2). Similar features were observed in gastric mucosae from 24-month-old histamine H_2 receptor-null mice (data not shown). Unlike histamine H_2 receptor-null mice, there were no significant differences in oxyntic mucosae between 10-week-old and 12-month-old double null mice (data not shown).

4. Discussion

Oxyntic mucosal atrophy in double-null mice confirms the oxyntic mucosal hypertrophy observed in histamine H_2 receptor-null mice to be due to stimuli delivered via gastrin receptors. In double-null and gastrin receptor-null mice, numbers of gland cells as a whole (downward migrating cells) were decreased. However, despite gastric mucosal atrophy surface mucous cell number was moderately but significantly increased in gastrin receptor-null and double-null mice as compared with wild-type mice (Table 1). Turnover of surface mucous cells is far faster than that of downward migrating cells (Karam and Leblond, 1992, 1993a,b,c,d, 1995). Thus, it is likely that most of the increases in BrdU labeling in oxyntic mucosae in gastrin receptor-null and double-null mice are attributable to increased growth and differentiation into surface mucous cells. In the case of gastrin-null mice, the percentage of BrdU positive cells in oxyntic mucosa was not different from that in wild-type mice and there was a marked decrease in the surface mucous cells in gastrin-null mice as compared with wild-type mice (Koh et al., 1997). Thus, gastric mucosae from gastrin receptor-null and double-null mice and those from gastrin-null mice are different in terms of number of surface mucous cells. Post-translational modification of preprogastrin yields progastrin and glycine-extended gastrin as well as gastrin-17 (Dockray et al., 2001). In G-cells, gastric mucosal processing of preprogastrin yields gastrin and glycine-extended gastrin (Dockray et al., 2001). Glycine-extended gastrin reportedly has very low affinity for the gastrin receptor and has been suggested to interact with a novel receptor, which remains to be identified (Dockray et al., 2001). Thus, serum and oxyntic mucosal levels of glycine-extended gastrin may well be elevated, like those of gastrin-17, in gastrin receptor-null and double-null mice. In a study using gastrin-null mice, infusion of gastrin-17 and glycine-extended gastrin had distinct effects on gastric acid secretion, via different signal transduction pathways (Chen et al., 2000; Hollande et al., 2001; Stepan et al., 1999). Thus, the absence of glycine-extended gastrin effects in gastrin-null mice and possible hyperstimulation of the glycine-extended gastrin receptor in gastrin receptor-null mice might account for the difference in surface mucus cells in these mice. The finding of similar surface mucous cell increases in double-null mice indicates that a glycine-extended gastrin-dependent increase in surface mucous cells in the absence of gastrin receptors is not dependent on the histamine H_2 receptor. We speculate that a similar increase in surface mucous cell number in histamine H_2 receptor-null mice was caused by such a glycine-extended gastrin effect. Taken together, our results show gastrin and glycine extended-gastrin to have distinct roles in the growth of gastric mucosa.

We previously reported that maturation of the chief cell lineage was impaired in gastric mucosa from histamine H_2 receptor-null mice (Fukushima et al., 2003). In this report, mature chief cells, which we define as being positive for pepsinogen and negative for type III mucin, were present gastrin receptor-null mouse. In contrast, in histamine H_2 receptor-null mice and double-null mice expression levels of pepsinogen per cell are very low and mature chief cells were very scarce. Considering the marked difference in pH values in histamine H_2 receptor-null mice and double-null mice (Fig. 6), the difference in chief cells in these mice is not attributable to low acidity but rather to disruption of the histamine H_2 receptor itself. Genetic ablation of parietal cells with $H^{(+)}/K^{(+)}$-ATPase promoter resulted in loss of mature chief cells, which can be taken as evidence that parietal cells are involved in chief cell maturation (Canfield et al., 1996; Li et al., 1996). However, it has been suggested

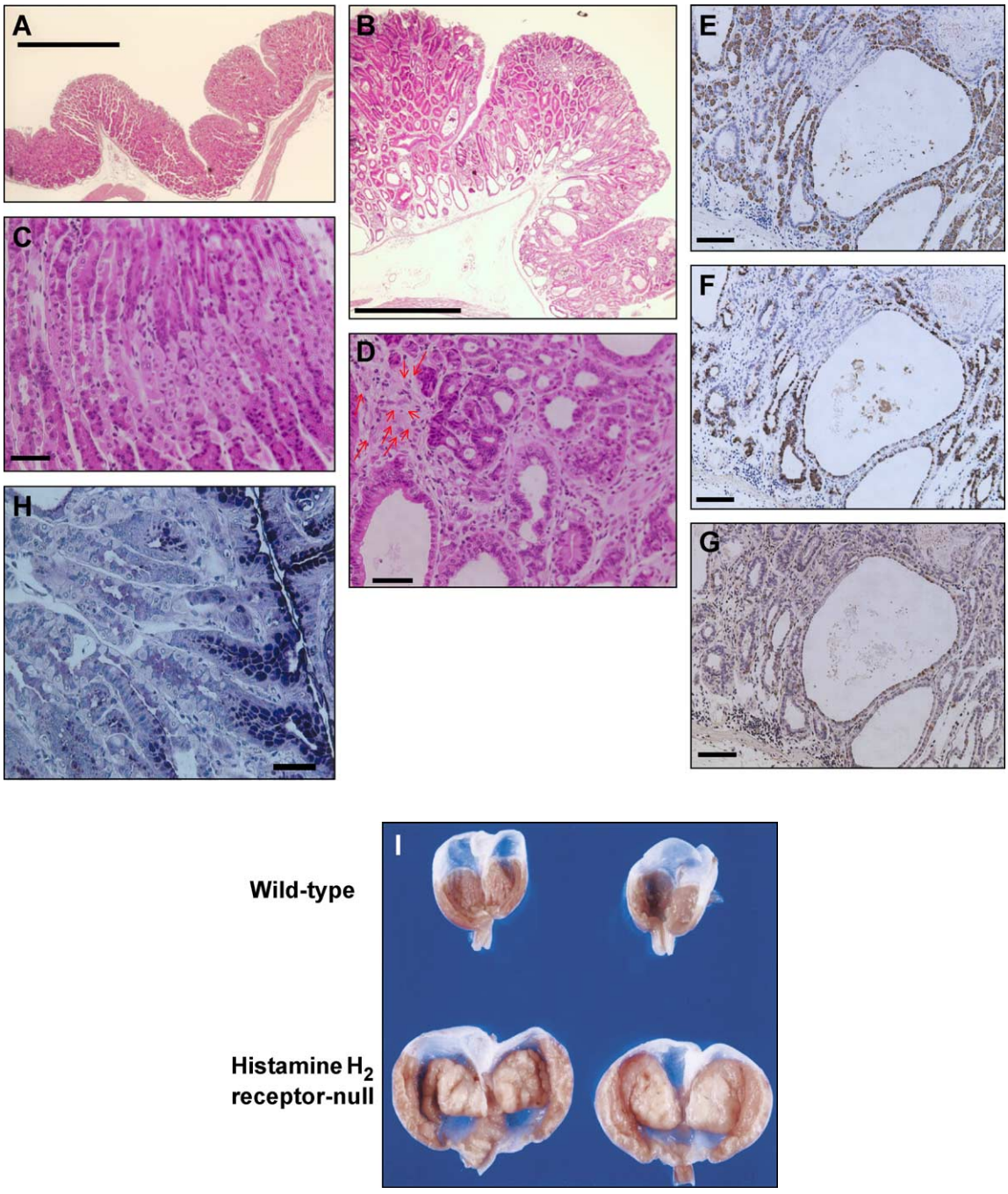

Fig. 7. Oxyntic mucosa from 12-month-old wild-type and histamine H_2 receptor-null mice. A, B, C, D, E, F, G and H Sections of oxyntic mucosa from histamine H_2 receptor-null mice (B, D, E, F, G, H) and wild-type (A, C) mice were stained with hematoxylin and eosin (A, B, C, D) and with anti-$H^{(+)}/K^{(+)}$-ATPase antibody (E), anti-pepsinogen antibody (F) or with anti-HDC antibody (G) and PAS (H). Arrows indicate interstitial cells. Scale bars, 1000 μm (A, B); 100 μm (E, F, G); 50 μm (C, D, H). (I) Macroscopic views of stomachs from wild-type and histamine H_2 receptor-null mice. The excised stomachs from 12-month-old mice were opened along the greater curvature.

that chief cell precursor cells express $H^{(+)}/K^{(+)}$-ATPase (Mutoh et al., 2002). Thus, it is likely that ablation of chief cell precursors rather than ablation of parietal cells resulted in the loss of chief cells observed in the study (Canfield et al., 1996; Li et al., 1996). In contrast, our pepsinogen and type III mucin findings show that the histamine H_2 receptor per se is involved in production and/or secretion of pepsinogen in chief cells. Thus, the histamine H_2 receptor is indispensable for chief cell maturation at least in terms of pepsinogen secretion.

Even in double-null mice, with severely impaired acid production, parietal cells and $H^{(+)}/K^{(+)}$-ATPase were present (Table 1). In addition, electron microscopic analysis of parietal cells from double-null, gastrin receptor-null and histamine H_2 receptor-null mice revealed no essential ultrastructural differences as compared to wild-type mice

(data not shown). Thus, there is no apparent structural alteration in gastric acid secretion mechanisms in double-null mice. However, gastric pH values were higher than in double-null mice than in the other three kinds of mice studied and were unresponsive even to carbachol. In histamine H_2 receptor-null mice, carbachol-induced acid production was mostly preserved (Fukushima et al., 2003; Kobayashi et al., 2000). Thus, considering the loss of the in vivo acid production response in gastrin receptor-null mice, acid production via cholinergic stimuli is largely dependent on the gastrin receptor. The finding that gastrin receptor disruption in histamine H_2 receptor-null mice, i.e. double-null mice, resulted in marked elevation of gastric pH (Fig. 6) reinforces the notion that gastrin receptors in parietal cells function in gastric acid secretion (Fukushima et al., 2003). In any case, it is noteworthy that disrupting histamine H_2 and gastrin receptors resulted in loss of response to secretagogues, even in terms of gastric pH, confirming the pivotal roles of these receptors in gastric acid production and secretion.

Recently, Ogawa et al. (2003) reported that findings in the stomachs of aged histamine H_2 receptor-null mice were compatible with Menetrier's disease. Menetrier's disease is characterized by hyperplasia of oxyntic mucosa which is attributable to hyperplasia of surface mucous cells and is often accompanied by hypoplasia of gland cells and low gastric acidity (Wolfsen et al., 1993; Yamada et al., 1995). As we previously reported, oxyntic mucosa from histamine H_2 receptor-null mice is characterized by marked hyperplasia of downward migrating cells, while hyperplasia of surface mucous cells is negligible (Fukushima et al., 2003). In 12-month-old mice, marked gastric mucosal hypertrophy was observed. However, as shown in Fig. 7, the extremely hypertrophic gastric mucosa consists of markedly elongated glands, cysts which originated from dilated gastric glands and increased interstitial tissues. The contribution of surface mucous cells is minimal. Thus, we consider it difficult to conclude that the gastric mucosal findings of aged histamine H_2 receptor-null mice are compatible with Menetrier's disease.

Rather, histological findings in aged histamine H_2 receptor-null mice can be fully explained by the findings in their 10-week-old counterparts. Oxyntic mucosal stem cells reside in the upper one-third of the mucosa away from the basal region and differentiate, growing upward or downward (Karam and Leblond, 1993a). In histamine H_2 receptor-null mice, marked hyperplasia of downward migrating cells results in unlimited movement of stem cells away from the basal region of the gastric mucosa (Fukushima et al., 2003). In addition, in the mid-portion of gastric glands both the number and mucous content of mucous neck cells are increased, which can lead to increased viscosity of the gastric juice retained in the mid-portions of gastric glands. Thus, due to this marked elongation of gastric glands together with the increased viscosity of gastric juice, gastric glands in histamine H_2 receptor-null mice would presumably be susceptible to occlusion. Once occlusion occurs, secretions from gland cells, even if impaired, promote the formation of cysts. Since gastric pH values per se are essentially preserved in histamine H_2 receptor-null mice (Fukushima et al., 2003; Kobayashi et al., 2000), leakage of contents and cystic rupture are expected to induce inflammation and an increase in interstitial tissues. Therefore, although the phenotype of stomachs from aged histamine H_2 receptor-null mice appears to be quite unusual, there is no essential difference between gastric mucosae from young and aged histamine H_2 receptor-null mice.

In conclusion, we have used double-null mice to show that (1) gastrin and histamine H_2 receptors are both essential in gastric acid production and secretion, (2) the histamine H_2 receptor plays a pivotal role in chief cell maturation, (3) gastrin gene products other than gastrin-17, such as glycine-extended gastrin, might be involved in surface mucous cell proliferation and (4) hypertrophy of gastric mucosa from histamine H_2 receptor-null mice is due to hyperstimulation of gastrin receptors via marked hypergastrinemia. Since gastric oxyntic mucosa is quite unique in that different cell types interact with each other both structurally and functionally, our murine models are potentially valuable for further analyzing differentiation of gastric mucosa and gastric acid secretion mechanisms.

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