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Structural and functional characterization of gastric mucosa and central nervous system in histamine H_2 receptor-null mice

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Abstract

To examine the physiological role of the histamine H_2 receptor, histamine H_2 receptor-null mice were generated by homologous recombination. Histamine H_2 receptor-null mice, which developed normally and were fertile and healthy into adulthood, exhibited markedly enlarged stomachs and marked hypergastrinemia. The former was due to hyperplasia of gastric gland cells (small-sized parietal cells, enterochromaffin-like cells and mucous neck cells which were rich in mucin), but not of gastric surface mucous cells, which were not increased in number as compared with those in wild-type mice despite the marked hypergastrinemia. Basal gastric pH was slightly but significantly higher in histamine H_2 receptor-null mice. Although carbachol but not gastrin induced in vivo gastric acid production in histamine H_2 receptor-null mice, gastric pH was elevated by both muscarinic M_3 and gastrin antagonists. Thus, both gastrin and muscarinic receptors appear to be directly involved in maintaining gastric pH in histamine H_2 receptor-null mice. Interestingly, gastric glands from wild-type mice treated with an extremely high dose of subcutaneous lansoprazole (10 mg/kg body weight) for 3 months were very similar to those from histamine H_2 receptor-null mice. Except for hyperplasia of gastric surface mucous cells, the findings for gastric glands from lansoprazole-treated wild-type mice were almost identical to those from gastric glands from histamine H_2 receptor-null mice. Therefore, it is possible that the abnormal gastric glands in histamine H_2 receptor-null mice are secondary to the severe impairment of gastric acid production, induced by the histamine H_2 receptor disruption causing marked hypergastrinemia. Analyses of the central nervous system (CNS) of histamine H_2 receptor-null mice revealed these mice to be different from wild-type mice in terms of spontaneous locomotor activity and higher thresholds for electrically induced convulsions. Taken together, these results suggest that (1) gastrin receptors are functional in parietal cells in histamine H_2 receptor-null mice, (2) abnormal gastric glands in histamine H_2 receptor-null mice may be secondary to severe impairment of gastric acid production and secretion and (3) histamine H_2 receptors are functional in the central nervous system.

Keywords: Gastric mucosa; Central nervous system; Histamine H_2 receptor

1. Introduction

The gastric mucosa contains several cell types (Karam and Leblond, 1992, 1993, 1995) that interact both structurally and functionally. Among them, parietal cells are unique in that they secrete acid in response to various stimuli, thereby performing a major function of the stomach, gastric acid secretion (Karam, 1993; Soll, 1980). Gastric acid secretion is stimulated by three G protein-coupled receptor ligands: histamine, acetylcholine and gastrin (Soll, 1978, 1980, 1982). Histamine acts via histamine H_2 receptors on parietal cells (Fukushima et al., 1999; Soll, 1980; Soll and Wollin, 1979), the importance of which in mediating acid production is con-

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firmed by the use of histamine H_2 receptor antagonists as successful treatment for peptic ulcers (Black et al., 1972; Fukushima et al., 2001a; Jensen et al., 1994; Taha et al., 1996).

Studies of the histamine H_2 receptor have often been carried out in isolated parietal cells (Soll et al., 1984), an approach that has yielded a great deal of information about the mechanism of gastric acid secretion. Moreover, the cloning of the histamine H_2 receptor gene has made it possible to study histamine H_2 receptors expressed in cultured cell lines (Gantz et al., 1991a,b), which allowed detailed analysis of the histamine H_2 receptor protein and yielded much information about its signaling and regulation (Alewijnse et al., 2000; Fukushima et al., 1993, 1996, 1997, 2001b,c; Saitoh et al., 2002; Smit et al., 1994, 1996a,b). However, these systems are limited in that interactions between parietal cells and other cell types are difficult to study when using isolated parietal cells or histamine H_2 receptor expressing cells. In addition, because cell lines lack an acid secreting apparatus, signaling via expressed histamine H_2 receptors is not linked to acid secretion (Arima et al., 1991). Consequently, information gained by expressing histamine H_2 receptors has been limited to analyses of the histamine H_2 receptor itself.

To circumvent these difficulties and to investigate the physiological functions of histamine H_2 receptors in vivo, we used gene targeting to generate a line of histamine H_2 receptor-null mice characterized by significantly elevated gastric pH, hypertrophy of gastric fundic mucosa and high serum gastrin levels. Recently, Kobayashi et al. (2000) reported on gastric mucosal hypertrophy in histamine H_2 receptor-deficient mice, concluding that gastrin receptors on parietal cells do not directly participate in acid secretion. In this report, we analyzed gastric mucosa from histamine H_2 receptor-null mice in more detail and found striking similarities between gastric mucosa from histamine H_2 receptor-null mice and gastric mucosa from high-dose lansoprazole-treated wild-type mice. Furthermore, we assessed the effect of histamine H_2 receptor disruption on the central nervous system (CNS) and recognized differences between wild-type mice and histamine H_2 receptor-null mice in spontaneous locomotor activity and electrically induced convulsion threshold, evidence which shows that histamine H_2 receptor is also functional in the CNS.

2. Materials and methods

2.1. Materials

YM022 was a generous gift from Yamanouchi Pharmaceutical Company. Rat gastrin-17, pirenzepine, famotidine, histamine dihydrochloride and lansoprazole were purchased from Sigma. All animal experiment procedures were reviewed and approved by the Institutional Animal Care and Research Advisory Committee of University of Tokyo.

2.2. Construction of the histamine H_2 receptor gene-targeting vector

To isolate the mouse histamine H_2 receptor gene, a mouse 129/Sv genomic library was screened with a canine histamine H_2 receptor cDNA probe (Fukushima et al., 1999). Two overlapping clones, which contained an 18-kb genomic region including the mouse histamine H_2 receptor exon, were obtained and then subcloned into pBluescript (Stratagene). The targeting vector was constructed by replacing the 2.0-kb KpnI/*Hin*dIII fragment, which contains most of the mouse histamine H_2 receptor exon (1–913), with the neomycin-resistance gene. The resultant plasmid was linearized with NotI and introduced into 129/Sv-derived SM-1 embryonic stem (ES) cells by electroporation (Shindo et al., 2000). The ES cells were selected in medium containing G418 and ganciclovir. Homologous recombinants were identified by polymerase chain reaction and Southern blot analysis. Of the 350 G418 resistant ES clones screened, 22 were homologous recombinants. Eight independent ES clones were microinjected into C57BL/6 blastocysts to generate chimeric mice. Male chimeras were then crossed with C57BL/6 females, and germline transmission was obtained for two of the eight independent ES clones. Littermates obtained by crossing heterozygotes with the genetic background of the 129/Sv × C57BL/6 hybrids were used for phenotypic analysis. For analysis of the CNS, only male mice were used.

2.3. Histological examination and immunohistochemistry

Gastric specimens were fixed in 10% phosphate-buffered formalin (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. The avidin–biotin–peroxidase complex method with specific antibodies against histidine decarboxylase (HDC), $H^{(+)}/K^{(+)}$-ATPase (Fukushima et al., 1999) and pepsinogen (Ichinose et al., 1988) was used to detect the cells expressing these gastric mucosa cell-specific markers. Paradoxical concanavalin A staining was used to detect mucosal cells synthesizing class III mucin (Katsuyama and Spicer, 1978). For studies of the proliferation of mucosal cells, we used antibodies against proliferating cell nuclear antigen (PCNA).

2.4. Northern blot analysis

Total RNAs isolated from fundic mucosa were subjected to an RNA blot analysis. The probes used were a 650-bp fragment of mouse histamine H_2 receptor cDNA and a 450-bp fragment of mouse G3PDH cDNA.

2.5. 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 body weight (BW) of famotidine, 10 mg/kg BW of pirenzepine (a muscarinic M_1 receptor antagonist) or 10 mg/kg BW of YM022 (a gastrin receptor antagonist) mice were killed and their stomachs were immediately excised. Gastric pH was measured using an ultrathin pH monitor (Horiba, Japan).

2.6. Measurement of secretagogue-induced acid secretion

Mice were maintained on anesthesia separately 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 PS three times. After extraction of the tube and ligation of the pylorus, PS or a secretagogue solution was administered peritoneally. BW (10 mg/kg) of histamine dihydrochloride, BW (0.05 mg/kg) of carbachol or BW (0.1 mg/kg) of rat gastrin-17 was administered, i.e. 50 μl/20 g BW of PS as a control, histamine dihydrochloride solution (4 mg/ml), carbachol solution (0.02 mg/ml) or gastrin-17 solution (0.04 mg/ml). We had confirmed that these doses of secretagogues induced significant increases in acid production in wild-type mice with no apparent adverse effects. Thirty minutes after administration, the mice were killed and their stomachs were excised. Gastric juice was collected with 1.5 ml of PS. Secreted gastric acid was measured by titrating the collected gastric juice to pH 7.0.

2.7. Analyses of the CNS

2.7.1. Spontaneous locomotor activity

Spontaneous locomotor activity (horizontal and vertical movements) was measured automatically over a 32-h period (8-h light/12-h dark/12-h light) using a locomotor activity measuring system (SCANET MV-10, Toyosangyou, Japan) (Kitaichi et al., 1994). An inexperienced mouse was housed in a polycarbonate cage (45 × 45 × 35 cm) that was placed in the measuring system. Both horizontal and vertical (rearing) movements were measured every 10 min. Ten 7- to 14-week-old male wild-type mice and ten 7- to 14-week-old male histamine H_2 receptor-null mice were used for the experiments.

2.7.2. Shuttle avoidance

The shuttle avoidance test was performed using experimental boxes for shuttle avoidance (Neuroscience, Japan). The temporal parameters of the discrete avoidance schedule were as follows: an intertrial interval of 25 s and a warning duration of 5 s. The warning stimulus was a 90-dB tone accompanied by light. The shock was an electric current of 100 V, 0.3 mA, 60 Hz AC, and was given to the mouse through the stainless steel floor grid of the experimental box. The maximum shock presentation was 3 s, and shuttling during the warning period led to avoidance of the electrical shock. Six sessions, consisting of 20 trials, were performed with each mouse for 3 successive days. The indices of the avoidance response were response rate (shuttlings/min) and avoidance rate (number of avoidance responses/number of avoidance trials) (Kuribara and Tadokoro, 1986). Ten male mice from each genotype group were used.

2.7.3. Convulsion thresholds for electrically and pentylenetetrazole-induced convulsions

Electrical shocks (5, 10 and 20 mA of 60 Hz AC) with a duration of 0.15 s were delivered through corneal electrodes (Toman et al., 1946), or 40, 70 or 120 mg/kg of pentylenetetrazole was administered subcutaneously (Everett and Richards, 1944). Mice were watched for 60 min after the shock or the drug administration to register tonic extensor seizures. Ten wild-type and 10 histamine H_2 receptor-null mice were used for this experiment.

2.7.4. Responses to pain and anesthetics

For the analysis of pain perception, 200 μl of 0.6% acetic acid was administered peritoneally to male wild-type mice and histamine H_2 receptor-null mice. Writhing behavior was counted for 10 min, starting 5 min after acid administration (Koster et al., 1959). Susceptibility to anesthetics was assessed by measuring the duration of anesthesia in response to 40 mg/kg of peritoneally administered pentobarbital sodium (Tanabe et al., 1991).

2.8. Statistical analysis

Quantitative values are expressed as means ± S.E. Statistical significance was tested using the unpaired *t*-test (two tailed), except for the convulsion threshold test experiment in which the χ^2-test was used. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Generation of histamine H_2 receptor-null mice

A targeting DNA construct designed to replace most of the mouse histamine H_2 receptor gene exon, together with the neomycin-resistance gene (Fig. 1A), was introduced into 129/Sv-derived ES cells (Fig. 1B), which were then injected into C57BL/6 blastocysts. Southern blotting confirmed that 10 chimeras derived from 2 independent ES clones showed germline transmission of the targeted allele (Fig. 1C). Among the offspring of these clones, the histamine H_2 receptor heterozygotes were normal in appearance and were fertile. By crossing heterozygotes, we obtained live histamine H_2 receptor-null mice, which was confirmed by the absence of histamine H_2 receptor mRNA in their gastric mucosa (Fig. 1D). Of the 343 neonates obtained from crosses between heterozygotes, 97 (28.3%) were wild-type,

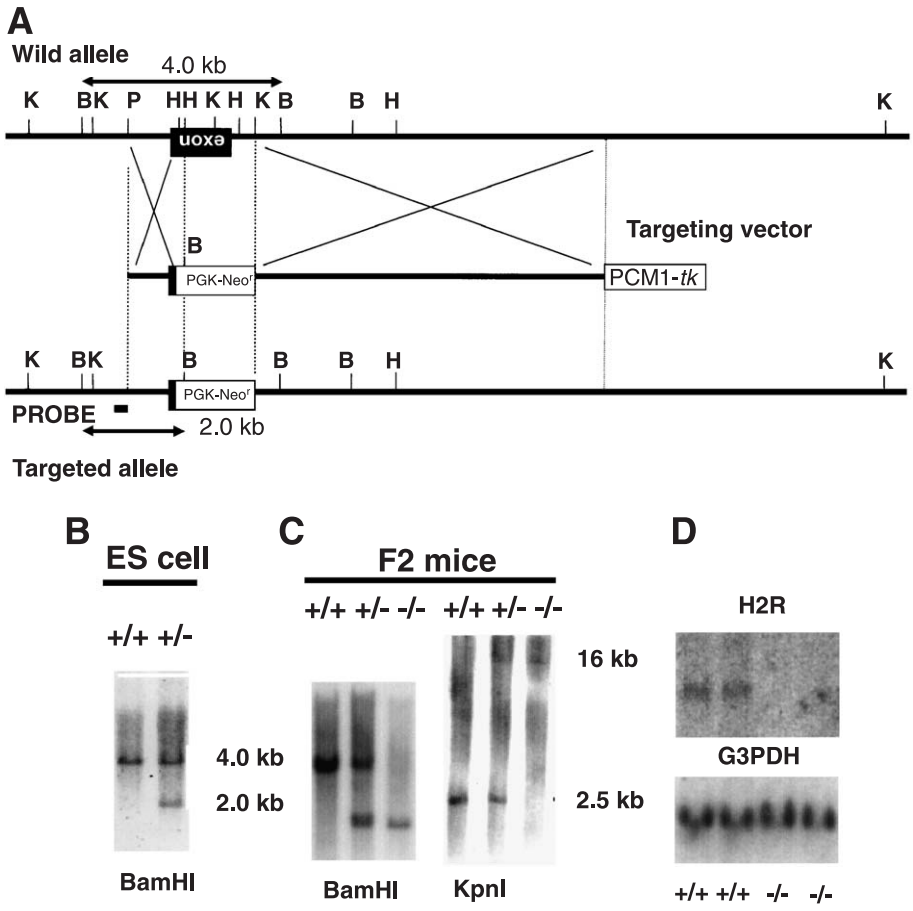

Fig. 1. Targeted disruption of the murine histamine H_2 receptor gene. (A) Diagram of the wild allele showing the targeting vector and predicted targeted allele; the exon is shown as a black box. The probe for Southern blot analysis is indicated, as are the BamHI (B), *Hin*dIII (H), KpnI (K) and PstI (P) sites. (B and C) Southern blot analyses of a homologous recombinant clone (B) and the offspring from a heterozygote intercross (C). The 2.0-kb BamHI fragment denotes the homologous recombinant allele. (D) Expression of murine histamine H_2 receptor and G3PDH mRNAs in the gastric mucosa of wild-type (+/+) and histamine H_2 receptor-null (− / −) mice.

175 (51.0%) were heterozygous and 71 (20.7%) were homozygous. This distribution conforms to Mendelian rules and indicates that targeted disruption of the histamine H_2 receptor gene did not impair embryogenesis. Histamine H_2 receptor-null mice were fertile and their growth (evaluated in terms of weight gain) was the same as that of wild-type littermates (data not shown).

3.2. Morphological and immunohistochemical analyses

The stomachs from histamine H_2 receptor-null mice were significantly larger and heavier than those from wild-type mice (Fig. 2, Table 1). For the most part, the increase in size was due to mucosal hyperplasia, which was reflected by the increase in the weight of the fundic mucosa being more pronounced than that of the stomach as a whole (Figs. 4 and 5, Table 1). Indeed, as shown in Table 2, the number of cells present in a gastric gland was increased in histamine H_2 receptor-null mice (111.2 ± 4.3 arbitrary units/gland) as compared with wild-type mice (46.8 ± 1.7 units/gland). Since gastrin has been implicated in the promotion of mucosal cell growth (Friis-Hansen et al., 1998; Ito et al., 1993; Langhans et al., 1997; Nagata et al., 1996; Wang et al., 1996), serum gastrin levels were measured and found to

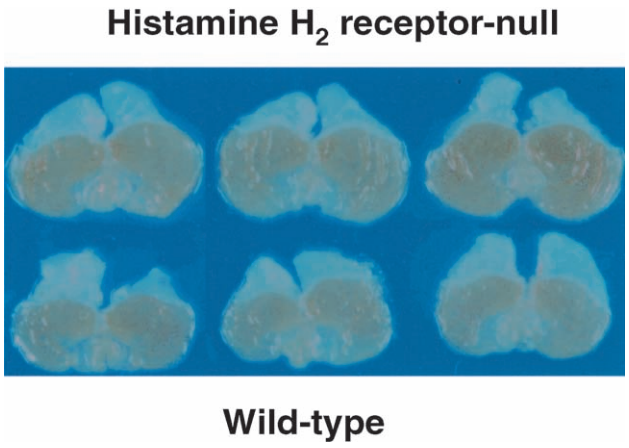

Fig. 2. Macroscopic view of stomachs from 12-week-old wild-type and histamine H_2 receptor-null mice. The excised stomachs were opened along the greater curvature.

Table 1
Weights of stomach and fundic mucosa from 12-week-old wild-type, heterozygous and histamine H_2 receptor-null mice

Genotype	Whole stomach (g/20 g BW)	Fundic mucosa (g/20 g BW)
Wild-type	0.150 ± 0.006	0.075 ± 0.061
Heterozygous	0.135 ± 0.011	0.074 ± 0.004
Histamine H_2 receptor-null	0.217 ± 0.010[a]	0.164 ± 0.017[b]

[a] $P < 0.02$ vs. corresponding values of wild-type and heterozygous mice.
[b] $P < 0.001$ vs. corresponding values of wild-type and heterozygous mice.

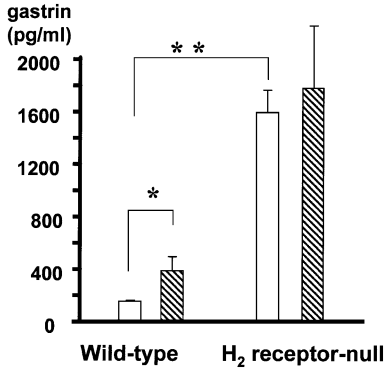

Fig. 3. Serum gastrin levels in wild-type and histamine H_2 receptor-null mice. Serum gastrin levels were measured in fasted (open bars) and fed (hatched bars) 12- to 16-week-old mice. Data are presented as means ± S.E. ($n = 15$). *$P < 0.01$, **$P < 0.001$.

be significantly higher in histamine H_2 receptor-null mice than in wild-type mice (Fig. 3). In addition, feeding stimuli induced an elevation in serum gastrin levels in wild-type mice, but not in histamine H_2 receptor-null mice (Fig. 3). This indicates that stimuli for gastrin secretion in histamine H_2 receptor-null mice are already saturated in the fasting state.

Antibodies against $H^{(+)}/K^{(+)}$-ATPase and HDC were used to identify parietal and ECL cell lineages, respectively. Parietal cells were evenly distributed throughout the entire mucosa of histamine H_2 receptor-null mice (Fig. 4A), whereas their distribution in wild-type mice was limited to the lower three-fourths of the mucosa (Fig. 4D). Parietal cell number was increased in histamine H_2 receptor-null mice as compared with wild-type mice (55.0 ± 2.2 and 21.6 ± 0.5 arbitrary units/gland, respectively). However, the percentage of parietal cells was not increased in histamine H_2 receptor-null mice as compared with wild-type mice (49% and 46%, respectively). In contrast to the increase in parietal cell number, parietal cell size, defined as the longitudinal sectional area, was significantly smaller in histamine H_2 receptor-null mice than in wild-type mice (Table 2). The upper region of the fundic mucosa, which was free of parietal cells, was composed of gastric surface mucous cells, and it thus appears that there was no increase in this cell lineage in gastric mucosa from histamine H_2 receptor-null mice (Fig. 4D). ECL cells were more abundant in the stomachs of histamine H_2 receptor-null mice (9.5 ± 1.1 units/gland) than in those of wild-type mice (2.6 ± 0.2 units/gland), and they were more widely distributed throughout the gastric mucosa than in wild-type mice (Fig. 4B,E).

To further examine the effect of histamine H_2 receptor disruption on growth and differentiation of the gastric mucosa, immunohistochemistry using antibodies against pepsinogen and mucin histochemistry with concanavalin A and PAS staining were carried out. Concanavalin A-positive cells are considered to represent mucous neck cells, which normally proliferate and then differentiate into chief cells, identifiable by their diminished expression of class III mucins and enhanced expression of pepsinogen. Higher numbers of cells positive for concanavalin A were found in the gastric glands of histamine H_2 receptor-null mice than in those of wild-type mice (Figs. 5C and 6D), and they were distributed deeper in the glands than in wild-type mice (Figs. 5E and 6B). In wild-type mice, mucous neck cells fully differentiate into chief cells before reaching the termini of the gastric glands; consequently, concanavalin A-positive cells were distributed exclusively in the mid-portion of gastric glands in wild-type mice (Fig. 5E). Staining with anti-pepsinogen antibody revealed the number of pepsinogen-positive cells to be diminished in histamine H_2 receptor-null mice (not shown), reflecting a decrease in the number of mature chief cells. PAS staining confirmed the number of surface mucous cells not to be increased in histamine H_2 receptor-null mice (Fig. 5A,D). In concert with the concanavalin A staining data, mucous neck cells in histamine H_2 receptor-null mice were increased in number (Fig. 6A,C). In addition, in histamine H_2 receptor-null mice, there were mucous neck cells in the neck region of the gastric glands, which differed from those in wild-type mice in that they contained an unusually large amount of mucin and protruded into the gastric gland lumen (Fig. 6E). These

Table 2
Quantitative analyses of gastric glands from wild-type and histamine H_2 receptor-null mice

Genotype	Total cell number/gland	Number/gland (arbitrary units/gland) and size (arbitrary units) of parietal cells		ECL cell number/gland (arbitrary units/gland)
Wild-type	46.8 ± 1.7	21.6 ± 0.5	37.5 ± 1.9	2.6 ± 0.2
Histamine H_2 receptor-null	111.2 ± 4.3[a]	55.0 ± 2.2[a]	24.8 ± 1.3[a]	9.5 ± 1.1[a]

The total cell number and number of parietal and ECL cells were counted in gastric glands sectioned centrally and in a manner parallel to their longitudinal axes. Parietal cell size was determined by measuring the longitudinal cross-sectional area of parietal cells from these gastric glands. Data are expressed as arbitrary units per gland or parietal cell since the data obtained are proportional but not equivalent to the real cell numbers or parietal cell mass.
[a] $P < 0.001$ vs. corresponding values of wild-type mice.

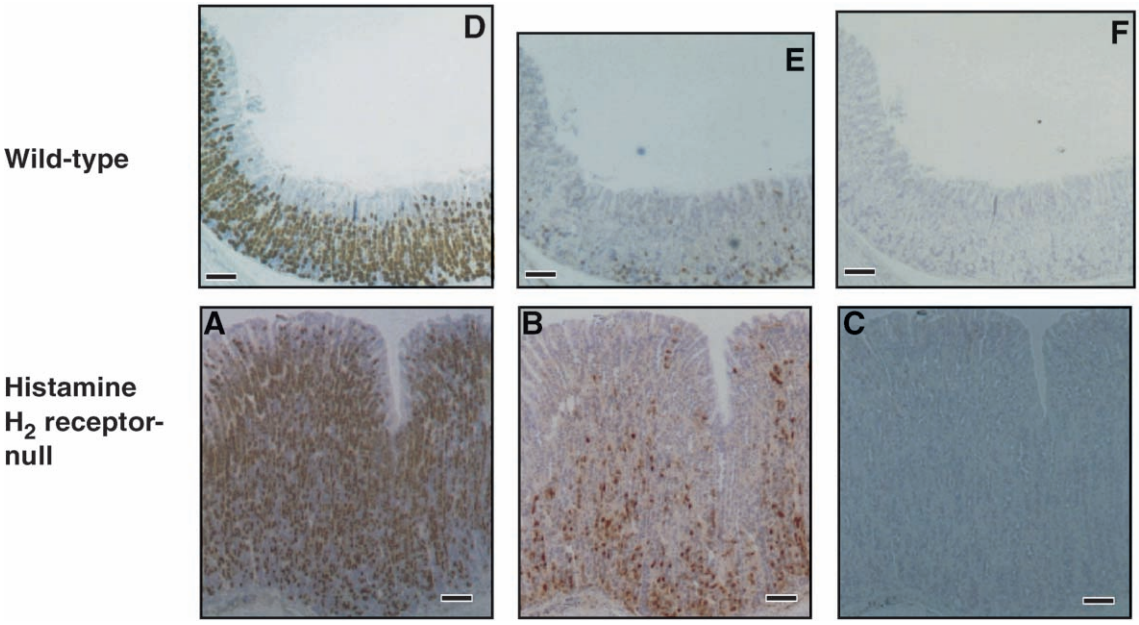

Fig. 4. Immunohistological localization of $H^{(+)}/K^{(+)}$-ATPase and HDC in fundic mucosa from wild-type and histamine H_2 receptor-null mice. Sections of fundic mucosa from 12- to 16-week-old histamine H_2 receptor-null (A, B, C) and wild-type (D, E, F) mice were stained with anti-$H^{(+)}/K^{(+)}$-ATPase (A, D), anti-HDC (B, E) or control antibody (C, F). Scale bars, 250 μm.

cells appeared white when stained with hematoxylin and eosin (not shown) and were mainly present in the antral regions of the fundic mucosa. Apparently, differentiation, but not growth, of the chief cell lineage is impaired in histamine H_2 receptor-null mice.

3.3. Secretagogue-induced acid secretion and gastric pH in wild-type and histamine H_2 receptor-null mice

Measurement of gastric acid secretion elicited in vivo by histamine (10 mg/kg BW), carbachol (0.05 mg/kg BW)

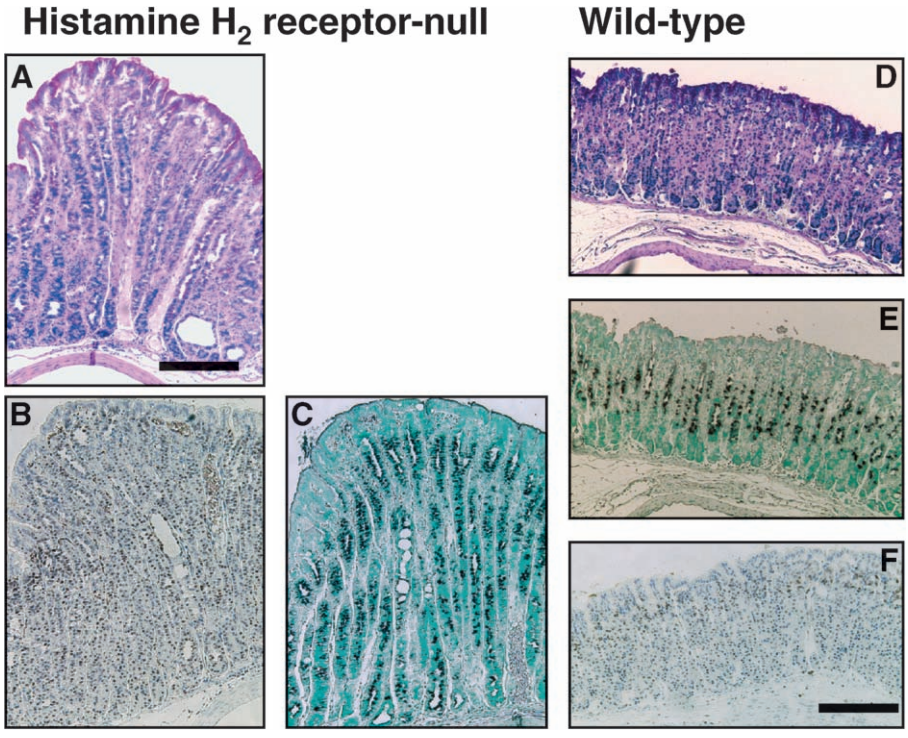

Fig. 5. PAS, concanavalin A and PCNA staining of fundic mucosa from wild-type and histamine H_2 receptor-null mice. Sections of fundic mucosa from 12- to 16-week-old histamine H_2 receptor-null (A, B, C) and wild-type (D, E, F) mice were stained with PAS (A, D), concanavalin A (B, E) or anti-PCNA (C, F). Scale bars, 200 μm.

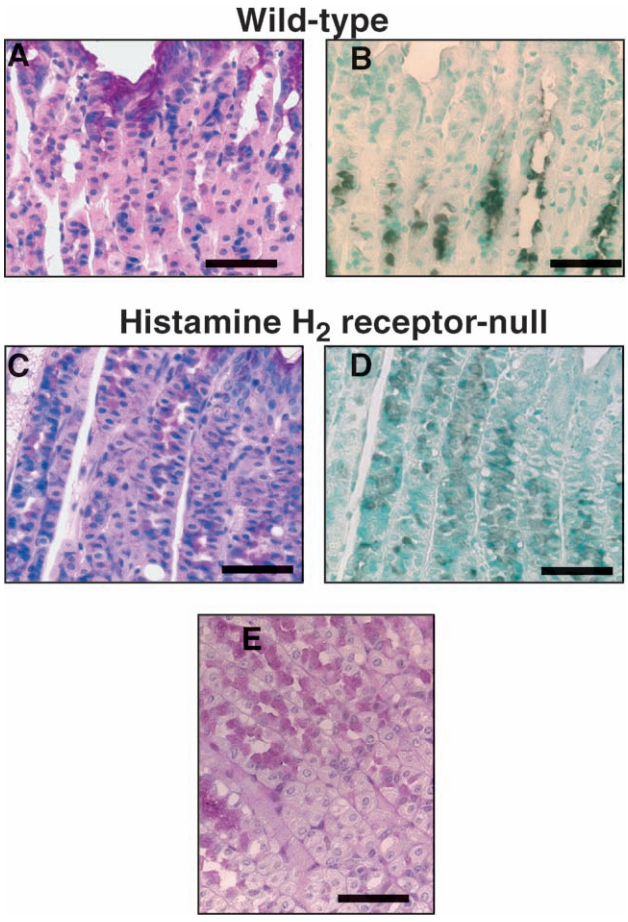

Fig. 6. PAS and concanavalin A staining of fundic mucosa from wild-type and histamine H_2 receptor-null mice. Sections of fundic mucosa from 12- to 16-week-old histamine H_2 receptor-null (C, D, E) and wild-type (A, B) mice were stained with PAS (A, C, E) and concanavalin A (B, D). Scale bars, 50 μm.

or gastrin-17 (0.1 mg/kg BW) revealed no significant difference in basal acid secretion in anesthetized wild-type mice and histamine H_2 receptor-null mice (Fig. 7). Histamine administration elevated gastric acid secretion in wild-type mice by approximately 20-fold (20.5 ± 3.4 μEq/h, $P = 0.002$ vs. PS), but had no effect in histamine H_2 receptor-null mice (0.67 ± 0.15 μEq/h, NS vs. PS) (Fig. 7). Likewise, gastrin stimulated gastric acid secretion in wild-type mice (6.2 ± 0.75 μEq/h, $P < 0.001$ vs. PS) but not in histamine H_2 receptor-null mice (1.5 ± 0.31 μEq/h, NS vs. PS) (Fig. 7). Although carbachol was effective in both wild-type and histamine H_2 receptor-null mice, the effect was significantly greater in the former (9.64 ± 0.84 μEq/h vs. 4.60 ± 0.30 μEq/h, $P < 0.001$) (Fig. 7). Thus, in addition to a loss of histamine sensitivity, gastrin-dependent acid secretion was also severely impaired in histamine H_2 receptor-null mice.

Basal gastric pH was slightly but significantly higher in fasted histamine H_2 receptor-null mice than in wild-type mice (2.41 ± 0.21 vs. 1.86 ± 011, $P = 0.024$). In addition,

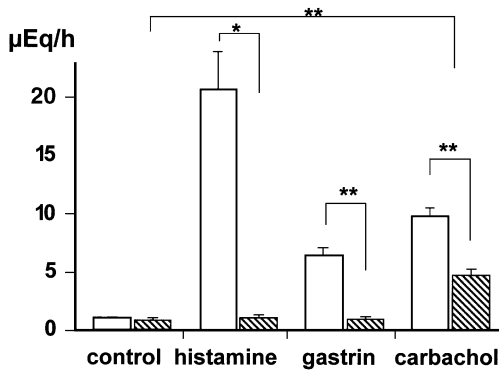

Fig. 7. Secretagogue-induced gastric acid secretion in wild-type and histamine H_2 receptor-null mice. Mice (12- to 16-week-old) were anesthetized with diethylether, after which the stomach and duodenum were exposed via an epigastric midline incision. A tube was inserted from the duodenum into the gastric lumen, and the stomachs were washed three times with 1 ml of prewarmed PS. After extraction of the tube, the pylorus was ligated and PS or secretagogue solution was administered peritoneally. Histamine dihydrochloride (10 mg/kg BW), carbachol (0.05 mg/kg BW) or rat gastrin-17 (0.1 mg/kg BW) was administered in a volume of 50 μl/20 g BW of PS. Thirty minutes after administration, the mice were killed, their stomachs excised and the gastric juice was collected with 1.5 ml of PS. Secreted gastric acid was measured by titrating the collected gastric juice to pH 7.0. Wild-type mice, open bars; histamine H_2 receptor-null mice, hatched bars. Data are presented as means ± S.E. ($n = 15$). *$P < 0.001$, **$P < 0.01$ vs. respective control values.

Fig. 8 shows that the histamine H_2 receptor antagonist famotidine (10 mg/kg BW) had no effect on gastric pH in histamine H_2 receptor-null mice, but significantly elevated gastric pH in wild-type mice. The muscarinic antagonist pirenzepine significantly elevated gastric pH in both wild-type mice and histamine H_2 receptor-null mice. Importantly,

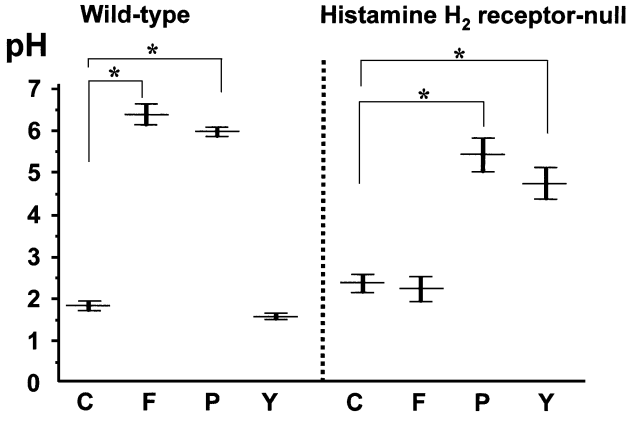

Fig. 8. Effects of histamine H_2 receptor, muscarinic M_1 receptor and gastrin receptor antagonists on gastric pH in wild-type and histamine H_2 receptor-null mice. Wild-type and histamine H_2 receptor-null mice (12- to 16-week-old) were fasted overnight with free access to water. At 1.5 h after subcutaneous injection of vehicle (0.5% methylcellulose) ($n = 30$), 10 mg/kg body weight famotidine ($n = 30$), 10 mg/kg body weight pirenzepine ($n = 30$) or 10 mg/kg body weight YM022 ($n = 30$), the mice were killed and their stomachs immediately excised. Gastric pH was measured using an ultrathin pH monitor. C, vehicle; F, famotidine; P, pirenzepine; Y, YM022. Data are presented as means ± S.E. *$P < 0.001$ vs. respective values.

the gastrin antagonist YM022 significantly elevated gastric pH in histamine H_2 receptor-null mice but not in wild-type mice, indicating that gastrin receptors on parietal cells do indeed participate directly in maintaining gastric pH in parietal cells.

3.4. Effect of lansoprazole administration on stomachs from wild-type and histamine H_2 receptor-null mice

Treatment with proton pump inhibitors leads to hypertrophy of the gastric mucosa, possibly via the proliferative effect of gastrin (Langhans et al., 1997; Larsson et al., 1986; Nagata et al., 1996). We treated 4-week-old wild-type and histamine H_2 receptor-null mice with an extremely high dose of lansoprazole (10 mg/kg BW) for 12 weeks in order to block acid production completely. Significant increases in stomach weight in both wild-type and histamine H_2 receptor-null mice were observed (Table 3). The increases in stomach weight were more prominent in histamine H_2 receptor-null mice (49%) than in wild-type mice (15%). Histological examination revealed mucous neck cells to be increased in number in both wild-type mice and histamine H_2 receptor-null mice. The increase in these mucous neck cells in wild-type mice was predominantly in the fundic mucosa near the antral region (data not shown). The mucous neck cells in lansoprazole-treated mice looked very similar to those in both nontreated and treated histamine H_2 receptor-null mice, i.e. they were full of mucin and protruded into the gastric gland lumen. Lansoprazole treatment induced an increase in parietal cell number in wild-type mice but not in histamine H_2 receptor-null mice (Table 3). Interestingly, lansoprazole treatment brought about a reduction in parietal cell size both in wild-type mice and histamine H_2 receptor-null mice (Table 3). The size of parietal cells from lansoprazole-treated wild-type mice was comparable to that of parietal cells from nontreated histamine H_2 receptor-null mice. Serum gastrin levels in wild-type mice were significantly elevated, but those in histamine H_2 receptor-null mice were unaffected by lansoprazole treatment (Table 3). In accordance with the elevated serum gastrin levels, gastric surface mucous cell number increased in wild-type mice treated with lansoprazole. In contrast, no further increase in gastric surface mucous cell number was observed in histamine H_2 receptor-null mice treated with lansoprazole. These findings for gastric mucosa from lansoprazole-treated wild-type mice are noteworthy. Except for the increase in gastric surface mucous cells, gastric glands from lansoprazole-treated wild-type mice were very similar to those from histamine H_2 receptor-null mice, an increase in the number of gastric gland cells, parietal cells and mucous neck cells and a decrease in parietal cell size. With

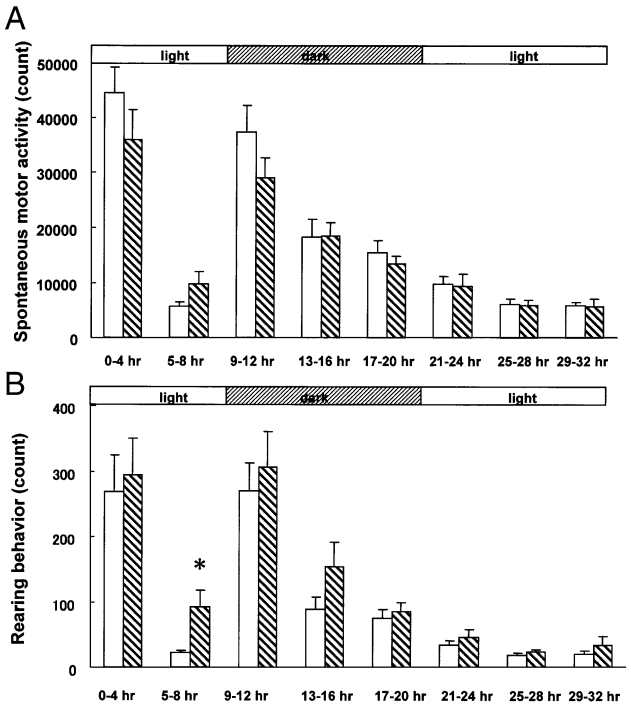

Fig. 9. Locomotor activity of wild-type and histamine H_2 receptor-null mice. Spontaneous locomotor activity was measured automatically over a 32-h period (8-h light/12-h dark/12-h light) using a locomotor activity measuring system. An inexperienced mouse was housed in a polycarbonate cage (45 × 45 × 35 cm) that was placed in the measuring system. Both horizontal (A) and vertical (rearing) (B) movements were recorded every 10 min. Ten 7- to 14-week-old male wild-type mice (open bars) and ten 7- to 14-week-old male histamine H_2 receptor-null mice (hatched bars) were used for this experiment. Data are presented as means ± S.E. of counts for each 4-h time period. *$P < 0.05$ vs. wild-type mice.

Table 3
Effects of lansoprazole treatment on wild-type and histamine H_2 receptor-null mice

Genotype	Treatment	Stomach weight (g/20 g BW)	Number/gland (arbitrary units/gland) and size (arbitrary units) of parietal cells		Serum gastrin (pg/ml)
Wild-type	vehicle	0.167 ± 0.051	19.5 ± 0.8	11.4 ± 0.5	163 ± 37
	lansoprazole	0.193 ± 0.090[a]	34.7 ± 1.6[b]	6.2 ± 0.2[b]	1117 ± 136[b]
Histamine H_2	vehicle	0.222 ± 0.063	70.0 ± 2.0	7.8 ± 0.4	1620 ± 209
receptor-null	lansoprazole	0.331 ± 0.021[c]	68.0 ± 2.5	4.9 ± 0.2[b]	2025 ± 409

Mice were treated with 10 mg/kg of subcutaneous lansoprazole for 12 weeks and fasted overnight.
[a] $P < 0.02$ vs. corresponding values of vehicle.
[b] $P < 0.001$ vs. corresponding values of vehicle.
[c] $P = 0.001$ vs. corresponding values of vehicle.

this high dose of lansoprazole, gastric acid production was completely blocked. Thus, the findings for the gastric glands from histamine H_2 receptor-null mice might not be a result of the histamine H_2 receptor deficiency itself but rather of the completely blocked acid production due to a lack of histamine H_2 receptor.

3.5. Effect of histamine H_2 receptor deficiency on the CNS

Histamine H_2 receptors are abundantly expressed in the CNS as well as in the stomach. To explore the possible role of histamine H_2 receptors in the CNS, several experiments were performed. Shuttle test avoidance, locomotor activity, electrically and drug-induced convulsion thresholds, pain perception and susceptibility to anesthetics were analyzed.

No significant difference was found between wild-type and histamine H_2 receptor-null mice ($n = 10$) in the shuttle avoidance test (data not shown). This indicates that learning and memory are not affected by histamine H_2 receptor gene disruption. In contrast, there was a difference in the locomotor activity between wild-type mice and histamine H_2 receptor-null mice. In terms of horizontal movements, although not significant, histamine H_2 receptor-null mice were less active than wild-type mice especially in new environments (Fig. 9A, 0–4 h, 9–12 h). However, there was tendency for histamine H_2 receptor-null mice to be more active than wild-type mice during the 5- to 8-h observation period, indicating that histamine H_2 receptor-null mice were more restless even after adaptation to a new environment. As for rearing behavior, histamine H_2 receptor-null mice tended to be more active than wild-type mice during the entire observation period. A significant difference in rearing behaviors was recognized during the 5- to 8-h observation period (wild-type 22 ± 4 counts/4 h, histamine H_2 receptor-null 89 ± 18 counts/4 h, $P < 0.05$).

Susceptibility to anesthetics and responses to pain did not differ between wild-type mice and histamine H_2 receptor-null mice (data not shown). However, thresholds for electrically induced convulsions, but not for pentylenetetrazole-induced convulsions, differed significantly between wild-type mice and histamine H_2 receptor-null mice (Table 4). Histamine H_2 receptor-null mice were significantly less prone to electrically induced convulsions than were wild-type mice. However, no difference was found between histamine H_2 receptor-null mice and wild-type mice in terms of pentylenetetrazole-induced convulsions.

Table 4

A. Susceptibility to electrically induced convulsion

Genotype	Number of mice tested	Number of mice with tonic extensor convulsion		
		5 mA	10 mA	20 mA
Wild	10	0	10	10
Histamine H_2 receptor-null	10	0	5[a]	10

B. Susceptibility to pentylenetetrazole-induced convulsion

Genotype	Number of mice tested	Number of mice with tonic extensor convulsion		
		40 mg/kg	70 mg/kg	120 mg/kg
Wild	10	0	4	10
Histamine H_2 receptor-null	10	0	5	10

[a] $P < 0.05$ (χ^2-test).

4. Discussion

We have shown that targeted disruption of the mouse histamine H_2 receptor gene results in a marked increase in stomach size and elevated serum gastrin levels. The former is attributable to hypertrophy of the fundic mucosa due to hyperplasia of parietal cells, mucous neck cells and ECL cells. The percentage of mucous neck cells and ECL cells per gland was increased as well. In addition to the increased numbers of these gland cells, there were morphological abnormalities in parietal and chief cell lineages. First, as Kobayashi et al. reported, parietal cells in histamine H_2 receptor-null mice were smaller than those in wild-type mice. Second, peculiar mucous neck cells full of mucin and protruding into the glands were present in the upper portion of the neck region of gastric glands in histamine H_2 receptor-null mice. These cells were present mainly in the fundic mucosa near the pyloric region in histamine H_2 receptor-null mice. In addition, even in glands without these peculiar neck cells, increased proliferation of mucous neck cells, deeper in the gastric glands was observed, indicating a maturation impairment of chief cell lineages. It is very important to determine whether this mucosal phenotype is directly attributable to the disruption of the histamine H_2 receptor gene itself or secondary to the changes in parietal cells and acid secretion induced by the mutation.

Despite prominent hypergastrinemia, there was no increase in the number of gastric surface mucous cells in histamine H_2 receptor-null mice. This is in contrast to transgenic mice with comparable serum gastrin levels, which exhibit hyperplasia of gastric surface mucous cells (Konda et al., 1999). Similarly, elongation of the pit region has been observed in other murine models overexpressing gastrin (Wang et al., 1996). By contrast, not only did 6-month-old histamine H_2 receptor-null mice show a similar hyperplasia, they actually did not show an increase in the gastric surface mucous cell layer either (data not shown). Since increased proliferation from stem cells in both luminal and basal directions was observed with PCNA staining in histamine H_2 receptor-null mice, it is unlikely that gastric surface mucous cells stop proliferating just after their differentiation. In fact, we observed an increase in apoptotic cells in gastric surface mucous cell layers, but not in other cell types, in histamine H_2 receptor-null mice as compared with wild-type mice (data not shown). Thus, the finding that gastric surface mucous cells were not increased in number in

histamine H_2 receptor-null mice might be due to the susceptibility of gastric surface mucous cells to apoptosis. It may be that a small number of histamine H_2 receptors, possibly expressed on gastric surface mucous cells, or intercellular signals from histamine H_2 receptors in parietal cells might be important for protecting gastric surface mucous cells from apoptosis. Taking these observations together, we can conclude that in histamine H_2 receptor-null mice hyperplasia of the downward growing components, which are morphologically abnormal, contributes to hypertrophy of the gastric mucosa.

Gastric acid secretion in histamine H_2 receptor-null mice was impaired, as shown by the absence of gastrin and histamine-induced acid production in vivo. In contrast, carbachol-induced gastric acid production was present in histamine H_2 receptor-null mice, as previously reported. Interestingly, in the absence of histamine H_2 receptor-mediated acid production, gastric pH in histamine H_2 receptor-null mice, although slightly but significantly higher than that in wild-type mice, was essentially preserved. Administration of a gastrin or muscarinic antagonist significantly elevated gastric pH in histamine H_2 receptor-null mice, indicating that stimuli delivered via gastrin and muscarinic receptors can maintain gastric pH in histamine H_2 receptor-null mice and that gastrin receptors in parietal cells stimulate acid production. Gastric pH can affect gastrin release from gastric G-cells (Walsh et al., 1975). However, the markedly elevated serum gastrin levels in histamine H_2 receptor-null mice, which maintain gastric pH in these mice, cannot be explained by the slightly higher gastric pH. In histamine H_2 receptor-null mice, in accordance with the markedly increased serum gastrin levels, an increase in gastrin immunoreactivity in the pyloric mucosa of the antral region was observed (data not shown). However, there was no gastrin production in the fundic mucosa from histamine H_2 receptor-null mice. Anatomically, the fundic mucosa and pyloric mucosa are discrete. Thus, stimuli other than gastric pH changes for gastrin production and secretion, if present at all, are not conveyed directly from parietal cells but via other humoral factors. Using AGS cells that express endogenous gastrin, we examined the possibility that specific humoral factors present in the serum of histamine H_2 receptor-null mice might stimulate gastrin synthesis and secretion. However, serum from histamine H_2 receptor-null mice did not increase levels of gastrin mRNA, as determined by reverse transcriptase polymerase chain reaction (data not shown). This method might not be sensitive enough to detect the presence of possible humoral factors. Another possible explanation for the increased gastrin production is that gastric pH during feeding might be affected more severely than fasting gastric pH in histamine H_2 receptor-null mice.

As for the impairment in gastrin-induced gastric acid production, three explanations can be considered. First, most acid-producing stimuli acting via gastrin are mediated by the release of histamine from ECL cells. Second, histamine H_2 receptor disruption might directly affect a gastrin receptor signaling pathway leading to acid production, though this possibility is less likely because muscarinic signaling, which also elevates intracellular Ca^{2+}, is preserved in histamine H_2 receptor-null mice. Third, high serum gastrin concentrations might desensitize and/or downregulate gastrin receptors, disrupting gastrin-dependent gastric acid secretion in histamine H_2 receptor-null mice. Desensitization of histamine H_2 receptors has been observed (Fukushima et al., 1993; Smit et al., 1994), and both phenomena are well characterized for other G protein-coupled receptors (Lefkowitz, 1998; Pitcher et al., 1998). Gastric acid secretion in gastrin transgenic mice, which exhibit serum gastrin levels similar to those of our histamine H_2 receptor-null mice, is highly responsive to gastrin (Konda et al., 1999). In either case, it can be concluded that although gastrin-induced acid production in histamine H_2 receptor-null mice is impaired, gastric receptors on parietal cells are directly involved in mediating acid secretion. Whatever the cause of the hypergastrinemia in histamine H_2 receptor-null mice, it is important to determine what percentage of the gastric hypertrophy is caused by hypergastrinemia.

The gastric mucosa findings for lansoprazole-treated wild-type and histamine H_2 receptor-null mice were very surprising. With 10 mg/kg BW of lansoprazole, which is 20 times the dose used in humans, acid production in wild-type and histamine H_2 receptor-null mice should be completely blocked. Interestingly, fundic mucosa from lansoprazole-treated wild-type mice was very similar to that from histamine H_2 receptor-null mice except for the increase in surface mucous cells due to hypergastrinemia. First, there were increased numbers of parietal cells, ECL cells and mucous neck cells in wild-type mice after lansoprazole treatment. The parietal cells were smaller than those in wild-type mice treated with vehicle alone. In addition, the mucous neck cells were full of mucin and looked very similar to those in histamine H_2 receptor-null mice. It is significant that these characteristics, small parietal cells and peculiar mucous neck cells, were seen in lansoprazole-treated wild-type mice, suggesting that the mucosal phenotype in histamine H_2 receptor-null mice is secondary to impaired acid production and secretion by parietal cells. The nature of the impairment in gastric acid production and secretion by parietal cells from histamine H_2 receptor-null mice remains to be revealed. In histamine H_2 receptor-null mice, lansoprazole treatment induced a further increase in the number of mucous neck cells and very interestingly a further reduction in parietal cell size. This further reduction in parietal cell size, with lansoprazole treatment, supports the possibility that the mucosal findings were secondary to impaired acid production and secretion. The reason for the greater increase in stomach weight in histamine H_2 receptor-null mice than in wild-type mice remains to be determined.

Histamine H_2 receptors are also abundantly expressed in the CNS (Vizuete et al., 1997). The histamine H_2 receptor

was recently implicated in the pathogenesis of schizophrenic disorders (Martinez, 1999). Although we observed no obvious abnormalities in either the morphology or the histology of the brains of histamine H_2 receptor-null mice (data not shown), we performed several experiments to examine the possible role of histamine H_2 receptors in the CNS. Locomotor activity, shuttle test avoidance, electrically induced convulsion threshold, pentylenetetrazole-induced convulsion threshold, pain perception and susceptibility to anesthetics were analyzed. In two of these experiments, we detected significant differences in locomotor activity and electrically induced convulsion threshold between wild-type mice and histamine H_2 receptor-null mice. During the 5- to 8-h observation period, when wild-type mice were accustomed to the new environment and horizontal movements had decreased, although not statistically significant, histamine H_2 receptor-null mice were more active than wild-type mice. That is, histamine H_2 receptor-null mice were restless at rest. In addition, during the entire observation period, histamine H_2 receptor-null mice were more active in terms of rearing behavior. A significant difference was observed during the 5- to 8-h observation period. Histaminergic and dopaminergic systems are suggested to interact with each other in the CNS (Paul et al., 2000). Rearing behavior is thought to represent motivation, which involves dopaminergic systems. Thus, the present findings might be attributable to effects of the histamine H_2 receptor gene disruption on the dopaminergic system. As for the convulsion threshold, histamine H_2 receptor-null mice were less prone to electrically induced convulsions than wild-type mice. In contrast, there was no difference in pentylenetetrazole-induced convulsions, indicating that disruption of the histamine H_2 receptor does not affect the gamma-aminobutyric acid-benzodiazepine system. Although the mechanisms underlining these findings in histamine H_2 receptor-null mice remain to be determined, these findings provide strong evidence that histamine H_2 receptors in the CNS are functional. These findings may represent only a small portion of the CNS changes resulting from the loss of histamine H_2 receptors, and further studies are needed on the role of histamine H_2 receptors in the CNS.

In conclusion, we have shown here that histamine H_2 receptor disruption affects the gastric mucosa both functionally and structurally. In addition, significant differences were observed in the CNS functions of wild-type mice and histamine H_2 receptor-null mice. Thus, the histamine H_2 receptor-null mouse is a potentially valuable tool for further analysis of the gastric mucosa and the CNS.

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