

Impaired gastric acid secretion in gastrin-deficient mice

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Departments of ¹Physiology, ⁴Medicine, and ⁵Pathology, The University of Michigan, Ann Arbor, Michigan 48109; ²Department of Clinical Biochemistry, Rigshospitalet, DK-2100 Copenhagen, Denmark; and ³Department of Physiology and Neuroscience, Lunds Universitet, Lund, Sweden

Friis-Hansen, Lennart, Frank Sundler, Ying Li, Patrick J. Gillespie, Thomas L. Saunders, Joel K. Greenson, Chung Owyang, Jens F. Rehfeld, and Linda C. Samuelson. Impaired gastric acid secretion in gastrin-deficient mice. *Am. J. Physiol.* 274 (*Gastrointest. Liver Physiol.* 37): G561–G568, 1998.—To further understand the role of the peptide hormone gastrin in the development and function of the stomach, we have generated gastrin-deficient mice by gene targeting in embryonic stem cells. Mutant mice were viable and fertile, without obvious visible abnormalities. However, gastric function was severely affected by the loss of gastrin. Basal gastric acid secretion was abolished and could not be induced by histamine, carbachol, or gastrin. Histological analysis revealed alterations in the two cell types primarily involved in acid secretion, parietal and enterochromaffin-like (ECL) cells. Parietal cells were reduced in number with an accumulation of immature cells lacking H⁺-K⁺-adenosinetriphosphatase (H⁺-K⁺-ATPase). ECL cells were positioned closer to the base of the gastric glands, with markedly lower expression of histidine decarboxylase. Gastrin administration for 6 days reversed the effects of the gastrin deficiency, leading to an increase in the number of mature, H⁺-K⁺-ATPase-positive parietal cells and a partial restoration of acid secretion. The results show that gastrin is critically important for the function of the acid secretory system.

achlorhydria; gastric mucosa; gastrointestinal hormones; knockout mice; parietal cells

GASTRIN is the principal hormonal regulator of gastric acid secretion. Eating a meal stimulates gastrin release from G cells in the antral part of the stomach, the primary site of gastrin synthesis in the adult. The acid-producing portion of the stomach responds to rises in circulating gastrin by increasing acid output (34). Gastrin stimulation of acid secretion is mediated through the gastrin/cholecystokinin-B receptor (CCK-B), which has almost equal affinity for gastrin and the related intestinal hormone CCK (35). Gastrin receptors are found on the two cell types primarily responsible for the secretion and regulation of gastric acid, parietal and enterochromaffin-like (ECL) cells (32). In addition to its role as a regulator of acid secretion, gastrin also functions as a growth factor for the gastric mucosa. Elevated gastrin levels increase both parietal and ECL cell numbers (4, 20), and sustained hypergastrinemia can be associated with the development of ECL cell carcinoid tumors (11).

Acid secretion by the parietal cell is stimulated by gastrin, histamine, and acetylcholine (ACh). Gastrin or ACh activation of the parietal cell can occur by two pathways: directly by activating receptors on the parietal cell surface, and indirectly by stimulating ECL

cells to release histamine, which then activates parietal cells in a paracrine fashion. The importance of direct stimulation of the parietal cell vs. indirect stimulation via histamine is not fully known. However, a central role for histamine in acid secretion is demonstrated by the potent inhibitory effect of histamine H₂ receptor antagonists on secretory responses (2). ACh release by vagal cholinergic pathways stimulates parietal cells by interacting with muscarinic M₃ receptors (14). Activation of gastrin and M₃ receptors results in increased intracellular Ca²⁺ (24), whereas the H₂ receptor couples to the adenosine 3',5'-cyclic monophosphate (cAMP) signal-transduction pathway (1). Therefore, activation of parietal cells can involve at least two different second messenger pathways.

Recently, genetic models have been useful to gain insight into the physiological function of peptide hormones. In the present study a gastrin-deficient mouse strain was produced by gene targeting in embryonic stem (ES) cells. Analysis of gastric physiology and mucosal cellular development in the mutant demonstrates that gastrin is required for the functional maturation of the acid secretory system.

METHODS

Gene targeting and generation of mice. The targeting vector contained fragments from upstream (8-kb *Not* I-*Xho* I) and downstream (1.2-kb *Xba* I) of the mouse gastrin gene cloned into the pPNT vector (Fig. 1). Gene targeting in R1 ES cells (26) was performed as previously described (17) with the use of recombinant leukemia inhibitory factor (ESGRO; Life Technologies, Gaithersburg, MD). Targeted ES clones were identified by polymerase chain reaction (PCR) screening with primers TS-NEO (5'-CGCCTTCTATCGCCTTCTTGACGAGTTCTT) and GS (5'-CCTGCCTCTCAGCATCCCATCCAAAGT) and verified by genomic Southern analysis and long-range PCR (21). Four clones were microinjected into C57BL/6 blastocysts, with two clones yielding chimeric mice that transmitted the targeted mutation through the germ line. Male chimeras were crossed with female C57BL/6 mice to generate a 129/Sv × C57BL/6 heterozygous founder generation. Mice used in experiments were obtained from intercrossing heterozygous founders. Genotypes were determined using multiplex PCR with primers TS-NEO, GS, and CA (5'-TCTTCTCATTTCCTTGGACATCTGTA) and verified by Southern analysis (Fig. 1).

Gastric acid secretion. Mice 10–16 wk old were fasted overnight with free access to water, anesthetized with a mixture of xylazine and ketamine (11.3 and 8.7 mg/kg im, respectively), and maintained at 37°C on an electric heated pad. The abdomen was opened and the esophagus and pylorus were ligated. PE-50 tubing (0.58 mm ID, 0.965 mm OD) was inserted through an incision in the forestomach to infuse normal saline (0.2 ml/min), and PE-240 tubing (1.67

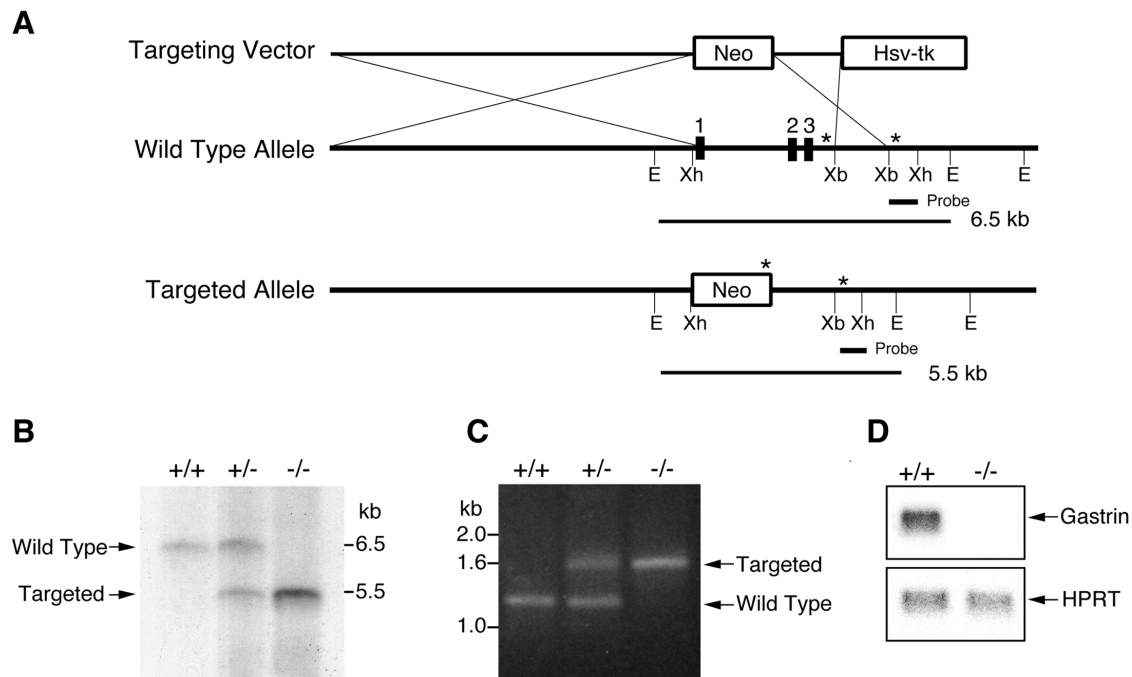


Fig. 1. Targeted deletion of the mouse gastrin gene. *A*: a diagram of the targeting vector is shown above the wild-type gastrin gene with its 3 exons. After homologous recombination, the neomycin cassette (neo) replaces all 3 exons, resulting in complete deletion of the gastrin gene from the targeted allele. Polymerase chain reaction (PCR) screening primers (*) and probe and diagnostic restriction fragments for Southern analysis are shown. E, *EcoR* I; Xh, *Xho* I; Xb, *Xba* I. *B*: Southern blot analysis of wild-type (+/+), heterozygous (+/-), and gastrin-deficient (-/-) mice. Genomic DNA was digested with *EcoR* I, and blot was hybridized with probe shown in *A*. *C*: PCR genotyping of wild-type (+/+), heterozygous (+/-), and gastrin-deficient (-/-) mice. Gastrin alleles were amplified with primers TS-NEO, GS, and CA and resolved by agarose gel electrophoresis followed by ethidium bromide staining. Multiplex PCR simultaneously amplifies wild-type (1.2 kb) and targeted (1.6 kb) gastrin alleles. *D*: Northern blot analysis of gastrin gene expression in wild-type (+/+) and gastrin-deficient (-/-) mice. A filter containing stomach RNA [1 μ g poly(A⁺)] was first hybridized with a mouse gastrin probe, washed, and then hybridized with a mouse hypoxanthine-guanine phosphoribosyl transferase (HPRT) probe as a loading control.

mm ID, 2.42 mm OD) was inserted in the antrum to collect stomach secretions. Samples were collected every 10 min, and acid output (μ mol H⁺) was determined by titration. Saline, histamine (20 mg/g body wt; Sigma Chemical, St. Louis, MO), carbachol (2 μ g/mouse), or rat gastrin-17 (1 μ g/g body wt; Peninsula Laboratories, Belmont, CA) was injected subcutaneously.

Peptide quantitation. Blood and tissues were collected from mice that had been fasted overnight with free access to water. Peptides were extracted (5) and measured by radioimmunoassay (RIA), using the following antisera: gastrin 2604 (28); sulfated CCK-92128 (J. F. Rehfeld, unpublished observations); and COOH-terminal chromogranin A (CgA)-95037 (J. F. Rehfeld, unpublished observations). Histamine was extracted from the stomach as described previously (20) and measured by enzyme-linked immunosorbent assay (Immuno-technique, Westbrook, ME).

Histochemical analysis. For hematoxylin-eosin staining, stomachs from freely fed mice (8–16 wk old) were dissected, rinsed in phosphate-buffered saline (PBS; 0°C), fixed in 4% paraformaldehyde in PBS (4–6 h), embedded in paraffin, and sectioned (3 μ m). Tissues for immunohistochemistry were fixed overnight in 2% formaldehyde and 0.2% picric acid phosphate buffer (pH 7.2), washed in buffer with 10% sucrose (wt/vol), and frozen on dry ice. Sections (10 μ m) were then cut and mounted on chrome alum-coated slides (9). Immunostaining was done by indirect immunofluorescence and viewed with a Leica Aristoplan microscope. Fluorescein isothiocyanate (FITC)-labeled swine anti-rabbit immunoglobulin G

(IgG; Dako, Carpinteria, CA) or FITC anti-mouse IgG (Jackson ImmunoResearch Laboratories; West Grove, PA) were used as secondary antibodies. Controls included immunoadsorption of primary antigens before application to the slides or omission of the primary antibody when purified antigen was not available. For monoclonal antibody staining, endogenous mouse antibodies were blocked with a rabbit anti-mouse antibody (Dako). Morphometric analysis was performed by counting cells in transverse sections as described previously (33). Primary antibodies included the mouse H⁺-K⁺-adenosinetriphosphatase (H⁺-K⁺-ATPase) monoclonal antibody (31) and rabbit polyclonal antibodies against CgA (C-SP-1 and 1:160; INCSTAR, Stillwater, MN) and histidine decarboxylase (HDC) (36).

Northern blot analysis. Stomachs were removed from adult mice that had been fasted overnight, total RNA was isolated after homogenization in guanidine thiocyanate followed by centrifugation through 5.7 M CsCl (27), and poly(A⁺) RNA was isolated with the PolyATtract mRNA System (Promega, Madison, WI). For Northern blots, poly(A⁺) RNA was electrophoresed in agarose gels containing 2.2 M formaldehyde and transferred to Zeta-Probe nylon membrane (Bio-Rad, Hercules, CA). For preparation of hybridization probes, mouse cDNA fragments were isolated from agarose gels using the Qiaex gel extraction kit (QIAGEN, Chatsworth, CA), ³²P-labeled, and used as previously described (29). Final wash conditions were 0.5 \times SSC (0.075 M NaCl, 7.5 mM trisodium citrate, pH 7.0) and 0.1% sodium dodecyl sulfate at 60°C. Imaging and quantitation were performed on a GS-250

Table 1. Peptide concentrations in mouse plasma and tissue extracts

Genotype	Treatment	Plasma		Stomach		Duodenum
		Gastrin, pmol/l	CCK, pmol/l	Gastrin, pmol/g	CgA, pmol/g	CCK, pmol/g
+/+	None	51 ± 25	3.8 ± 1.4	641 ± 186	23.7 ± 2.1	59 ± 24
+/-	None	53 ± 27	3.6 ± 1.0	339 ± 94	21.7 ± 3.5	71 ± 27
-/-	None	0	3.8 ± 0.8	0	12.1 ± 3.5	73 ± 36
-/-	Gastrin	347 ± 157	ND	0	26.8 ± 3.7	ND
-/-	BSA	0	ND	0	12.0 ± 1.7	ND

Gastrin, CCK, and chromogranin A (CgA) peptide concentrations (means ± SD) in plasma and tissue extracts from normal (+/+), heterozygous (+/-), and gastrin-deficient (-/-) mice as determined by radioimmunoassay. Treatment groups include gastrin-deficient mice given continuous minipump infusions of gastrin or bovine serum albumin (BSA) for 6 days; *n* = 9 for untreated groups and 5 for treated groups. ND, not determined.

Molecular Imager (Bio-Rad). The gastrin (422 bp) and hypoxanthine-guanine phosphoribosyl transferase (HPRT) (249 bp) mouse cDNA probes were previously described (6, 16). The HDC probe (312 bp) was a fragment of the mouse cDNA, from nucleotides 1717 to 2028 (37). The mouse H₂ receptor probe (738 bp) was reverse transcription-PCR (RT-PCR) amplified with primers 5'-ATCGATGTCAACGAGGTATATGACTGG and 5'-GAGCTCCTCTGGAGTGAGTAACATTCT (GenBank Accession no. D50096). The mouse M₃ receptor probe (630 bp) was RT-PCR amplified with rat primers 5'-CTGCAGCAGAGAGTCACAATGACCTTGC and 5'-GTCGACGCCAGAACAAGATGGCAGGAG (GenBank Accession no. M62826). The mouse gastrin/CCK-B receptor probe (542 bp) was RT-PCR amplified with primers 5'-CTCGAGCAGAGAGTCACAATGACCTTGC and 5'-TGCATGCACTGCAGTATTCGA (L. C. Samuelson, unpublished observations). RT-PCR amplification used the Superscript kit (Life Technologies) and adult mouse stomach RNA (5 µg), with the following cycle parameters: 1) 94°C for 5 min; 2) 35 cycles of 94°C for 45 s, 60°C for 45 s, and 72°C for 1.5 min; and 3) 72°C for 4 min. RT-PCR-amplified fragments were verified by nucleotide sequence analysis.

Gastrin replacement. Mice 7 wk old were implanted subcutaneously with osmotic minipumps (Alzet no. 2001; Alza, Palo Alto, CA) containing rat gastrin-17 (5 µg·kg⁻¹·h⁻¹), or vehicle (saline with 1% bovine serum albumin). Minipumps were removed after 6 days, and acid secretion was measured the following day. Blood and tissues for histology and peptide analysis were isolated from mice at *day 6*, before removal of the minipumps, or after removal, at the completion of the acid secretion studies.

RESULTS

Gastrin-deficient mice are viable. A mouse strain with a complete gastrin gene deletion was generated by gene targeting in mouse ES cells (Fig. 1). Southern blot analysis of genomic DNA isolated from the targeted

mouse strain confirmed the structure of the gene deletion (Fig. 1B). Heterozygous mutant mice were intercrossed to obtain homozygotes for the gastrin deletion. The ratio of genotypes did not deviate significantly from the expected 1:2:1 distribution of homozygous mutants, heterozygotes, and wild-type progeny, with equal proportions of male and female homozygotes. The gastrin-deficient mice developed without gross abnormalities and exhibited normal weight gain and fertility in comparison to their wild-type littermates. The total absence of gastrin transcripts was confirmed by Northern blot analysis (Fig. 1D). RIA of plasma and tissue extracts confirmed the absence of gastrin peptides in the mutant (Table 1). Interestingly, heterozygotes (+/-) had normal concentrations of plasma gastrin in spite of the twofold reduction of gastrin in the stomach, indicating that concentrations of circulating gastrin are tightly regulated. Concentrations of the related peptide CCK were unchanged in plasma and duodenal extracts from mutant mice (Table 1). Thus CCK is not upregulated to compensate for the loss of gastrin, even though both CCK and gastrin activate the gastrin receptor with equal affinity (35).

Abolishment of gastric acid secretion in gastrin-deficient mice. Since gastrin is thought to be the primary hormonal regulator of gastric acid secretion, we examined the consequences of gastrin deficiency on stomach secretory function in anesthetized mice. Basal and agonist-induced acid secretion were measured over a 60-min period. Gastrin-deficient mice exhibited a complete loss of gastric acid secretion. Basal acid secretion was undetectable, and there was no response to stimulation by histamine, carbachol, or gastrin (Fig. 2). In contrast, basal acid secretion in normal mice was

Table 2. Morphometric analysis of the oxyntic mucosa of gastrin-deficient mice

Genotype	Treatment	Parietal		ECL	
		H&E, cells/gland	H ⁺ -K ⁺ -ATPase, cells/gland	HDC, cells/mm	CgA, cells/mm
+/+	None	24.3 ± 3.8	20.3 ± 1.5	34.8 ± 10	89.2 ± 13
-/-	None	18.8 ± 2.4*	9.4 ± 1.5*	10.0 ± 3.0*	87.5 ± 26
-/-	Gastrin	17.8 ± 2.9	17.1 ± 1.6	ND	81.4 ± 12
-/-	BSA	16.8 ± 3.4	12.8 ± 2.5*	ND	77.3 ± 12

Values are means ± SD. Cells were counted after hematoxylin and eosin (H&E) staining of paraffin sections or immunostaining of cryosections as described in METHODS. Treatment groups include gastrin-deficient mice given continuous minipump infusions of gastrin or BSA for 6 days; *n* = 4–6 mice for each genotype; 3–4 visual fields were counted for each mouse. ECL, enterochromaffin-like; HDC, histidine decarboxylase. ND, not determined. **P* < 0.05.

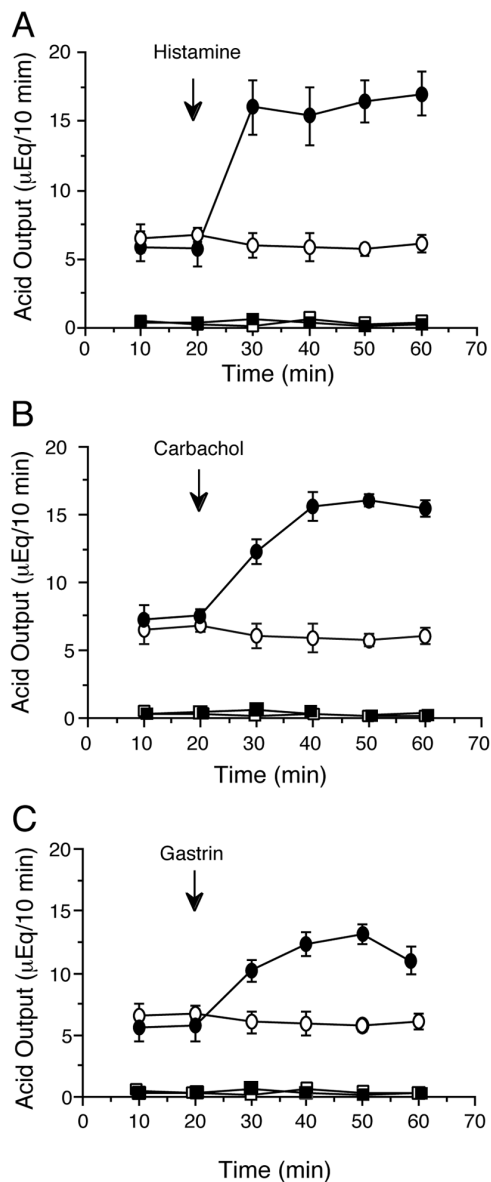


Fig. 2. Gastric acid secretion in wild-type (circles) and gastrin-deficient (squares) mice. Basal secretion was monitored for 60 min in one group of mice (open symbols). Other groups (filled symbols) were stimulated by subcutaneous injection of histamine (A), carbachol (B), or gastrin (C) as indicated; $n = 6$ mice/group.

6 $\mu\text{Eq}/10$ min and increased to 12.5–17.5 $\mu\text{Eq}/10$ min after subcutaneous injection of histamine, carbachol, or gastrin (Fig. 2). The lack of response to the three primary stimulators of gastric acid secretion suggests that the acid secretory machinery requires gastrin to function.

Northern blot analysis of total stomach RNA showed that the genes for the receptors for histamine (H_2), ACh (M_3), and gastrin (CCK-B) were expressed in the mutant (Fig. 3). Quantitation of the abundance of these transcripts by PhosphorImager analysis showed that the levels of expression of the H_2 and M_3 receptors were unchanged in the mutant. In contrast, gastrin receptor transcripts, although readily detectable, were reduced

approximately fourfold in comparison to wild-type controls.

Parietal and ECL cell defects in gastrin-deficient mice. Histological analysis of the oxyntic mucosa showed that all of the major epithelial cell types were present in gastrin-deficient mice, including parietal, ECL, chief, and mucous cells. However, there were marked changes in the number and properties of the two cell types of primary importance for the synthesis and secretion of gastric acid, parietal and ECL cells. Morphometric analysis of hematoxylin-eosin-stained sections showed that gastrin-deficient mice exhibit a 23% reduction in parietal cell number (18.8 vs. 24.3 cells per gland, $P < 0.05$; Table 2). Moreover, when analyzed by H^+K^+ -ATPase immunostaining, the number of parietal cells was decreased to 46% of normal, with unstained parietal cells interspersed between stained cells in the gastric glands (Table 2 and Fig. 4). We interpret the H^+K^+ -ATPase-negative parietal cells to be functionally immature.

ECL cells in the oxyntic mucosa of gastrin-deficient mice were analyzed by immunohistochemistry, using HDC and CgA as markers. HDC-positive cells were markedly reduced to 29% of control and stained very weakly in mutant mice (Table 2 and Fig. 4). The

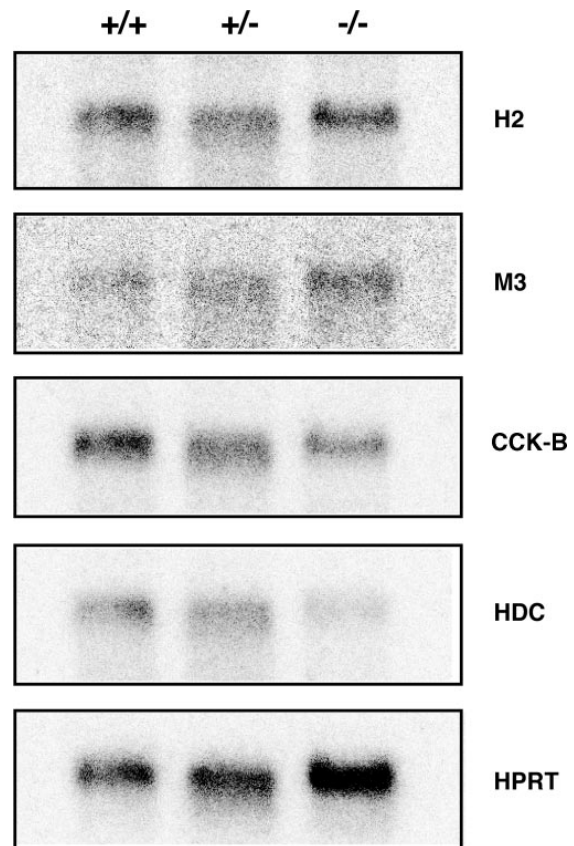


Fig. 3. Northern blot analysis of gene expression in stomachs of gastrin-deficient mice. A single filter containing 5 μg poly(A^+) stomach RNA samples from wild-type (+/+), heterozygous (+/-), and gastrin-deficient (-/-) mice was hybridized sequentially with probes, including probes for the histamine receptor H_2 , the muscarinic receptor M_3 , the gastrin receptor (CCK-B), histidine decarboxylase (HDC), and HPRT as a loading control.

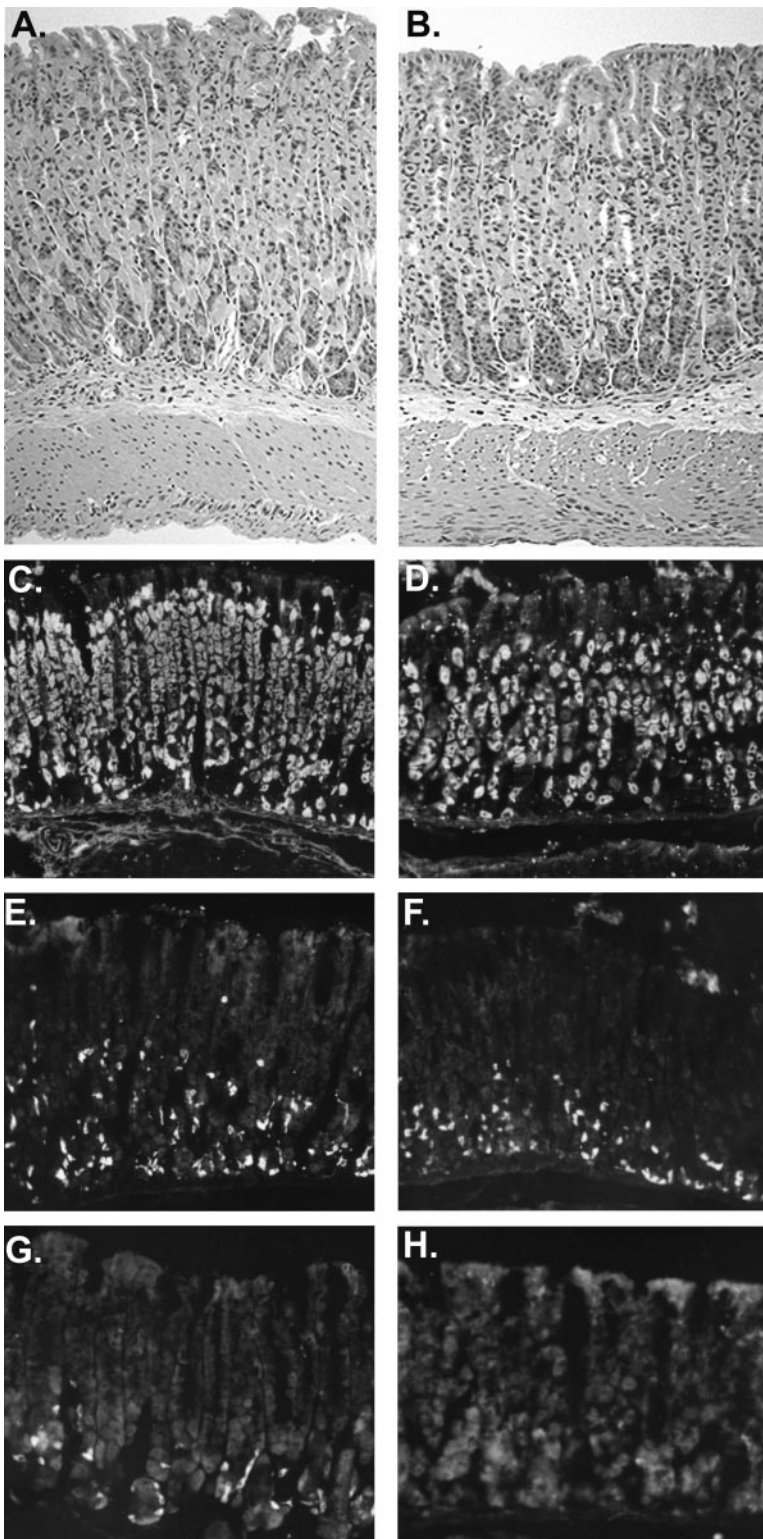


Fig. 4. Histologic comparison of the gastric mucosa in the fundic region of wild-type (*A, C, E, G*) and gastrin-deficient mice (*B, D, F, H*). *A* and *B*: hematoxylin-eosin-stained paraffin sections demonstrate that the overall morphology is not markedly different in gastrin-deficient mice, although morphometric analysis revealed a reduction in parietal cell number (Table 2). *C–H*: immunohistochemical staining of parietal cells with antibodies to H^+K^+ -ATPase (*C* and *D*) and of ECL cells with antibodies to chromogranin A (CgA) (*E* and *F*) and HDC (*G* and *H*) demonstrated marked changes in these 2 cell populations, as described in the text.

reduction in HDC immunoreactivity agrees with the marked sevenfold reduction in HDC mRNA abundance in the mutant (Fig. 3). In addition to the reduction in HDC, CgA peptide concentrations were reduced by 50% (Table 1), and histamine concentrations were reduced 40%, from $15.6 \pm 3.1 \mu\text{g/g}$ stomach (wet weight) in wild-type mice to $9.1 \pm 0.9 \mu\text{g/g}$ in gastrin-deficient

mice ($P < 0.05$). However, contrary to these observations, we were not able to detect differences in the total number of CgA-positive cells in the oxyntic mucosa of mutant and wild-type mice, although the positive cells were clustered nearer to the base of the gastric glands in mutant mice (Fig. 4, *E* and *F*). Moreover, staining ECL cells with an antibody to vesicular monoamine

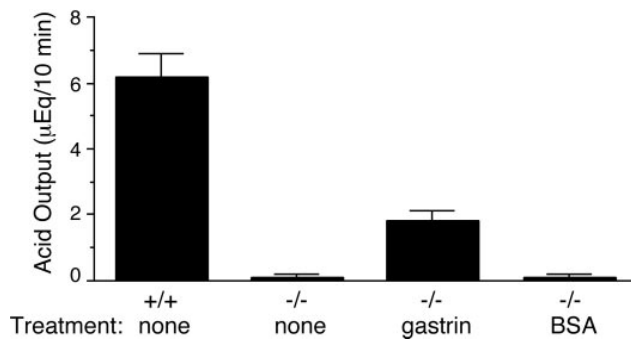


Fig. 5. Partial restoration of basal acid secretion in gastrin-deficient mice by gastrin infusion at $5 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ for 6 days with osmotic minipumps. Untreated and treated gastrin-deficient mice are compared with untreated wild-type controls. Acid secretion was measured 1 day after removal of minipumps; $n = 6$ mice/group.

transporter 2 (7) yielded similar results to the CgA staining (data not shown). Thus lack of gastrin did not appear to reduce the total number of ECL cells but rather altered their position and reduced the level of expression of ECL cell markers.

Gastrin replacement partially repairs acid secretion. We tested whether the defects in acid secretion and alterations in the gastric mucosa could be corrected by continuous infusion of gastrin. Gastrin was administered for 6 days with the use of osmotic minipumps, which provided supraphysiological concentrations of circulating gastrin, as determined by RIA (Table 1). Gastric acid secretion was measured in anesthetized mice 1 day after removal of the minipumps, when the concentration of circulating gastrin was undetectable. The gastrin infusion markedly increased basal acid secretion in the mutants (Fig. 5), indicating that the defect in acid secretion is at least partially correctable in adults. The restoration of acid secretion was paralleled by an increase in $\text{H}^+\text{-K}^+\text{-ATPase}$ -positive parietal cells, although there was no increase in the total number of parietal cells, as determined by hematoxylin-eosin-stained sections (Table 2). In contrast, gastrin treatment did not alter the number of CgA-positive cells (Table 2). However, gastrin did restore CgA peptide to the wild-type concentration (Table 1).

DISCUSSION

This study demonstrates that gastrin deficiency affects both the normal development and physiology of the stomach in mice. Lack of gastrin disrupts basal gastric acid secretion and renders the system unresponsive to histaminergic, cholinergic, and gastrinergic stimulation. Many components of the acid-secretory system were affected by the loss of gastrin: parietal cell numbers were reduced with an accumulation of $\text{H}^+\text{-K}^+\text{-ATPase}$ -negative cells, and ECL cells showed reduced HDC expression and histamine content. Although the cellular changes in the gastric mucosa of the mutant were significant, acid secretion was impaired much more extensively than would be predicted from the reduction in parietal cell number. Fewer gastrin receptors may contribute to the block in gastrin-stimulated acid secretion, since receptor mRNA levels were four-

fold lower in the mutant. The lack of response to histamine and ACh does not appear to be a receptor defect, since the expression of both H_2 and M_3 receptors is unchanged in the mutant. Instead, we conclude that the absence of gastrin causes an intrinsic defect in the parietal cell. The results of the gastrin replacement study indicate that the parietal cell defect can be at least partially corrected in adult mice. Basal acid secretion was restored to one-third of normal levels after 1 wk of gastrin infusion. This restoration is more pronounced when it is taken into account that these mice still have 25% fewer parietal cells than normal (Table 2). The results suggest that gastrin is required for development of the acid-secretory machinery.

Gastrin or ACh stimulation of acid secretion may occur via direct activation of the parietal cell or indirectly by triggering histamine release from ECL cells, because histamine acts as a paracrine stimulator of the parietal cell. Both parietal and ECL cells contain receptors for gastrin and ACh (12). There has been extensive discussion concerning the relative importance of direct gastrin and ACh stimulation of the parietal cell vs. indirect stimulation via histamine release. The hypothesis that histamine is the final common stimulator of gastric acid, developed by Code in 1965 (3), is supported by the potent inhibitory action of H_2 -receptor antagonists (2). The alternative view put forward by Grossman and Konturek (8) proposed that acid release is regulated by the interactions of gastrin, ACh, and histamine binding to the parietal cell. Our results suggest that histamine may not be the final mediator of acid secretion, since histamine was unable to evoke a response in gastrin-deficient mice. Instead, input from two or more stimuli may be necessary for acid secretion from parietal cells. In vitro studies have shown that secretion may require both activation of the cAMP signal-transduction cascade (histaminergic) and the Ca^{2+} pathway (gastrinergic/cholinergic) (23). A lack of gastrin potentially affects both pathways. The Ca^{2+} pathway is directly affected due to a reduction in gastrin receptor agonist concentration. The cAMP pathway is indirectly affected, since lack of gastrin leads to a reduction in histamine in the ECL cell. It remains to be determined whether gastrin and histamine stimulation of the parietal cell might be required simultaneously or if gastrin functions to prime the parietal cell to respond to histamine stimulation. One advantage of our mutant model is that gastrin replacement can be used in the future to dissect the molecular mechanisms of parietal cell maturation and agonist activation.

Koh et al. (18) recently reported the phenotype of an independently derived gastrin-deficient mouse mutant. Their results, as well as the abnormalities reported in two studies of gastrin receptor-deficient mice (19, 25), agree with our data showing that disruption of gastrin signaling leads to gastric mucosal atrophy characterized by reductions in the number of parietal cells, as well as increased stomach acid pH. The similar phenotype exhibited by receptor-deficient and hormone-deficient mutants confirms that gastrin action in the stomach is mediated by the gastrin/CCK-B receptor.

Our study extends the characterization of gastric physiology to show that the abnormalities in parietal and ECL cells are associated with a severe impairment in both basal and agonist-induced acid secretion.

The lack of gastrin did not seem to grossly affect the number of ECL cells, as measured by CgA immunostaining, although CgA peptide concentration in the stomach was reduced twofold. The observation of normal ECL cell numbers in the mutant differs from the gastrin-deficient mouse study by Koh et al. (18) as well as the gastrin receptor-deficient models (19, 25), which described a reduction in CgA-positive cells. Whether this difference might be due to the use of different CgA antibodies remains to be resolved. However, our results clearly demonstrate that ECL cell function is diminished in our gastrin-deficient mouse strain, since the CgA-immunoreactive cells are clustered closer to the base of the gastric glands, HDC is markedly reduced, and histamine concentration is twofold lower compared with wild-type mice. The marked reduction in HDC expression agrees with results in other studies showing that HDC is regulated by circulating gastrin (10, 13, 30). Our study demonstrates that gastrin is not necessary for HDC expression as such, since, although markedly reduced, HDC mRNA is still detected.

Previous studies have demonstrated that gastrin operates as a trophic factor for both the parietal and ECL cell lineages (4, 20). It is assumed that all the cells in the gastric glands are derived from stem cells in the neck region (15). However, little is known about the factors controlling the rate of cell proliferation and differentiation into the different cell lineages. Lack of gastrin reduces the number of parietal cells, and the accumulation of H⁺-K⁺-ATPase-negative parietal cells suggests delayed parietal cell maturation. Recently Li et al. (22) described increased proliferation of preparietal cells in mice in which the parietal cells were ablated. Gastrin was not measured in these mice, but without mature parietal cells and no acid secretion, it is likely they were hypergastrinemic, and thus gastrin could be responsible for the accelerated entry of progenitor cells into the parietal cell lineage. Furthermore, the present data indicate 1 wk of gastrin infusion can drive the maturation of H⁺-K⁺-ATPase-negative parietal cells into H⁺-K⁺-ATPase-positive parietal cells. Taken together, these studies suggest that gastrin induces the expansion and maturation of the parietal cell lineage. Koh et al. (18) showed that there was no difference in the labeling index in the fundic mucosa of gastrin-deficient and wild-type mice after 1-h 5'-bromodeoxyuridine (BrdU) treatment. However, they did not report the specific labeling index for parietal cells. Since only 10% of the stem cells in the isthmus eventually differentiate into parietal cells (15), the BrdU labeling results (18) do not contradict a modest reduction in the generation of this cell type in the mutant. The gastric cell lineages may be differentially affected by the absence of gastrin, with some lineages increasing (mucous neck cells) and others decreasing (parietal cells) in number in the mutant.

In conclusion, this study demonstrates that gastrin deficiency results in reduced parietal cell numbers as well as defective maturation of parietal and ECL cells. These abnormalities may be responsible for the lack of an acid-secretory response to gastrin, histamine, and ACh. Thus gastrin appears to be a key player for developing and maintaining normal gastric morphology and physiology. We also demonstrate that maturation of the acid-secretory system can be induced in gastrin-deficient mice by hormone replacement. This model provides an important tool for future analysis of the molecular basis for parietal cell maturation and function.

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