Enhanced calcium signaling and acid secretion in parietal cells isolated from gastrin-deficient mice

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Hinkle, Karen L., Gina C. Bane, Ali Jazayeri, and Linda C. Samuelson. Enhanced calcium signaling and acid secretion in parietal cells isolated from gastrin-deficient mice. Am J Physiol Gastrointest Liver Physiol 284: G145–G153, 2003. First published September 11, 2002; 10.1152/ajpgi.00283.2002.—Gastrin-deficient mice have impaired basal and agonist-stimulated gastric acid secretion. To analyze whether an intrinsic parietal cell defect contributed to the reduced acid secretion, we analyzed parietal cell calcium responses and acid secretory function in vitro. Parietal cells were purified by light-scatter cell sorting and calcium responses to gastrin, histamine, and carbachol were measured in gastrin-deficient and wild-type mice cell preparations. Surprisingly, basal and histamine-induced calcium concentrations were higher in the mutant cell preparations. [14C]aminopyrine uptake analysis in acutely isolated gastric glands revealed that basal acid accumulation was enhanced in gastrin-deficient cell preparations as well as on treatment with carbachol or histamine. These results suggested that an intrinsic parietal cell defect was not responsible for the reduced acid secretion in gastrin-deficient mice. Flow cytometric analysis of dispersed, H⁺-K⁺-ATPase-immunostained gastric mucosal preparations revealed a marked increase in parietal cell number in gastrin-deficient mice, which may have accounted for the enhanced in vitro acid secretion detected in this study. Parietal cells were found to be significantly smaller in the mutant cell preparations, suggesting that gastrin stimulation modulates parietal cell morphology.

STIMULATED ACID SECRETION from the gastric parietal cell is an elegant and dynamic cellular phenomenon regulated by a complex network of endocrine, paracrine, and neural factors. The major endocrine stimulator of gastric acid secretion is the peptide hormone gastrin, which is released upon ingestion of a meal (13, 33). Gastrin is capable of stimulating parietal cells directly through gastrin/cholecystokinin B (CCKB) receptors or indirectly by activating enterochromaffin-like (ECL) cells to release the paracrine stimulator histamine. Acetylcholine (ACh), the third major acid stimulant, is released from neurons to activate parietal cells via muscarinic 3 (M₃) receptors. Although the ability of gastrin, histamine, and ACh to stimulate acid secretion is well documented, the relative importance of each of these regulators for normal parietal cell development and function is not clearly understood.

There is evidence that increases in both intracellular cAMP and calcium are required for parietal cells to secrete acid (23, 32). Gastrin and ACh stimulate CCKB receptors, respectively, to primarily evoke increases in intracellular calcium (6, 26), whereas stimulation of histamine-2 (H₂) receptors appears to increase both intracellular calcium and cAMP (7). Because gastrin stimulates ECL cell histamine release, gastrin treatment in vivo most likely results in activation of both parietal cell calcium and cAMP pathways. It is debated whether direct gastrin stimulation of the parietal cell is required for acid secretion or whether subsequent histamine release is sufficient to activate parietal cells (3). However, the recent finding that H₂ receptor-deficient mice maintain normal basal acid secretion (17) suggests that gastrin can play a primary role.

In addition to regulating acid secretion in response to a meal, gastrin is a growth factor for the gastric mucosa (34). High gastrin levels produced experimentally or naturally result in a thickening of the gastric mucosa due to increased numbers of gastric epithelial cells, including parietal and ECL cells (8, 21). CCKB receptors were recently identified on cells within the proliferative zone of the gastric glands that were not characteristic of parietal or ECL cells, suggesting that gastrin may directly stimulate the development or maturation of progenitor cells within this region (16). In addition, it has been shown that gastrin stimulates the expression of several growth factors, including the regenerating gene protein (12), and the EGF receptor ligands amphiregulin and heparin-binding EGF (31).

The importance of gastrin for parietal cell function has been addressed most recently in studies using gene-deficient mouse models, including gastrin-deficient (11, 18) and CCKB receptor-deficient strains (22, 25). Despite the important trophic effect of gastrin, parietal cells are formed in gastrin-deficient mice and express their functional marker H⁺-K⁺-ATPase. However, in both gastrin- and CCKB receptor-deficient mice, there is a severe impairment in basal acid secretion, suggesting a requirement for gastrin in the nor-
ENHANCED FUNCTION IN GASTRIN−/− PARIETAL CELLS

Becton, Dickinson FACS Vantage SE cell sorter. Light-scatter parameters were used to sort parietal cells on the basis of large size (side scatter) and structural complexity (forward scatter). Cell viability was determined by trypan blue staining to be 80%.

Immunocytochemistry. Isolated cell preparations were cytospun onto microscope slides (2 × 10⁵ cells), air-dried, and fixed in 4% paraformaldehyde for 10 min, followed by three 5-min washes in PBS. To identify parietal cells in the sorted populations, cells were immunostained with a monoclonal antibody for the α-subunit of H+-K+-ATPase (1:500, Medical & Biological Laboratories). This was followed by treatment with Cy2-conjugated donkey anti-mouse IgG (1:200; Jackson ImmunoResearch Laboratories) that was used as a secondary antibody. Controls in which the primary antibody was omitted were included for each experiment. The nuclear stain 4,6-diamidino-2-phenylindole (DAPI, 1 μg/ml, Sigma) was added to the mounting media Fluoromount-G (Southern Biotechnology Associates). Immunofluorescence was detected with a Leica Aristoplan microscope. Digital images of H+-K+-ATPase- and DAPI-stained cells were captured, and overlay images were produced using Adobe Photoshop 5.5 software. The ratio of H+-K+-ATPase-positive cells to total cells (DAPI positive) was calculated to obtain the proportion of parietal cells in each population.

Calcium imaging. Sorted parietal cells from mice aged 2–4 mo were plated onto 22-mm round glass coverslips coated with growth factor-reduced Matrigel (1:6; BD Biosciences) at the density of 5 × 10⁶ cells per well. Cells were incubated with the calcium-sensitive dye fura-2 AM (1 μg/ml; Molecular Probes). Calcium responses were analyzed using a method adapted from Del Valle et al. (6). Briefly, coverslips were placed into a specialized airtight chamber mounted onto the stage of a Zeiss Axiovert 35 microscope maintained at 37°C, with media and treatments perfused at 1 ml/min. Fluorescence intensity of multiple resting and stimulated parietal cells was recorded at the excitation wavelengths of 340 and 380 nm with emission at 510 nm using an Attofluor RatioVision 6.0 microspectrofluorometer system. Medium C was used for all calcium experiments: 1× Earle’s balanced salt solution (Life Technologies), 1 mg/ml BSA, 2.2 mg/ml NaHCO₃, and 10 mM HEPES. Intracellular calcium levels were calculated using the fura-2 AM dissociation constant (224 nM). Cells were treated with rat gastrin-17 (100 nM; Bachem), histamine (100 μM; Sigma), carbachol (10 μM; Sigma), or cimetidine (200 μM; Sigma). Responses were measured from 7–14 independently isolated wild-type and gastrin-deficient mouse parietal cell preparations.

[^1] Captoprinate uptake analysis. Acid secretory function was analyzed by[^1] C captoprinate uptake in isolated gastric gland preparations using a modified protocol of a method described by Chew (4). Glands were prepared from gastrin-deficient and wild-type mice aged 2 mo as described in Mouse parietal cell isolation and purification. Dispersed glands were washed twice and resuspended in 5 ml pronase-free medium A, after which[^1] C captoprinate (0.1 μCi/ml) was added. Aliquots (0.5 ml) were incubated for 30 min at 37°C (90 rpm) in airtight plastic vials before treatment with gastrin (100 nM), carbachol (100 μM), histamine (100 μM), or vehicle (PBS) for 30 min. Gland suspensions were then transferred to 1.5-ml microcentrifuge tubes and pelleted at 4,000 g for 15 s. Glands were washed once with PBS and lysed with 500 μl 0.5 M NaOH + 0.5% Triton X-100 (Sigma). Radioactivity in gland and immunoassay for acid was determined by liquid scintillation counting and normalized to protein levels. Protein was quantitated using a Lowry assay (Bio-Rad).

Flow cytometric and size analysis of parietal cells. Three wild-type or gastrin-deficient mice aged 2 mo were fasted overnight, and dispersed gastric mucosal cells were isolated
as described in Mouse parietal cell isolation and purification. Gastric epithelial populations were quantitated by flow cytometry using a method developed by Zavros et al. (35). After being washed in pronase-free medium A, cells were fixed and permeabilized using the Cytofix/Cytoperm kit (Pharmingen) and immunostained with either an H+-K+-ATPase a-subunit monoclonal antibody (1:100), a keratin 18 polyclonal antibody (1:10; ICN), or a polyclonal antibody-to-rat intrinsic factor (1:1,000; David Alpers, Washington University, St. Louis, MO) at 4°C. In the murine gastric mucosa, intrinsic factor is expressed exclusively in chief cells and thus is a specific marker for this cell type (28). After being washed, cells were incubated with appropriate Cy2-conjugated donkey anti-IgG (1:250; Jackson ImmunoResearch Laboratories). Cell quantitation was performed with a Becton, Dickinson Elite flow cytometer on samples containing 5 × 10^4 cells. Samples in which the primary antibodies were omitted were used to determine background fluorescence. Cells positive for keratin 18 represented the total number of epithelial cells in the preparation, and the percent parietal (H+-K+-ATPase-positive) or chief (intrinsic factor-positive) cells/total epithelial cells was then calculated (35). To verify that H+-K+-ATPase-immunostained cells were indeed parietal cells, they were sorted on the basis of fluorescence, cytospun onto slides, and analyzed microscopically for characteristic parietal cell morphology.

Parietal cell area was measured using nonsorted cell preparations. Mixed mucosal cells were cytospun onto slides and H+-K+-ATPase-immunostained. Digital images were analyzed using NIH Image 1.62 software.

Statistical analysis. All statistical tests were performed using GraphPad In-Stat 2.04 software. A two-tailed Student’s t-test was used to analyze differences in the cell number, size, and maximum calcium responses between gastrin-deficient and wild-type cells. A one-way ANOVA followed by a Bonferroni test was used for [14C]aminopyrine uptake differences in the number of cells that showed calcium responses on agonist stimulation.

RESULTS

Purification of mouse parietal cells. A mixed gastric mucosal cell preparation was obtained by protease digestion of the luminal-side of the stomach fundus. This preparation contained ~25% parietal cells, as determined by immunostaining for the parietal cell marker H+-K+-ATPase (Fig. 1C). Flow cytometry was used to purify parietal cells from the mixed cell population on the basis of the unusually large size and structural complexity that these cells exhibit. Light-scatter analysis revealed a population of cells that had high degrees of both side scatter and forward scatter (Fig. 1A, “large”). Microscopic inspection revealed that the large-sorted cell population exhibited characteristics of parietal cells, including large size, a centrally located nucleus, large cytoplasm, and H+-K+-ATPase expression (Fig. 1D). Parietal cells consistently made up >90% of the large cell-sorted population.

Another population with low degrees of side and forward scatter was sorted for comparison (Fig. 1A, small). This population was composed of smaller cells that were primarily nonparietal, as only 10% of the sorted cells expressed H+-K+-ATPase (Fig. 1E). The few H+-K+-ATPase positive cells in this population were markedly smaller than the H+-K+-ATPase positive cells in the large-sorted group (compare Fig. 1, D and E), consistent with the notion that the degree of light scatter is directly related to cell size.

Light-scatter flow cytometry of mixed gastric mucosal cell preparations from gastrin-deficient mice yielded similar profiles to wild-type mice, with two populations (large and small) recognizable (Fig. 1B). Proportions of H+-K+-ATPase-positive cells in both large- and small-sorted populations in gastrin-deficient mice were similar to wild-type (Fig. 1, G and H). Parietal cell purity in the mutant cell was >90% in the large-sorted population as was observed for wild-type controls.

Calcium signaling in parietal cells from gastrin-deficient mice. Previous in vivo studies of gastric acid secretion showed that gastrin-deficient mice have impaired basal secretion and lack responses to agonist stimulation compared with wild-type controls (11). To determine whether the acid secretory defect in these mice stems from an intrinsic defect in parietal cell calcium signaling, responses to agonist stimulation were measured in purified parietal cells cultured for 48 h. After loading with the calcium-sensitive dye fura-2 AM, cells from wild-type and gastrin-deficient mice were stimulated with carbachol, gastrin, or histamine, and increases in fluorescence intensity were...
measured. Increases in intracellular calcium were observed after treatment with all three agonists in both gastrin-deficient and wild-type parietal cells (Fig. 2). Carbachol produced the most frequent response with at least 80% of the cells responding (Table 1). Gastrin and histamine also consistently evoked responses, although less frequently than carbachol. Responses to gastrin stimulation were only observed when a recovery period of 24–48 h in culture was allowed (data not shown).

Interestingly, the gastrin-deficient parietal cells had markedly increased calcium levels compared with cells from wild-type mice. Resting calcium concentration in the mutant cells was 1.7-fold higher, whereas the histamine-stimulated calcium concentration was 2.3-fold higher than in wild-type cells (Table 1).

To determine whether the calcium responses evoked by gastrin were due to direct gastrin stimulation of the parietal cell vs. indirect stimulation via histamine release from possible contaminating ECL cells, parietal cells were treated with gastrin in the presence of cimetidine, an H2 receptor antagonist. Cimetidine effectively blocked histamine-stimulated increases in intracellular calcium but was unable to block the gastrin response (Fig. 3), indicating that the gastrin-evoked calcium responses seen in this study were not due to histamine release.

Enhanced [14C]aminopyrine uptake in parietal cells of gastrin-deficient mice. To test whether the parietal cells of gastrin-deficient mice were capable of secreting acid, [14C]aminopyrine uptake was measured in acutely prepared gastric glands. In parietal cells studied in vitro, acid is trapped inside secretory canaliculi, and therefore the amount of the weak base aminopyrine that partitions into the cells is directly proportional to secreted acid (4). In both wild-type and gastrin-deficient gland preparations, histamine and carbachol treatment significantly increased the amount of trapped [14C]aminopyrine over untreated controls (Fig. 4). This was consistent with the ability of these two agonists to stimulate intracellular signaling responses. In contrast, there was no increase in [14C]aminopyrine uptake in response to gastrin stimulation in wild-type or gastrin-deficient mice. Moreover, there was no augmentation of the histamine response when gastrin was added at the same time (data not shown). These results were consistent with the lack of a calcium response upon stimulation with gastrin in isolated parietal cells that were not given time to recover.

Interestingly, basal and stimulated [14C]aminopyrine values were markedly increased in glands prepared from gastrin-deficient mice compared with glands prepared from wild-type mice (Fig. 4). Basal [14C]aminopyrine uptake was 707 ± 49 dpm/mg protein for wild-type glands vs. 970 ± 120 dpm/mg protein for mutant glands (P = 0.02). The carbachol- and histamine-stimulated responses in gastrin-deficient gland preparations were ~2- and 2.5-fold higher, respectively, than wild-type controls.

Increased number of parietal cells in gastrin-deficient mice. We consistently obtained an increased number of cells in the large population sorted from gastrin-deficient mice relative to wild-type mice. On average, the number of large cells isolated from five gastrin-deficient mice was 1.77 ± 10^6 ± 0.3 × 10^6, whereas the average number purified from five wild-type mice was 0.96 × 10^6 ± 0.1 × 10^6, a 1.8-fold increase (n = 11; P = 0.02). To verify that gastrin-deficient mice had a greater number of parietal cells, the proportion of parietal cells in mixed gastric mucosal cell preparations

![Fig. 2. Calcium responses to agonist stimulation are observed in parietal cells from wild-type (A and C) and gastrin-deficient (B and D) mice. Intracellular calcium levels are indicated by fluorescence intensity ratio (530/380 nm) over time (s). Each tracing depicts one representative cell of an experiment. A and B: cells were treated with gastrin (100 nM, open bars) for 60 s followed by a 30-s stimulation with carbachol (10 μM, filled bars). C and D: cells were treated with histamine (100 μM, shaded bars) for 30 s, also followed by carbachol stimulation.](G148_AJP-GastrointestLiverPhysiol-VOL284-JANUARY2003-CellRes2003-03-023.png)

Table 1. Intracellular calcium concentrations in parietal cells from gastrin-deficient and wild-type mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Carbachol (10 μM)</th>
<th>Gastrin (100 nM)</th>
<th>Histamine (100 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G+/+</td>
<td>35 ± 9</td>
<td>238 ± 58</td>
<td>57/66</td>
</tr>
<tr>
<td>G−/−</td>
<td>57 ± 7*</td>
<td>313 ± 45</td>
<td>45/56</td>
</tr>
</tbody>
</table>

Intracellular calcium concentrations are presented as means ± SE. G+/+, wild-type; G−/−, gastrin-deficient. For each genotype, responses to carbachol, gastrin and histamine were measured from 14, 9 and 7 cell preparations, respectively. The number of cells responding to agonist treatment with an increase in intracellular calcium is shown. For carbachol, the number of responders is shown over the number of cells examined; for gastrin and histamine, responder numbers are shown over the number of cells that subsequently responded to carbachol (see Fig. 2). *P ≤ 0.02 vs. G+/+.
was directly measured by quantitative flow cytometry. Samples were immunostained for H⁺K⁺-ATPase and analyzed by fluorescence (Fig. 5). Cells were also immunostained with an antibody to keratin 18, a global epithelial cell marker, to determine the total number of epithelial cells in the preparation. Results showed that the proportion of parietal cells in the epithelial population was nearly doubled in gastrin-deficient preparations compared with wild-type preparations (Fig. 5E; wild-type, 18.7 ± 3.0%; gastrin-deficient, 29.5 ± 0.9%; \( P < 0.03 \)).

To test whether the increase in parietal cells seen in gastrin-deficient mice was specific for this cell type, we also quantitated chief cells by flow cytometry. In contrast to parietal cells, chief cell numbers were unchanged in gastrin-deficient mice (Fig. 6), indicating that the increased proportion of parietal cells was not associated with a change in proportion of this other major cell type in the epithelium.

**Parietal cell size is decreased in gastrin-deficient mice.** Visual comparison of H⁺K⁺-ATPase-immunostained preparations indicated that parietal cells from gastrin-deficient mice were smaller in size than wild-type parietal cells (Fig. 1, compare D and G). To determine parietal cell size, we used NIH Image software to calculate the cell area from digital microscopic images of H⁺K⁺-ATPase-immunostained mixed mucosal cell preparations. The area was normalized to protein (mg) and expressed as means ± SE. (*P < 0.03, **P < 0.003; \( n = 8 \) preparations for each group.)

**Fig. 4.** \(^{14}C\)aminopyrine uptake is enhanced in isolated gland preparations from gastrin-deficient mice. Gastric glands isolated from wild-type (filled bars) and gastrin-deficient (shaded bars) mice were treated with PBS (basal), gastrin (10 nM), carbachol (100 µM), or histamine (100 µM), and \(^{14}C\)aminopyrine uptake was measured. Values were normalized to protein (mg) and expressed as means ± SE. (*P < 0.03, **P < 0.003; \( n = 8 \) preparations for each group.)

**Fig. 5.** Parietal cell number is increased in gastrin-deficient mice. To calculate the proportion of parietal cells in the mixed mucosal preparations, quantitative flow cytometry was performed on H⁺K⁺-ATPase-immunostained cells. A and B: representative histograms show the fluorescence distribution of H⁺K⁺-ATPase-immunostained cells. Positive parietal cell fractions from wild-type (A) and gastrin-deficient (B) mice are indicated (P). Note that the peak size for H⁺K⁺-ATPase-positive cells is markedly increased in the cells prepared from gastrin-deficient mice. C and D: representative histograms show the keratin 18-positive epithelial fraction (E) for wild-type (C) and gastrin-deficient (D) preparations. E: average number of parietal cells (%) normalized to the number of epithelial cells in wild-type (filled bars) and gastrin-deficient (shaded bars) mucosal preparations is shown in the bar graph. Values are expressed as means ± SE of four independent experiments (*P = 0.03).
preparations. Wild-type parietal cells ranged from \(200\) to \(800\) \(\mu\)m\(^2\) and were distributed in a bell-shaped curve (Fig. 7A, solid line). As predicted, the parietal cells from gastrin-deficient mice were shifted to smaller sizes, ranging in area from \(150\)–\(700\) \(\mu\)m\(^2\). The average size of parietal cells from gastrin-deficient mice was markedly smaller than the parietal cells of wild-type mice (Fig. 7B; wild-type, \(447 \pm 8.1\) \(\mu\)m\(^2\); gastrin-deficient, \(375 \pm 7.4\) \(\mu\)m\(^2\); \(P < 0.0001\)).

**DISCUSSION**

Previous studies demonstrated that the loss of gastrin signaling results in a marked reduction in in vivo gastric acid secretion. Studies using genetically engineered mouse mutants (11, 18, 22, 25) generally supported the previous literature on experimental models of hypogastrinemia developed by pharmacological blocking of gastrin receptor activation (9, 27), surgical excision of the gastrin-producing portion of the stomach (1, 2), or immunoneutralization of gastrin with specific antibodies (19, 20, 24). However, genetic ablation of gastrin has more serious consequences on the acid secretory system than was seen in the other hypogastrinemia models (14). For example, acid secretion in gastrin-deficient mice was unresponsive to histamine and ACh stimulation (11), whereas these responses were maintained in rats treated with a gastrin-specific neutralizing antibody (24). Loss of agonist responsiveness suggested that the constitutive loss of gastrin in gastrin-deficient mice might result in an intrinsic parietal cell defect.

In this study, parietal cells from gastrin-deficient mice were analyzed in vitro to determine whether the reduction in acid secretion seen in vivo is due to a functional defect in parietal cells. Our study showed that parietal cells from gastrin-deficient mice were responsive to agonist stimulation and were capable of secreting acid. Thus the impairment in acid secretion seen in vivo is not due to an inherent defect in these cells. These results show that gastrin is not required for the development of the acid secretory machinery in parietal cells. However, dramatic changes were detected in both the number and size of parietal cells, indicating that gastrin plays an important role in regulating some aspects of parietal cell physiology.

Calcium signaling has been shown to be important for the morphologic rearrangements characteristic of parietal cells when transforming from the resting to the stimulated state (23, 32). Therefore, we originally suspected that the impairment in acid secretion in gastrin-deficient mice might be due to a defect in receptor-stimulated calcium signaling. To analyze cal-

![Fig. 6](image)

**Fig. 6.** Chief cell number is normal in gastrin-deficient mice. Quantitative flow cytometry was performed on intrinsic factor- and keratin 18-immunostained cells. A and B: representative histograms show the intrinsic factor-positive chief cell fractions (C) from wild-type (A) and gastrin-deficient (B) mice. C: bar graph shows the average number of chief cells (%) normalized to the number of epithelial cells in wild-type (filled bars) and gastrin-deficient (shaded bars) mucosal preparations. Values are expressed as means ± SE of three independent experiments.

![Fig. 7](image)

**Fig. 7.** Parietal cells are smaller in size in gastrin-deficient mice. A: histogram shows the distribution in area (\(\mu\)m\(^2\)) of parietal cells from wild-type (solid line) and gastrin-deficient (dashed line) mice (A). B: average area of parietal cells isolated from wild-type (filled bar) and gastrin-deficient (shaded bar) mice are shown. Values are expressed as means ± SE (*\(P < 0.0001\); wild-type, \(n = 182\); gastrin-deficient, \(n = 210\)).
cium responses to agonist stimulation in parietal cells from gastrin-deficient mice, we developed a new method of purifying mouse parietal cells by light-scatter cell sorting. This method takes advantage of the large size and high degree of structural complexity of parietal cells and allows purification to 90% of viable parietal cells that can survive in culture for 3–4 days. The ease and purity of the light-scatter sorting method make it an attractive way to collect these cells for molecular or biochemical analysis. We detected calcium responses to gastrin, carbachol, and histamine stimulation in gastrin-deficient and wild-type parietal cells. These results confirm that the CCKB, M2, and H2 receptors are intact in parietal cells of mutant mice and suggest that there is no defect in calcium release that might contribute to the reduction in acid secretion. Interestingly, resting and histamine-stimulated calcium concentrations were increased in gastrin-deficient mice, suggesting that signaling pathways may be upregulated to compensate for the loss of gastrin.

To analyze acid secretion, we measured [14C]aminopyrine uptake in isolated gland preparations in response to agonist treatment. In contrast to the reduced acid secretion observed in vivo, the in vitro [14C]aminopyrine uptake measurements showed that parietal cells in gastrin-deficient mice are capable of producing acid. Furthermore, basal and carbachol- and histamine-stimulated acid secretion were elevated in glands prepared from gastrin-deficient mice compared with wild-type mice. Increased parietal cell number in the gastric mucosa of the mutants could explain the increased acid output measured in the gastric glands. However, increased calcium signaling in parietal cells from gastrin-deficient mice may also contribute to the enhanced acid production observed in the mutant gland preparation in vitro. Likewise, although stimulated cAMP levels were not analyzed in this study, an upregulation of the H2 receptor signaling pathway could also contribute to the enhanced secretion detected in the mutant. In support of this hypothesis, a recent study showed increases in basal and histamine-stimulated cAMP levels in parietal cells isolated from rabbits treated with an H2 receptor antagonist (29). In addition, in histidine decarboxylase-deficient mice with low basal acid secretion, H2 receptor and Gs expression were increased, as was the acid secretory response on exogenous histamine stimulation (30). The observed increases in calcium in our study and cAMP signaling in the histamine pathway studies suggest there may be compensatory changes in response to achlorhydria that would upregulate acid stimulatory signaling pathways in parietal cells. Further investigation will be needed to determine whether there is a general enhancement in signaling pathways leading to acid secretion in parietal cells from gastrin-deficient mice.

The increased number of parietal cells in gastrin-deficient mice detected by quantitative flow cytometry was surprising, given that gastrin is known to be a growth factor for the gastric mucosa. Instead, we might have predicted reduced parietal cell numbers in gastrin-deficient mice, because many studies have demonstrated that high levels of gastrin result in a substantial increase in the thickness of the gastric mucosa, targeting both parietal and ECL cells (5, 8, 10). Previous studies of parietal cell number in gastrin-deficient mice have reported both a modest decrease (11, 18) and an increase (35) in cell number. The basis for the variability in parietal cell number in gastrin-deficient mice is not clear, although it is known that inflammation (35) and age can affect parietal cell number. Indeed, the increased parietal cell number detected in 8-wk-old gastrin-deficient mice in this study was not observed when older mice were examined (K. L. Hinkle and L. C. Samuelson, unpublished observation). Thus the composition of the gastric mucosa can vary depending on the age and health status of the animal.

An observed increase in proliferation rate in the gastric mucosa of gastrin-deficient mice, as reported by Zavros et al. (35) and observed in this laboratory (data not shown), is likely to account for the increased number of parietal cells in young adults compared with wild-type mice. We also showed that the increase in parietal cell number in gastrin-deficient mice might be specific for this cell type, as chief cell numbers were unchanged from controls. Because all gastric mucosal cell types arise from the same progenitor cell, the specific increase in parietal cells must arise after commitment to this lineage.

Differentiation and maturation of a parietal cell have been shown to include a 54-day life cycle during which maturing parietal cells become larger in size (15). Although parietal cells of gastrin-deficient mice were capable of secreting acid in vitro, they were found to be smaller in size, suggesting that gastrin is important for development of normal parietal cell morphology. It is intriguing to think that gastrin acts as a maturation signal and that despite the fact that parietal cells are functional in gastrin-deficient mice, their final stages of maturity are never reached in the absence of gastrin. The actions of gastrin on the parietal cell could be either direct or indirect, because histamine release occurs as a result of gastrin stimulation of the ECL cell. Histamine has also been implicated in regulating parietal cell size. For example, in H2 receptor-deficient mice, there is a decrease in parietal cell size despite high plasma gastrin levels, suggesting a role for histamine in the normal maintenance of parietal cell morphology (17). Moreover, it is known that histamine content is decreased in gastrin-deficient mice (11), suggesting that the smaller parietal cell size in gastrin-deficient mice may be due to the loss of histamine.

We have shown in this study that although an in vivo impairment in acid secretion exists in gastrin-deficient mice, parietal cells are responsive to agonist stimulation and are capable of producing acid. This suggests that a cell-extrinsic change is responsible for the re-
duced acid secretion observed in vivo. One possible explanation for these results is that there is an increase in the release of an acid inhibitor in vivo in mutant mice that is not present in the in vitro studies. Although tissue somatostatin levels in gastrin-deficient mice were shown to be unchanged from controls (36), increases or alterations in the secretion of this molecule might have resulted in the lower acid levels detected in vivo. Another possible explanation for the in vivo impairment in acid secretion in gastrin-deficient mice is that there may be an alteration in ECL cell function resulting in reduced levels of histamine. Indeed, there are marked reductions in the expression of the histamine biosynthetic enzyme histidine decarboxylase and in intragastic histamine levels in gastrin-deficient mice (11). It is likely that the loss of both gastrin and histamine stimulation on the parietal cell contributes to the in vivo acid secretory defect seen in gastrin-deficient mice.

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