Lessons From Genetically Engineered Animal Models
I. Physiological studies with gastrin in transgenic mice*

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Wang, Timothy C., and Graham J. Dockray. Lessons From Genetically Engineered Animal Models. I. Physiological studies with gastrin in transgenic mice. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G6–G11, 1999.—The role of gastrin in the regulation of gastrointestinal growth and acid secretion has been addressed through recent studies involving transgenic and knockout mice. The role of gastrin as a key modulator of parietal cell function and gastric acid secretion has been confirmed through studies in mice deficient in either gastrin or the gastrin/CCK-B receptor. However, although gastrin-deficient mice show no changes in gastric proliferation, they do show reduced colonic cell proliferation, and rates of colonic proliferation are increased in transgenic mice expressing glycine-extended gastrin or progastrin. This themes article highlights recent progress in our understanding of the biology of gastrin through studies in genetically modified mice.

acid secretion; gastrointestinal growth

GASTRIN IS A PEPTIDE hormone, produced primarily by G cells located in the gastric antrum, that is involved in the regulation of gastric acid secretion and oxyntic gland proliferation. It was discovered by J. S. Edkins in 1905, and its chemical nature was defined in 1964, when Gregory and Tracy isolated and characterized a COOH-terminal amidated 17-residue peptide. Subsequent studies involving infusion of gastrin into animals, and radioimmunoassays of amidated gastrin in blood, provided the basis for much of our initial understanding of the physiological properties of gastrin and the possible consequences of hypergastrinemic states (15). Early on, it was recognized that there might be a link between gastrin and peptic ulcer disease. The relationship is not, however, a simple one, and recent work in this area has focused on the interactions between Helicobacter pylori, inflammatory mediators, and gastric endocrine cells.

Until recently, physiological studies on gastrin were almost entirely focused on the amidated peptide, and little attention was given to the functions of biosynthetic intermediates and precursor peptides. Gastrin is initially synthesized as a larger peptide, preprogastrin; after cleavage of the signal peptide to yield progastrin, the product is subsequently processed to yield glycine-extended gastrin (G-34-Gly and G-17-Gly) before conversion to the amidated forms (G-34 and G-17) (1). Although a number of in vitro studies suggested the possibility that progastrin and the G-Gly peptides might possess biological activity, until recently these precursors were largely thought to be inactive. The results of studies both in vitro and in vivo (in transgenic animals) now suggest new physiological roles for these peptides.

The application of molecular biology techniques to gastrin research led to the cloning of the gastrin gene in the 1980s, followed by the cloning of the receptor (gastrin/CCK-B) for amidated gastrin in the 1990s. The molecular tools generated by these studies paved the way for the generation of mutant (transgenic and knockout) mice, which have provided further insights regarding the biology of gastrin. These mouse studies have included experiments involving 1) analysis of the expression patterns and regulation of gastrin promoter constructs, 2) generation of mice deficient in gastrin or gastrin/CCK-B-receptor expression, and 3) overexpression in mice of amidated gastrin, G-Gly, and progastrin and the gastrin/CCK-B receptor. The results of these studies have not only clarified biological roles for differently processed forms of gastrin but have raised new questions regarding links with a number of disease states.

TISSUE-SPECIFIC EXPRESSION OF THE GASTRIN GENE

In most adult mammals, the gastrin gene is expressed primarily in endocrine cells (G cells) located in the gastric antrum and to a lesser extent in the proximal small intestine (duodenum) (15). However, in the fetal mammal, the major site of gastrin gene expression is pancreatic islet cells. Other sites of gastrin gene expression include the pituitary cells and spermatozoa. The regulatory elements responsible for tissue-specific expression of the gastrin gene have been studied through several transgenic experiments utilizing both human (9, 16, 18, 20) and rat gastrin (16, 18) promoter constructs. Initial studies by Montag et al. (9) showed that a 1.3-kb human gastrin promoter construct was able to target large T antigen expression to the hepatobiliary tract and pancreatic islets but not to
physiological studies with gastrin in transgenic mice

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The role of gastrin in the pancreas

A number of observations raised the possibility that gastrin might regulate the growth of the pancreatic islets during development. These include the transient expression of gastrin in the neonatal pancreas during a period of rapid pancreatic growth and the expression of gastrin in at least one case of infantile nesidioblastosis. To address the possible significance of gastrin as an islet growth factor, a line of transgenic mice (INS-GAS) was generated that overexpressed amidated gastrin in adult pancreatic islets (17). The transgenic construct consisted of the rat insulin promoter joined to the coding sequences of the human gastrin gene and targeted human gastrin expression specifically to the β-cells of the mouse pancreatic islets. Overexpression of gastrin alone resulted in no significant stimulation of islet mass in these mice. However, when the INS-GAS mice were crossed to a second line of transgenic mice (MT-TGF-α) that overexpressed transforming growth factor-α (TGF-α) in the murine pancreas, the combination of gastrin and TGF-α expression resulted in a twofold increase in islet cell mass. Gastrin appeared to reduce the TGF-α-mediated increase in pancreatic ductular mass, suggesting the possibility that gastrin promoted the differentiation of metaplastic ductules and thus was acting more as a morphogen in this setting (17).

The role of fetal pancreatic expression of gastrin has also been assessed through studies involving targeted deletion of both the mouse gastrin/CCK-B-receptor gene (8, 10) and mouse gastrin gene (3, 6). Although sophisticated morphometric and/or functional studies of the pancreatic islets have yet to be reported, preliminary studies have indicated no gross pancreatic abnormalities in these knockout animals, suggesting that gastrin is not essential for normal pancreatic development.

More recently, a strain of mice expressing the human gastrin/CCK-B receptor in the exocrine pancreas was created, using a transgenic construct consisting of the elastase I promoter joined to the human gastrin/CCK-B-receptor gene (12). In these mice, the gastrin/CCK-B receptor was expressed in the exocrine pancreas and bound both CCK and sulfated gastrin. Initial studies with these mice showed that sulfated gastrin could stimulate exocytosis and enzyme (amylase) secretion by acinar cells through a phospholipase C-dependent pathway (12).

A number of studies have raised the possibility that gastrin acts as an autocrine growth factor in the pancreas. For example, a number of human pancreatic adenocarcinomas have been shown to express both the gastrin and the gastrin/CCK-B-receptor genes. In addition, transgenic mice bearing an elastase I promoter-SV40 T antigen transgene develop pancreatic adenocarcinomas, and these mice express gastrin (CCK-B) receptors in both the pancreatic carcinomas and dysplastic pancreas (11). This expression may provide a growth advantage to acinar cells as part of the multistage process of carcinogenesis. Long-term studies with the elastase I-gastrin/CCK-B-receptor transgenic mice may be helpful in addressing the role of the gastrin/CCK-B receptor in mediating pancreatic growth and possibly neoplasia.

Role of gastrin in the stomach

It has been well established that normal circulating gastrin plays an important role in the regulation of meal-stimulated acid secretion, but the role of normal...
plasma gastrin concentrations in the control of mucosal growth is less clear. The role of gastrin in the regulation of growth and secretion has been addressed recently through the generation of gastrin-deficient and gastrin receptor-deficient mice.

Initially, mice that lacked the gastrin/CCK-B receptor through targeted gene disruption were generated by two independent groups (8, 10). Homozygous gastrin/CCK-B receptor knockout mice were viable, fertile, and appeared grossly normal into adulthood. The receptor-deficient mice exhibited a marked increase in basal gastric pH (from 3.2 to 5.2) and an ~10-fold elevation in plasma gastrin concentration compared with wild-type controls. The homozygous mice showed a remarkable atrophy of the gastric mucosa macroscopically, even in the presence of severe hypergastrinemia. The atrophy and decreased acid secretion were due to a decrease in parietal cells expressing the H⁺-K⁺-ATPase and a decrease in enterochromaffin-like (ECL) cells expressing chromogranin A and histidine decarboxylase (HDC) genes. In the antrum, a decrease in somatostatin cell density and an increase in the gastrin cell number were observed, consistent with the concomitant elevation in circulating gastrin secondary to raised intragastric pH.

Similar physiological and histological findings were noted in mice deficient in gastrin generated through targeted disruption of the gastrin gene by two independent groups (3, 6). Gastrin-deficient mice were also viable and fertile and had no visible abnormalities. Gastrin-deficient mice also showed reduced (~25–30%) numbers of parietal cells, with an accumulation of immature cells lacking H⁺-K⁺-ATPase expression. ECL cells were not as reduced in gastrin-deficient compared with gastrin/CCK-B receptor-deficient mice but did show lower expression of HDC. The gastrin-deficient mice also exhibited an elevated basal gastric pH (5.77–0.32) similar to that seen in the gastrin/CCK-B-receptor-deficient mice.

The abnormality in parietal cell development and/or differentiation appeared to be the most dramatic phenotype in these mice, but Koh et al. (6) demonstrated that the reduction in parietal cells was not due to abnormalities in proliferation. Using the technique of bromodeoxyuridine (BrdU) incorporation, Koh et al. (6) showed that there was no difference in the proliferation labeling index of the stomach from gastrin-deficient mice (3.04%) compared with that from wild-type littermates (3.15%). In addition, gastrin-deficient mice showed an increase in the number of spasmolytic polypeptide-positive mucous neck cells (6). Thus gastrin does not appear to be essential for normal gastric epithelial proliferation but is instead important in the regulation of differentiation, particularly for the parietal cell.

Interestingly, gastric acid secretion in these gastrin-deficient mice could not be induced by gastrin, carbachol, or histamine (3). Acid secretion appears, therefore, to be more extensively impaired than might be predicted from the reduction in parietal cell numbers alone, suggesting a primary defect in parietal cell function. The role of amidated gastrin in this parietal cell defect was further addressed in the initial study by Friis-Hansen et al. (3) through short-term G-17 reconstitution. Infusion of amidated gastrin (rat G-17) for 6 days resulted in supraphysiological concentrations of circulating gastrin, and partial (~30%) restoration of basal acid secretion was achieved in the gastrin-deficient mice (3). Whether full restoration of the acid secretory defect requires longer infusions of gastrin or other factors was not addressed in the study by Friis-Hansen et al. (3).

In addition to gastrin deficiency, the role of gastrin in the stomach has been studied in transgenic mice overexpressing amidated gastrin. Subsequent studies with the INS-GAS mice revealed that expression of gastrin in pancreatic islets resulted in islet secretion of amidated gastrin and circulating levels of plasma gastrin that, at early time points (1–3 mo), were elevated approximately twofold compared with that in wild-type mice (20). Hypergastrinemia led to thickening of the fundic mucosa and multifocal hyperplasia that occurred in association with an increased BrdU proliferative index of the stomach from gastrin-deficient mice (3.04% vs. 3.15%)

Table 1. Summary of gastrin transgenic and knockout mouse studies

<table>
<thead>
<tr>
<th>Transgenic and/or Knockout Construct</th>
<th>Expression Pattern or Phenotype</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td><strong>Promoter studies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP1.5 Tag</td>
<td>Hepatobiliary and pancreatic islet tumors</td>
<td>9</td>
</tr>
<tr>
<td>GP10.5 Tag</td>
<td>Antral G cell hyperplasia</td>
<td>9</td>
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<tr>
<td>450 rGAS-hGAS</td>
<td>Antral G cell expression</td>
<td>16, 18</td>
</tr>
<tr>
<td>450 rGAS-hGH</td>
<td>No antral cell expression</td>
<td>16</td>
</tr>
<tr>
<td>hGAS-hGAS (hGAS)</td>
<td>Hepatobiliary and fetal islet expression</td>
<td>16, 20</td>
</tr>
<tr>
<td><strong>Knockout studies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrin/CCK-B receptor</td>
<td>Decreased parietal and ECL cells, achlorhydria</td>
<td>8, 10</td>
</tr>
<tr>
<td>Gastrin</td>
<td>Decreased parietal cells, achlorhydria, decreased colonic proliferation</td>
<td>3, 6</td>
</tr>
<tr>
<td><strong>Overexpression</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INS-GAS</td>
<td>Increased amidated gastrin, increased gastric proliferation, foveal hyperplasia, gastric atrophy/hyperplasia</td>
<td>17, 19, 20</td>
</tr>
<tr>
<td>Elastase I-CCK-B</td>
<td>Gastrin-mediated pancreatic exocrine secretion (amylase)</td>
<td>12</td>
</tr>
<tr>
<td>hGAS</td>
<td>Progastatin, colonic hyperplasia, increased ACFs with AOM</td>
<td>13, 20</td>
</tr>
<tr>
<td>MT1/G-Gly</td>
<td>Increased glycine-extended gastrin, colonic hyperplasia, lung tumors (??)</td>
<td>5</td>
</tr>
</tbody>
</table>

hGAS, human gastrin transgene; hGH, human growth hormone; rGAS, rat gastrin transgene; ECL, enterochromaffin-like; ACF, aberrant crypt foci; AOM, azoxymethane; MT, metallothionein.
tion index (20). These mice initially showed increased parietal and ECL cell numbers and increased gastric acid secretion. However, over time, hypergastrinemia in these mice resulted in gradual parietal cell loss, gastric atrophy, foveolar hyperplasia, and gastric metaplasia. When followed for >20 mo, the majority of INS-GAS mice were found to develop invasive glandular foci consistent with gastric carcinoma (19). Thus moderate elevations of amidated gastrin led to eventual parietal cell loss in mice and predisposed mice to the development of gastric cancer (19).

ROLE OF GASTRIN IN THE COLON

There has been considerable controversy regarding the possible trophic effects of gastrin outside of the stomach, particularly with respect to a possible role in colonic proliferation. A number of studies showed that amidated gastrin was able to stimulate proliferation of colon cancer cell lines in vitro, but the effects appeared small. In addition, most laboratories found that colon cancer cell lines do not contain gastrin/CCK-B receptors, although they often possess low-affinity gastrin receptors. Studies utilizing gastrin infusion or omeprazole-induced hypergastrinemia were unable to show effects on colonic proliferation. However, although the majority of colon cancer cell lines do produce gastrin mRNA, progastrin peptides are not processed to amidated gastrin in these cell lines. Instead, the majority of colon cancers express significant amounts of progastrin and glycine-extended gastrin. Thus these observations raised the possibility of a biological function for incompletely processed forms of gastrin, such as progastrin and/or glycine-extended gastrins.

Initial studies with progastrin-expressing transgenic mice confirmed a colonic trophic effect for human progastrin. Transgenic mice expressing a human gastrin transgene (hGAS) showed expression of the transgene in hepatocytes where it was largely unprocessed and secreted, resulting in circulating progastrin levels that were 10–1,000 times elevated but normal levels of amidated gastrin (20). The hGAS transgenic mice showed colonic hyperplasia and a marked increase in the BrdU labeling index of the colon (7.46 ± 1.90) compared with age-matched, wild-type control mice (4.01 ± 0.98; P < 0.05). Proliferative effects were observed in all portions of the colon, including the proximal colon, the distal colon, and the rectum. In addition to increased BrdU labeling, the proliferative zone in the colons of hGAS mice was expanded upward toward the tops of the glands, in contrast to that observed in wild-type mice in which the proliferative zone was polarized toward the base of the glands. The colonic glands of hGAS mice were elongated, with a mean crypt depth that was 30% greater than that of age-matched, wild-type mice (20).

A role for gastrin in colonic proliferation was strengthened through studies in mice deficient in gastrin through targeted disruption of the murine gastrin gene (6). These mice showed a decreased proliferation labeling index (2.97 ± 0.52%) compared with wild-type littermates (4.71 ± 0.44%). The decreased rate of proliferation was found throughout the colon, including the proximal colon, distal colon, and rectum (6). However, the height of the colonic crypts was not statistically different, the crypts had a normal histologic appearance, and the location of the proliferative zone was not changed.

More recently, we have addressed the role of glycine-extended gastrin in colonic proliferation through additional transgenic studies. Transgenic mice were generated carrying a mouse metallothionein-human gastrin transgene (MT1/G-Gly) that overexpress progastrin truncated at Gly-72 (5). The MT1/G-Gly showed elevated serum levels of G-34-Gly (~85 pM) compared with wild-type mice (<20 pM) as well as elevated colonic mucosal levels. Overexpression of G-Gly in these mice resulted in a significant increase in the colonic proliferation index (9.03%) compared with wild-type mice (4.14%) and also resulted in significant increases in colonic mucosal thickness (43%) and the percentage of goblet cells (41%) compared with wild-type controls (5). Additional evidence for the notion that G-Gly is an important colonic growth factor was provided through reconstitution studies in gastrin-deficient mice. Reinfusion of G-17-Gly into gastrin-deficient mice resulted in an 81% increase in the colonic proliferation and increased colonic mucosal thickness, whereas reinfusion of the amidated peptide (G-17) had no significant effect on colonic proliferation (5). Taken together, these studies provide strong support for a...
significant role for glycine-extended gastrins in the regulation of colonic growth.

These transgenic and knockout mice should prove useful in understanding the role of gastrin peptides in the susceptibility to colon cancer using the various models of colonic carcinogenesis mice treated with azoxymethane (AOM) or mice heterozygous for the multiple intestinal neoplasia (Min) mutation of the adenomatous polyposis coli (Apc) gene (Apcmin mice). Initial studies have shown that hGAS transgenic mice develop a higher number of aberrant crypt foci in response to studies have shown that hGAS transgenic mice develop a higher number of aberrant crypt foci in response to wild-type mice (13). Further studies should clarify the role of gastrin-dependent proliferation in progression to colon cancer.

SUMMARY AND FUTURE QUESTIONS TO BE ADDRESSED

Although the roles of gastrin in secretion and growth have been recognized for decades, recent associations with H. pylori infection and malignancies (in colon, gastric, pancreatic, and lung tissues) have raised new questions regarding the role of gastrin in the regulation of growth and differentiation. Many of these questions have been addressed through gastrin transgenic mouse studies (Table 1). Further studies with gastrin promoter constructs could generate new tools for analyzing the regulation of gastrin gene expression, not only in the gastric mucosa but also in extragastric sites such as the pancreatic islets, colon, and the central nervous system. In addition, further studies will be required to address the roles of various gastrin processing intermediates in the control of gastrointestinal growth and differentiation (Fig. 1). The notion that incompletely processed forms of gastrin have significant growth factor activity has now been supported by both knockout and transgenic overexpression studies: knockout of the gastrin gene leads to decreased colonic proliferation, whereas overexpression of progastrin and glycine-extended gastrin leads to increased proliferation and hyperplasia of the colon. In addition, the role of amidated gastrin in the regulation of stomach growth appears to be quite complicated. Although overexpression of amidated gastrin does stimulate proliferation in the oxyntic mucosa, knockout of the gastrin gene does not lead to a decrease in proliferation but instead leads to altered differentiation. In addition, increased amidated gastrin initially stimulates parietal cell growth but over time leads to a reduction in parietal cell number (atrophy). It is clear that analysis of the role of “the gastrin family of peptides” in growth requires an assessment of all the different products of progastrin processing. The availability of mouse models overexpressing various forms of gastrin and the ability to reconstitute different forms of gastrin in gastrin-deficient mice will help to further clarify the precise role of gastrins in growth and neoplasia of the gastrointestinal tract.

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