

Hypoglycemia, Defective Islet Glucagon Secretion, but Normal Islet Mass in Mice With a Disruption of the Gastrin Gene

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Background & Aims: Both cholecystokinin (CCK)-A and CCK-B receptors are expressed in the pancreas, and exogenous gastrin administration stimulates glucagon secretion from human islets. Although gastrin action has been linked to islet neogenesis, transdifferentiation, and beta-cell regeneration, an essential physiologic role(s) for gastrin in the pancreas has not been established. **Methods:** We examined glucose homeostasis, glucagon gene expression, glucagon secretion, and islet mass in mice with a targeted gastrin gene disruption. **Results:** Gastrin $-/-$ mice exhibit fasting hypoglycemia and significantly reduced glycemic excursion following glucose challenge. Insulin sensitivity was normal and levels of circulating insulin and insulin messenger RNA transcripts were appropriately reduced in gastrin $-/-$ mice. In contrast, levels of circulating glucagon and pancreatic glucagon messenger RNA transcripts were not up-regulated in hypoglycemic gastrin $-/-$ mice. Furthermore, the glucagon response to epinephrine in isolated perfused islets was moderately impaired in gastrin $-/-$ versus gastrin $+/+$ islets (40% reduction; $P < 0.01$, gastrin $+/+$ vs. gastrin $-/-$ mice). Moreover, the glucagon response but not the epinephrine response to hypoglycemia was significantly attenuated in gastrin $-/-$ compared with gastrin $+/+$ mice ($P < 0.05$). Despite gastrin expression in the developing fetal pancreas, beta-cell area, islet topography, and the islet proliferative response to experimental injury were normal in gastrin $-/-$ mice. **Conclusions:** These findings show an essential physiologic role for gastrin in glucose homeostasis; however, the gastrin gene is not essential for murine islet development or the adaptive islet proliferative response to beta-cell injury.

extended gastrins such as G34-Gly and G17-Gly exhibit mitogenic effects on normal colonocytes and promote proliferation of colon carcinoma cell lines.² The principal progastrin posttranslational product detected in human antral G cells, amidated gastrin (G-17), stimulates acid secretion in part via direct effects on parietal cells and acts on enterochromaffin-like cells to promote histamine release and up-regulation of genes important for histamine biosynthesis.³ Although the cholecystokinin (CCK)-B receptor mediates many of the actions ascribed to amidated gastrins G-17 and G-34, this receptor exhibits a low affinity for glycine-extended gastrins or progastrin⁴ and the specific mechanisms by which these larger gastrins exert their effects remain unclear.

Gastrin-deficient mice have been generated independently by different research groups and exhibit reduced numbers of parietal cells with deficient H^+-K^+ -adenosine triphosphatase expression in immature precursor cells, in association with increased basal gastric pH and reduced acid secretion.^{5–7} A similar phenotype was observed in mice with inactivation of the CCK-B receptor gene, which exhibit gastric atrophy despite a 10-fold increase in levels of circulating gastrin.^{8,9} Gastrin $-/-$ mice also exhibit decreased colonocyte proliferation,⁵ consistent with transgenic overexpression studies showing enhanced colonic cell proliferation in mice with increased levels of progastrin or G-Gly.^{1,10}

Although gastrin expression is predominantly restricted to endocrine G cells in the gastric antrum and proximal duodenum in the adult, high levels of gastrin expression are detected in the fetal pancreas, localized to

The human and murine gastrin genes encode a 101-amino acid precursor that gives rise to several biologically active, structurally distinct progastrin-derived peptides. Progastrin, the unprocessed prohormone, exerts proliferative effects in the colon.¹ Similarly, glycine-

Abbreviations used in this paper: BrdU, bromodeoxyuridine; R_a , rate of appearance of glucose; R_d , rate of total body glucose utilization; STZ, streptozotocin.

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islet beta cells.¹¹ Pancreatic gastrin expression remains detectable in the early neonatal period, followed by a progressive decrease in islet gastrin gene expression, which is extinguished in the adult pancreas.¹² The detection of both gastrin and the CCK-B receptor¹³ in the fetal pancreas during a time of rapid growth and differentiation has sparked interest into possible role(s) for one or more progastrin-derived peptides as islet growth factors. Transgenic expression of gastrin under the control of the insulin gene promoter produced no abnormalities in murine islet mass.¹⁴ However, double transgenic mice expressing both gastrin and transforming growth factor α in islet beta cells exhibit a doubling of islet mass and enhanced differentiation of metaplastic ducts¹⁴ when compared with transgenic mice expressing transforming growth factor α alone. These findings suggest that gastrin may act as a pancreatic growth modifier in the appropriate biological context. Consistent with this hypothesis, enhanced expression of the CCK-B receptor in pancreatic acinar cells leads to increased pancreatic weight in mice,¹⁵ and double transgenic mice that express both an insulin promoter-gastrin transgene and the CCK-B receptor under the control of the elastase promoter exhibit increased mass of the exocrine pancreas, with some mice developing pancreatic ductal adenocarcinoma.¹⁶

Although gastrin expression is extinguished in adult islets, CCK-B receptors are detected in the adult pancreas, suggesting that progastrin-derived peptides may regulate trophic or endocrine functions postnatally. Immunohistochemical analyses have localized the CCK-B receptor to both fetal and adult islet alpha cells^{13,17} and both gastrin and CCK-stimulated glucagon secretion from isolated human islets in a dose-dependent manner.¹³ More recent studies have shown induction of CCK-B receptor expression on rat ductal cells following pancreatic duct ligation,¹⁷ and infusion of gastrin for 3 days following ductal ligation markedly stimulated beta-cell mass via transdifferentiation of exocrine cells to duct-like cells and enhanced beta-cell neogenesis.¹⁸ Furthermore, exogenous administration of gastrin and epidermal growth factor lowered blood glucose levels and increased beta-cell mass in rats with streptozotocin (STZ)-induced diabetes.¹⁹

Despite increasing evidence that exogenous gastrin administration may regulate islet secretory function and/or pancreatic growth and differentiation, the physiologic importance of the gastrin gene for glucagon secretion or control of beta-cell mass remains unclear. Long-term administration of CCK-B receptor antagonists for 4–8 weeks produced hypoplasia of the rat

oxyntic mucosa, but no effect on the pancreas was reported.²⁰ Furthermore, mice with targeted disruption of genes for either gastrin or the CCK-A and CCK-B receptors develop normally; however, extensive studies of glucose homeostasis or pancreatic morphometry in these mice have not yet been reported.^{5,8,21,22} Given the potential biological complexity linking gastrin gene expression to multiple aspects of pancreatic growth and function, we examined whether progastrin-derived peptides exert essential roles in (1) glucose homeostasis, (2) the control of islet hormone secretion, and (3) islet growth and regeneration in gastrin $-/-$ mice with a targeted inactivation of the gastrin gene.

Materials and Methods

Animals

All experiments were performed in accordance with experimental guidelines approved by the animal care committee of the University Health Network. Gastrin $-/-$ mice originally generated in the C57BL/6 \times SV129 background were crossed to C57BL/6 mice and have been described.⁵ Initial studies of glucose homeostasis repeatedly compared $+/+$ littermates derived from mating gastrin $+/-$ heterozygotes with wild-type C57BL/6 mice purchased from Charles River Canada (Saint-Constant, Quebec, Canada). Fasting blood glucose level and glycemic excursion following glucose loading was indistinguishable in commercially purchased C57BL/6 wild-type mice and in $+/+$ littermate progeny derived from matings of gastrin $+/-$ mice and was always significantly greater compared with age- and sex-matched gastrin $-/-$ mice derived from the same litters. Hence, commercially available age- and sex-matched C57BL/6 mice were used as controls for the remainder of the studies.

Glucose Tolerance and Plasma Insulin and Glucagon Determination

Age- and sex-matched mice were fasted 15–16 hours before glucose tolerance testing, and glucose (1.5 mg/g body wt) was administered by oral gavage or via intraperitoneal injection.^{23,24} Blood was withdrawn from the tail vein at 0, 10, 20, 30, 60, 90, and 120 minutes and blood glucose levels determined using a One Touch Basic Glucometer (Lifescan Canada Ltd., Burnaby, British Columbia, Canada). Venous blood samples were collected from fasting (16 hours) mice and 20–30 minutes following intraperitoneal glucose injection in 10% vol/vol Trasylol/ethylenediaminetetraacetic acid/Diprotin A (5000 KIU/mL Trasylol, 1.2 mg/mL ethylenediaminetetraacetic acid, and 0.1 mmol/L Diprotin A). After centrifugation, plasma was collected and stored at -70°C before measuring levels of insulin and glucagon. Insulin was measured in duplicate using an insulin enzyme-linked immunosorbent assay kit (Crystal Chem, Chicago, IL) with mouse insulin as a standard.²⁴ Glucagon levels were measured using a glucagon radioimmunoassay kit (Linco Research Inc., St. Louis, MO).

RNA Isolation and Northern Blot Analysis

Total pancreatic RNA was isolated using a modified acid-ethanol guanidinium thiocyanate method.²⁵ For Northern blotting, 10 μ g of total RNA was size fractionated in a 1% formaldehyde-denaturing agarose gel, transferred to a nylon membrane, and immobilized with UV light. RNA integrity was assessed by examining 18S and 28S ribosomal bands on the ethidium bromide-stained gel before transfer. Nylon membranes were hybridized at 42°C for 18 hours in hybridization solution containing 1×10^6 cpm/mL of either ³²P-labeled rat insulin or mouse proglucagon complementary DNA as described.^{24,26} Blots were washed and either exposed to x-ray film with an intensifier screen at -80°C or a phosphorimaging cassette. Densitometry was performed using ImageQuant 4.1 software (Molecular Dynamics, Sunnyvale, CA) or performed on blots exposed to x-ray film (Kodak Diagnostic Film, X-OMAT AR; Kodak, Toronto, Ontario, Canada) using a Hewlett-Packard (Mississauga, Ontario, Canada) ScanJet 3p scanner and the NIH Image software. The housekeeping gene tubulin was used to monitor loading and transfer conditions.

Gastrin Replacement Experiments

Five weeks before gastrin administration, gastrin +/+ and gastrin -/- mice were fasted for 17 hours and administered 0.5 mL of saline via an intraperitoneal route. Blood was withdrawn from the tail vein at 0, 30, 60, and 90 minutes and blood glucose levels determined using a One Touch Basic Glucometer as previously described. This experiment was repeated (using saline injections) by the same investigator on a weekly basis for 5 weeks to minimize the stress invoked in response to human handling. In the sixth week, the same experiment was repeated using either human synthetic gastrin (G-17; Sigma Chemical Co., St. Louis, MO) diluted in saline to a final concentration of 30 μ g/kg or vehicle alone administered intraperitoneally.

Insulin-Induced Hypoglycemia

Eight- to 10-week-old male control C57BL/6 or gastrin -/- mice were fasted for 5 hours beginning at 8 AM. Fasting blood glucose was then measured ($t = 0$) using a Glucometer Elite (Bayer, Etobicoke, Canada). Immediately afterward, 1.49 U/kg of regular human insulin (Novo Nordisk, Bagsraerd, Denmark) was administered intraperitoneally and blood glucose level was then monitored at regular intervals. Mice that experienced seizures during this experiment were excluded from the data set.

Plasma Glucagon and Epinephrine Measurements

Eight- to 10-week-old male wild-type control or gastrin -/- mice were fasted for 5 hours beginning at 8 AM. Mice were then given an insulin dose of 1.49 U/kg administered intraperitoneally and anesthetized with a 100- μ L injection of Somnotol (MTC Pharmaceuticals, Cambridge, Ontario, Canada) administered intraperitoneally 40 minutes after the insu-

lin challenge. Anesthetized mice were then exsanguinated via cardiac puncture, and blood was collected in Eppendorf tubes containing Trasylol/ethylenediaminetetraacetic acid/Diprotin A (5000 KIU/mL Trasylol, 32 mmol/L ethylenediaminetetraacetic acid, and 0.1 nmol/L Diprotin A). Plasma was separated by centrifugation and stored at -80°C. Plasma concentrations of glucagon were determined in duplicate using a glucagon radioimmunoassay kit (Linco Research). Plasma concentrations of epinephrine (adrenaline) were determined in duplicate using a catecholamine enzyme-linked immunosorbent assay kit (IBL, Hamburg, Germany).

STZ-Induced Diabetes

Eight- to 10-week-old male wild-type control or gastrin -/- mice ($n = 12$ per group) were injected with STZ (Sigma Chemical Co.) once daily at 8 AM for 5 consecutive days. STZ was freshly dissolved each morning in 0.1 mol/L sodium citrate, pH 5.5, and administered intraperitoneally at a dose of 50 mg/kg. Five mice in each group received an injection of bromodeoxyuridine (BrdU) (Roche Diagnostics, Mannheim, Germany) administered intraperitoneally (100 mg/kg) approximately 24 hours after the final injection of STZ. Mice ($n = 5$) were killed 4 hours after the BrdU injection, and the pancreas was immediately removed and fixed in 10% formalin. The remaining mice were monitored daily during the next 2 weeks.

Hyperinsulinemic Clamp

Control mice were age-, sex-, and weight-matched C57BL/6 mice obtained from Charles River Laboratories (Wilmington, MA). Mice were fed Purina (St. Louis, MO) 5001 Laboratory Rodent Diet (23% protein, 5.5% fat, 49% carbohydrate, 5.3% fiber, 6.9% ash) and housed in an approved, temperature-controlled facility on a 6 AM/6 PM light/dark cycle with access to water ad libitum. All protocols were approved in advance by the Washington University Animal Studies Committee.

Clamp experiments were performed as previously described²⁷ with the following modifications. After a basal period of 60 minutes, an infusion of insulin (regular human; Eli Lilly, Indianapolis, IN) at a rate of $20 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ was started. Dextrose was begun at the same time and the infusion rate varied to maintain the blood glucose level at approximately 8.9 mmol/L, the average blood glucose level in a freely feeding, wild-type, conscious mouse. Blood samples for determination of specific activity were taken 15 and 7 minutes before and at the end of the basal period and the experimental period, when the blood glucose level was in steady state. Blood glucose level was measured using 5 μ L of whole blood in the Hemocue blood glucose meter (Mission Viejo, CA). Specific activity of glucose in whole blood was determined as described.²⁷ The rate of appearance of glucose (R_a), which equals the rate of total body glucose utilization (R_d) when the blood glucose level is in steady state, was calculated by dividing the infusion rate of 3-[³H]-glucose by the specific activity of glucose in the blood at the same time.

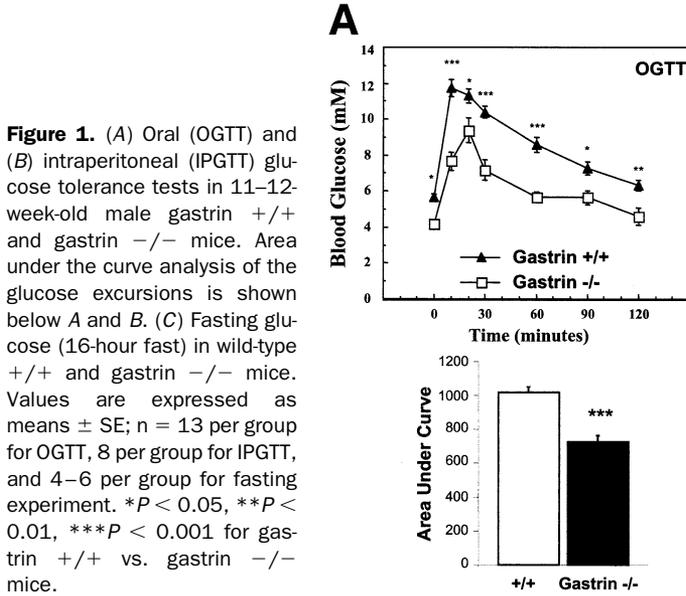


Figure 1. (A) Oral (OGTT) and (B) intraperitoneal (IPGTT) glucose tolerance tests in 11–12-week-old male gastrin +/+ and gastrin -/- mice. Area under the curve analysis of the glucose excursions is shown below A and B. (C) Fasting glucose (16-hour fast) in wild-type +/+ and gastrin -/- mice. Values are expressed as means ± SE; n = 13 per group for OGTT, 8 per group for IPGTT, and 4–6 per group for fasting experiment. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 for gastrin +/+ vs. gastrin -/- mice.

Measurement of Glucagon Release From Perfused Islets

Isolation of islets of Langerhans, overnight culture, and measurement of hormone release via perfusion experiments in a multiple microchamber module (Endotronics, Coon Rapids, MN) was performed as previously described.²⁸ Approximately 200 islets were loaded onto a Biogel P2 column and preperfused for 20 minutes in Ham’s F10 medium; supplemented with 0.5% bovine serum, 2 mmol/L glutamine, 2 mmol/L CaCl₂, and 1.4 mmol/L glucose; and equilibrated with 95% O₂/5% CO₂. At a flow rate of 0.5 mL/min, pulses of 15 minutes were given with either 1.4 mmol/L glucose and 20 mmol/L glucose with or without 0.1 nmol/L human gastrin I (Sigma, Bremen, Germany), 1 μmol/L epinephrine (Sigma), or 10 nmol/L somatostatin 14. Samples were collected every minute and assayed for immunoreactive glucagon with guinea pig anti-glucagon serum (Linco Research). Results for glucagon secretion were expressed as percentage of the glucagon content measured in each individual batch of islets after con-

clusion of the experiment by sonicating the Biogel P2 containing the islets in 5 mL of 2 mmol/L acetic acid and 0.25% bovine serum albumin. Cumulative glucagon release over the entire experiment amounted to <3% of glucagon recovered from the cells.

Measurement of Total Pancreatic Insulin and Glucagon Content

Pancreata were dissected from ad libitum-fed wild-type and gastrin -/- mice at different ages (neonatal 1 day old, 4 weeks, 8 weeks, and 16 weeks) and stored at -80°C

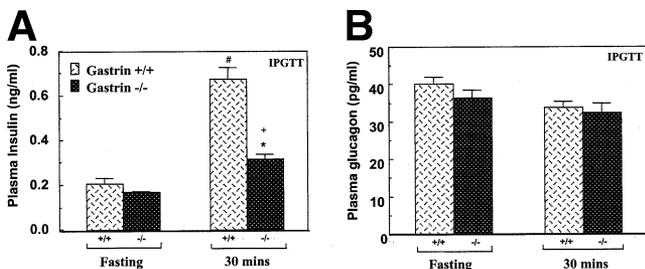


Figure 2. (A) Plasma insulin and (B) glucagon following a 16-hour fast and at the 30-minute time point during the intraperitoneal glucose tolerance test (IPGTT). #*P* < 0.001, gastrin +/+ mice fasting vs. 30-minute time point after intraperitoneal glucose loading; +*P* < 0.05, gastrin -/- mice fasting vs. 30-minute time point; **P* < 0.05, gastrin +/+ vs. gastrin -/- at the 30-minute time point.

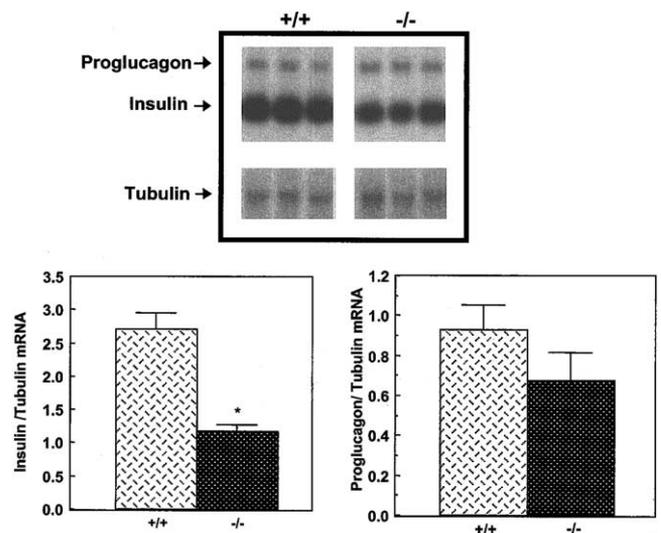


Figure 3. Northern blot analysis of pancreatic RNA from gastrin +/+ and gastrin -/- fasted male mice. The relative densitometric data in the panels below the Northern blot represent mean values for insulin and proglucagon (n = 8 per group). Values are normalized to signals obtained for tubulin in each sample. **P* < 0.05, gastrin +/+ vs. gastrin -/- mice.

Table 1. Islet Hormone Content in Gastrin +/+ and Gastrin -/- Mice

Age	Pancreatic glucagon content (ng/pancreas)		Pancreatic insulin content (μ g/pancreas)	
	Control	Gastrin -/-	Control	Gastrin -/-
16 wk	280.4 \pm 50.7 (n = 7)	293.7 \pm 43.4 (n = 9)	10.2 \pm 1.7 (n = 6)	9.9 \pm 1.8 (n = 6)
8 wk	89.3 \pm 7.3 (n = 5)	89.0 \pm 14.2 (n = 6)	6.3 \pm 0.4 (n = 6)	5.4 \pm 0.4 (n = 6)
4 wk	319.7 \pm 12.3 (n = 7)	332.1 \pm 10.8 (n = 11)	9.1 \pm 0.6 (n = 7)	9.6 \pm 0.4 (n = 9)
Neonatal	105.2 \pm 17.0 (n = 5)	178.9 \pm 67.6 (n = 3)	2.2 \pm 0.3 (n = 5)	2.6 \pm 0.4 (n = 3)

until analysis. The pancreata were homogenized in 15 mL of 2 mmol/L acetic acid with 0.25% bovine serum albumin, incubated for 2 hours on ice, and centrifuged at 8000g for 20 minutes. The supernatant fraction was collected while pellets were sonicated again and centrifuged at 8000g for 15 minutes. The second supernatant fraction was pooled with the first for analysis of insulin and glucagon content by radioimmunoassay.

Immunohistochemistry

Pancreata were dissected from ad libitum-fed wild-type and gastrin -/- mice at different ages (4, 8, and 16 weeks) and embedded in paraffin. Pancreas sections were stained for insulin, glucagon, somatostatin, and pancreatic polypeptide. Estimation of beta-cell and islet area was calculated as previously described.²⁹ Islet cell proliferation and the number of BrdU-positive islet cells was determined as previously described.³⁰

Statistical Analysis

Statistical difference between treatment groups was assessed using unpaired Student *t* test or by analysis of variance. Post-hoc testing was performed using the Bonferroni modification of the *t* test as appropriate. Statistical significance was calculated by analysis of variance using an SAS program (Statistical Analysis Systems, Cary, NC) for IBM computers. Data for clamp studies were analyzed using 2-tailed *t* tests calculated using StatView 4.51 software (Abacus Concepts, Berkeley, CA).

Results

To determine the consequences of gastrin gene disruption on glucose homeostasis, we assessed fasting glucose level and glucose clearance following oral glucose challenge in age- and sex-matched control and gastrin

-/- mice. Fasting glucose level was modestly but significantly lower in gastrin -/- mice (Figure 1). Similarly, glucose excursion was significantly lower following both oral and intraperitoneal glucose challenge in gastrin -/- versus control wild-type mice ($P < 0.05$ to $P < 0.001$ for wild-type vs. gastrin -/- blood glucose levels at multiple time points). Plasma levels of insulin were significantly and appropriately increased in both +/+ and gastrin -/- mice following glucose challenge (Figure 2). Consistent with lower levels of glucose in gastrin -/- mice, insulin levels were modestly lower in the fasting state and significantly lower following glucose challenge (Figure 2), suggesting that hypoglycemia was not attributable to inappropriate insulin secretion in gastrin -/- mice. In contrast, despite the lower levels of glucose in the fasting state and following glucose loading, glucagon levels were similar in gastrin -/- versus control mice (Figure 2). Levels of pancreatic insulin messenger RNA (mRNA) transcripts were significantly lower in hypoglycemic gastrin -/- mice compared with wild-type gastrin +/+ controls, whereas levels of pancreatic proglucagon mRNA transcripts were modestly lower in gastrin -/- mice despite lower levels of blood glucose (Figure 3).

No significant differences in pancreatic insulin or glucagon content were observed in wild-type versus gastrin -/- ad libitum-fed mice in the neonatal period or at 4, 8, or 16 weeks of age (Table 1). To ascertain whether hypoglycemia observed in gastrin -/- mice was attributable to changes in insulin sensitivity, we assessed R_a and R_d in response to insulin infusion in age- and sex-matched wild-type control and gastrin -/- mice. No

Table 2. Glucose Appearance and Utilization in Gastrin +/+ and Gastrin -/- Mice

Genotype	n	Wt (g)	Basal glucose (mg/100 mL)	Basal R_a ($mg \cdot kg^{-1} \cdot min^{-1}$)	Clamp glucose (mg/100 mL)	Clamp RD ($mg \cdot kg^{-1} \cdot min^{-1}$)	Clamp GIR ($mg \cdot kg^{-1} \cdot min^{-1}$)	Clamp HGP ($mg \cdot kg^{-1} \cdot min^{-1}$)	Insulin (μ U/mL)
Gastrin -/-	6	29.8 \pm 1.9	143 \pm 12	9.8 \pm 0.5	122 \pm 5	32.0 \pm 4.1	32.0 \pm 5.3	0.5 \pm 1.3	16.1 \pm 1.1
+/+ Control	6	31.9 \pm 1.3	133 \pm 6	11.0 \pm 0.4	119 \pm 4	26.5 \pm 5.0	28.0 \pm 3.8	-1.2 \pm 2.6	15.8 \pm 2.9

NOTE. Glucose production and disappearance in gastrin -/- mice and age- and sex-matched control (+/+ control) mice. The units for insulin values are as measured in a Linco radioimmunoassay using human insulin as a standard.

GIR, glucose infusion rate; HGP, hepatic glucose production.

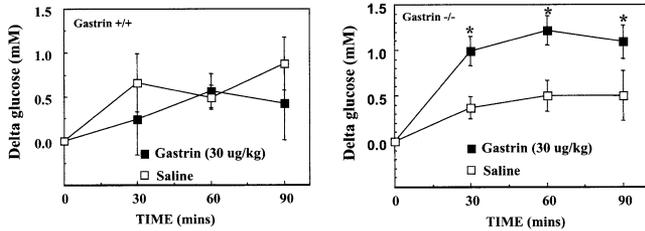


Figure 4. Change in plasma glucose level (delta glucose) following gastrin administration (30 µg/kg) in fasting male gastrin +/+ and gastrin -/- mice (n = 7–8 mice per group). *P < 0.05, gastrin vs. vehicle alone.

significant differences were observed in basal or insulin-stimulated R_a or R_d in control wild-type versus gastrin -/- mice (Table 2).

Previous studies have shown that the CCK-B receptor is expressed on human islet alpha cells and coupled to stimulation of glucagon secretion.¹³ Administration of gastrin to fasted mice produced a small but significant increase in blood glucose level in gastrin -/- but not in control gastrin +/+ mice in vivo (Figure 4). To determine whether gastrin stimulates glucagon secretion from wild-type or gastrin -/- islets, we perfused isolated islets in vitro with 100 pmol/L gastrin under conditions of low (1.4 mmol/L) or high (20 mmol/L) glucose levels. In contrast to findings with cultured human islets,¹³ gastrin-dependent stimulation of glucagon secretion was not observed in murine islets (Figure 5A). However, epinephrine markedly increased (between 5-fold and 6-fold) and somatostatin inhibited (approximately 75% decrease vs. control without somatostatin) glucagon secretion in the same islet preparations (Figure 5A and B, respectively, $P < 0.001$ and $P = 0.02$). Consistent with previous studies,³¹ the stimulatory effect of epinephrine on glucagon release was counteracted in part by high glucose levels (Figure 5A and B; $P < 0.01$). Under conditions of maximally stimulated glucagon secretion (1 µmol/L epinephrine in combination with 1.4 mmol/L glucose), a 40% loss of secretory function was observed in gastrin -/- islets (cumulative release over a 15-minute period: $0.84\% \pm 0.19\%$ of islet glucagon content in gastrin -/- islets) compared with $1.34\% \pm 0.17\%$ of islet glucagon content from gastrin +/+ islets that were perfused in parallel (mean \pm SEM; n = 9; $P = 0.006$). Thus, a mild but highly significant glucagon secretory defect was observed in islets isolated from gastrin -/- mice.

Because levels of circulating glucagon were not increased in the fasting state in gastrin -/- mice despite mild hypoglycemia but gastrin -/- islets exhibit a reduced maximal secretory response to epinephrine in vitro, we assessed whether a glucagon secretory defect in

gastrin -/- mice might be unmasked following insulin-induced hypoglycemia. Administration of insulin (1.49 U/kg) by intraperitoneal injection produced more profound hypoglycemia with significantly delayed glucose recovery in gastrin -/- versus wild-type control mice (Figure 6). Three gastrin -/- mice developed seizures during this experiment, whereas no seizures were observed in wild-type gastrin +/+ mice. Analysis of counterregulatory responses to hypoglycemia showed a significantly greater increment in circulating levels of epinephrine in gastrin -/- compared with wild-type control mice, consistent with the more severe hypoglycemia induced in gastrin -/- mice (Figure 6). In contrast, despite a greater and more prolonged hypoglycemic stimulus, levels of plasma glucagon were significantly lower in gastrin -/- mice (Figure 6; $P < 0.05$ for glucagon in wild-type +/+ vs. gastrin -/- mice).

Because gastrin is expressed at high levels in the fetal endocrine pancreas and has been postulated to play a role in islet growth or differentiation,^{14,17,18} we assessed islet histology and beta-cell area in wild-type control versus gastrin -/- mice. Islet histology appeared normal in gastrin -/- mice studied at 8 and 16 weeks of age, with normal numbers of beta, alpha, delta, and pancreatic

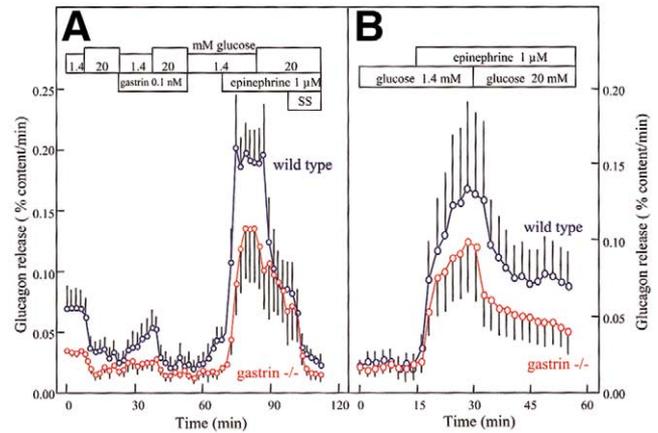


Figure 5. Glucagon release from perfused mouse islets in response to gastrin, epinephrine, or somatostatin. Islets isolated from gastrin +/+ wild-type mice (blue) and age- and sex-matched gastrin -/- mice (red) were perfused with the indicated concentrations of glucose (1.4 or 20 mmol/L) as well as gastrin (0.1 nmol/L), epinephrine (1 µmol/L), and somatostatin 14 (SS; 10 nmol/L). Data represent the mean fractional rates of glucagon release \pm SEM of (A) 6 and (B) 3 independent experiments. Rates of glucagon release per minute were expressed as percent of the islet glucagon content. In B, the second half of the experiment from A was repeated without addition of somatostatin 14 at the end of the experiment. The area under the curve for the epinephrine-induced stimulation of glucagon release at low glucose level was significantly lower in the gastrin -/- islets ($0.84\% \pm 0.19\%$ glucagon content) compared with gastrin +/+ islets ($1.34\% \pm 0.17\%$) (mean \pm SEM; n = 9; $P = 0.006$).

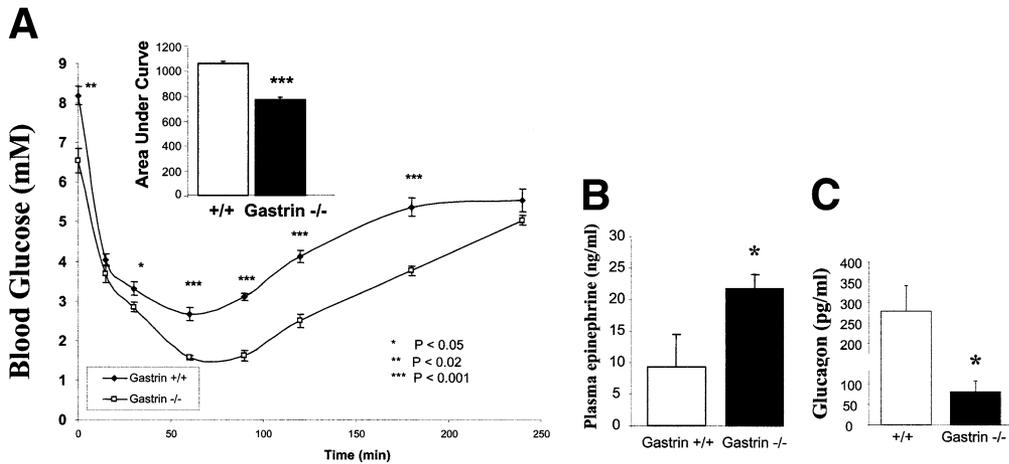


Figure 6. (A) Blood glucose levels during an insulin tolerance test (ITT) in gastrin +/+ and gastrin -/- mice. Statistical significance for glucose values from gastrin +/+ and gastrin -/- mice is shown. (B and C) Gastrin +/+ and gastrin -/- mice were subjected to an insulin tolerance test and were euthanized at t = 40 minutes and analyzed for (B) plasma epinephrine or (C) plasma glucagon levels. * $P < 0.05$ for gastrin +/+ vs. gastrin -/- mice.

polypeptide cells (Figure 7A). Similarly, assessment of relative beta-cell area at postnatal day 23, a time when islet neogenesis is high, showed no significant difference in percentage beta-cell area in control wild-type versus gastrin -/- male or female mice (data not shown).

Several lines of evidence suggest that exogenous gastrin administration activates pathways leading to islet growth or transdifferentiation in vivo.^{14,17-19} Accordingly, if the gastrin gene is an essential component of the islet regenerative response, induction of islet injury may potentially be associated with reduced beta-cell proliferation and more profound diabetes. To determine whether gastrin -/- mice exhibit abnormal susceptibility to or defective recovery from beta-cell injury, we treated control and gastrin -/- mice with daily injections of STZ for 5 days. Plasma glucose level was monitored daily, and the early histologic manifestation of islet injury was assessed approximately 28 hours after the last dose of STZ. Remarkably, gastrin -/- mice maintained significantly lower levels of blood glucose following STZ administration over the 2-week observation period (Figure 7B). Analysis of islet cell proliferation approximately 28 hours following the last dose of STZ showed no significant differences in the number of BrdU-positive islet cells in control versus gastrin -/- mice (Figure 7C).

Discussion

Genetic Disruption of the Gastrin or CCK Receptor Genes in Mice

Experiments directed at understanding the physiologic roles of murine progastrin-derived peptides have used genetic strategies to inactivate genes encoding gastrin and the CCK-A and CCK-B receptors.^{3,21} Pancreatic morphology and glucose homeostasis has been examined in mice with disruption of the CCK-A or CCK-B recep-

tor genes. Examination of the pancreas in CCK receptor mutant mice from 7 weeks to 21 months of age did not show evidence of islet fibrosis or differences in islet cell numbers.^{8,22} Furthermore, ambient blood glucose level was normal in freely fed CCK receptor knockout mice. Consistent with these findings, we did not detect abnormalities in pancreatic histology or blood glucose level in ad libitum-fed gastrin -/- mice. However, after overnight fasting or during both oral and intraperitoneal glucose challenge, blood glucose level was consistently lower in gastrin -/- mice.

Gastrin and Islet Glucagon Secretion

Although both gastrin and CCK directly stimulate glucagon secretion in isolated human islets,¹³ we did not observe a direct stimulatory effect of gastrin in perfused murine islets in vitro. This lack of response is unlikely to be an artifact of the islet isolation procedure because glucagon release from the same cultured islets was clearly responsive to epinephrine (6-fold stimulation) and somatostatin 14 (4-fold inhibition), and we have previously shown that these substances act directly on rodent alpha cells.^{32,33} A more likely explanation for the lack of significant gastrin-dependent activation of glucagon secretion in mice may be the very low levels of CCK-B receptor expression in murine islets. Consistent with this hypothesis, we detected only very low levels of CCK-B mRNA transcripts in RNA from both wild-type and gastrin -/- islets despite experiments using 40 polymerase chain reaction cycles (data not shown).

Glucagon release from rodent alpha cells is directly controlled by epinephrine³² and somatostatin.³³ Interestingly, the secretory behavior of the perfused gastrin -/- islets clearly shows that a moderate defect of glucagon release is present under conditions of alpha-cell stimulation (adrenergic activation in combination with

low glucose level). These data cannot be attributed to a loss of the glucagon storage pool in gastrin $-/-$ islets, because glucagon content in alpha cells was not statistically different in isolated islets or in whole pancreas from wild-type versus gastrin $-/-$ mice. Furthermore, the modest secretory defect observed in vitro is consistent

with the blunted alpha-cell response to insulin-induced hypoglycemia detected in vivo. Hence, the available data clearly show that the murine gastrin gene is an essential component of the islet glucagon response to hypoglycemia. It remains to be determined whether a progastrin-derived peptide is a direct regulator of islet glucagon secretion or perhaps acts indirectly to facilitate the alpha-cell response to hypoglycemia.

The Gastrin Gene and Glucose Homeostasis In Vivo

Although basal levels of fasting glucagon were not markedly different in wild-type versus gastrin $-/-$ mice, circulating glucagon levels might be predicted to be increased in gastrin $-/-$ mice due to the presence of mild hypoglycemia. Evidence for a modest alpha-cell secretory defect in gastrin $-/-$ mice was suggested by a reduced glucagon response to epinephrine in perfused islet experiments and by the deficient glucagon response to insulin-induced hypoglycemia. The defective glucagon response is unlikely due to a generalized inability to sense or respond to hypoglycemia, because levels of circulating insulin and insulin mRNA transcripts were appropriately reduced in gastrin $-/-$ mice and because glucose suppression of glucagon release from isolated islets was well preserved. Furthermore, gastrin $-/-$ mice exhibited a robust epinephrine response to hypoglycemia, consistent with the absence of a generalized defect in the counterregulatory response to hypoglycemia. Nevertheless, we cannot exclude the possibility that gastrin gene disruption may produce subtle developmental abnormalities leading to localized defects in alpha-cell function in the adult mouse.

The genetic determinants of normal glucagon secretion and control of the alpha-cell response to hypogly-

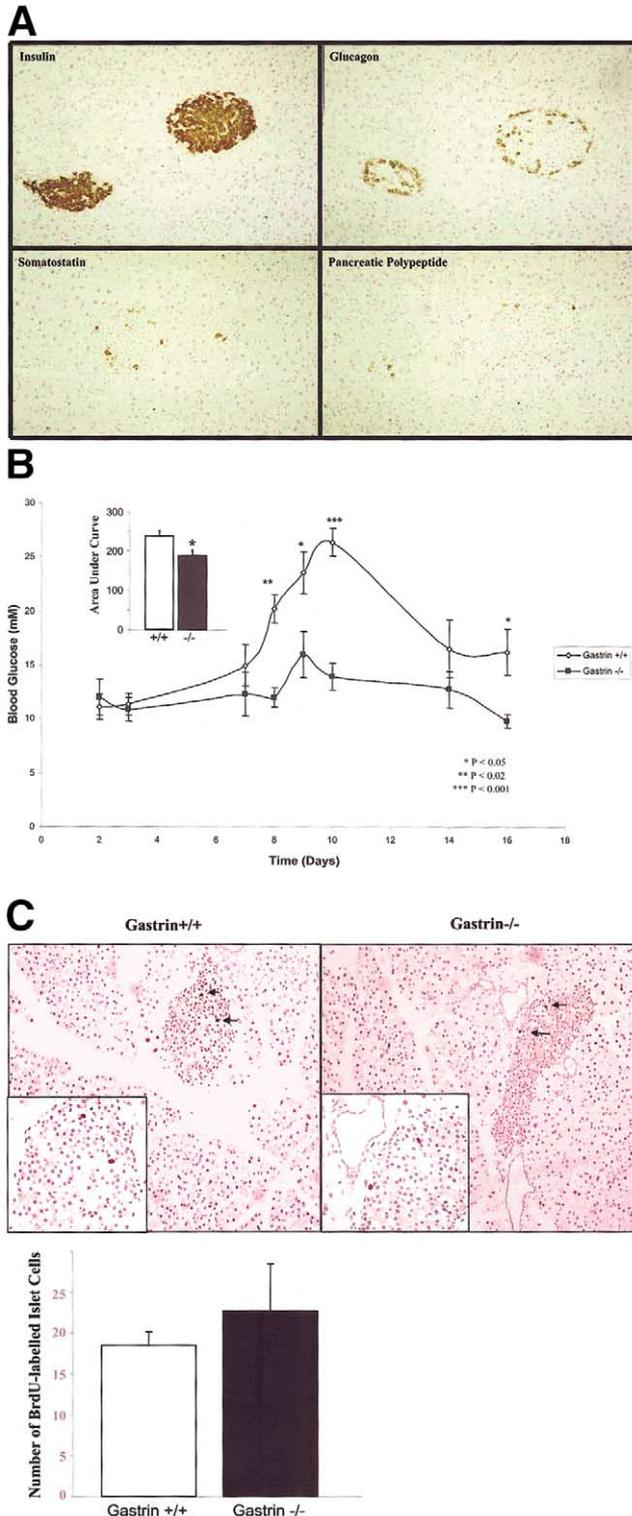


Figure 7. (A) Immunohistochemistry for islet hormones in gastrin $-/-$ pancreas. Pancreatic sections were stained with antisera against insulin, glucagon, somatostatin, and pancreatic polypeptide. The histologic appearance of pancreatic sections and islets from gastrin $+/+$ and gastrin $-/-$ mice at all ages examined was identical; hence, only a single representative panel is shown here from 16-week-old female gastrin $-/-$ mice. (B) Blood glucose level following STZ administration in gastrin $+/+$ and gastrin $-/-$ mice. Random blood glucose level was monitored daily at 1 PM for 2 weeks in ad libitum-fed mice starting 2 days after the final STZ injection. The level of statistical significance for values in gastrin $+/+$ vs. gastrin $-/-$ mice is shown. The area under the curve for the glucose determination is shown in the inset. (C) Islet proliferation in gastrin $+/+$ and gastrin $-/-$ mice treated with STZ. Mice received a single injection of BrdU approximately 24 hours after the final STZ injection; 4 hours later, pancreas was removed for histologic analysis. No significant differences were observed in the number of BrdU-positive cells detected in islets from gastrin $+/+$ vs. gastrin $-/-$ mice. Most BrdU-positive cells were islet beta cells, as assessed by analysis of serial sections stained with antisera against BrdU or insulin.

emia are poorly understood but highly relevant for the treatment of diabetes due to the critical importance of appropriate counterregulatory mechanisms for the defense against hypoglycemia.^{34,35} Mice with a targeted inactivation of the *Foxa1* (*HNF-3 α*) gene exhibit severe hypoglycemia, reduced pancreatic proglucagon mRNA transcripts, and a subnormal glucagon secretory response to hypoglycemia, implicating *Foxa1* as a determinant of both alpha-cell proglucagon gene expression and glucagon secretion.³⁶ Similarly, mice with disruption of the *Kir6.2* ion channel exhibit normal levels of basal glycemia and normal islet glucagon secretion in response to glucose yet subnormal glucagon responses to systemic hypoglycemia or neuroglycopenia,³⁷ strongly suggesting that the *Kir6.2* inward rectifier channel is an essential molecular component for counterregulation in response to hypoglycemia. The data from our studies clearly show that the murine gastrin gene is also an essential component of the normal islet glucagon counterregulatory response to hypoglycemia.

Gastrin, Islet Growth and Development, and the Response to Injury

Hypergastrinemia in human subjects with gastrin-producing tumors has been associated with greatly increased numbers of ducts and increased numbers of islets and endocrine cells scattered within acinar tissue.³⁸ Furthermore, double transgenic mice expressing gastrin and transforming growth factor α transgenes exhibit increased islet mass¹⁴ and diabetic rats treated with gastrin and epidermal growth factor exhibit lower blood glucose levels attributable to beta-cell regeneration.¹⁹ Nevertheless, we did not detect selective defects in the formation of islet cell types in gastrin $-/-$ mice, and the detection of normal islet topography and beta-cell area in gastrin $-/-$ mice is in keeping with the lack of histologic pancreatic abnormalities observed in mice with disruption of either the CCK-A or CCK-B receptors.^{8,22} Furthermore, transgenic overexpression of gastrin alone in murine beta cells does not result in abnormalities of islet growth or differentiation.¹⁴ Similarly, although CCK stimulates insulin³⁹ and glucagon secretion¹³ and CCK-8 has been localized to rat islet beta cells,³⁹⁻⁴¹ pancreatic weight, enzyme content, and cellular morphology are normal in mice with CCK deficiency.⁴² In contrast, rats with a genetic defect in the CCK-A receptor exhibit a reduced capacity for beta-cell proliferation following experimental 70% pancreatectomy.⁴³ Our data showing normal islet cell proliferation following experimental injury with STZ, taken together with normal islet histology and beta-cell mass

in gastrin $-/-$ mice, strongly suggest that the gastrin gene is not essential for islet development or the regenerative response to beta-cell injury. Nevertheless, our findings do not exclude a role for gastrin in islet neogenesis from ductular precursors as suggested by recent studies in gastrin-treated rats with ductal ligation.^{17,18}

Summary

Mice with gastrin gene disruption exhibit mild fasting hypoglycemia, reduced glycemic excursion following glucose challenge, impaired alpha-cell secretory responses to epinephrine, and a defective glucagon response to insulin-induced hypoglycemia. In contrast, islet development and the beta-cell response to injury are normal despite the absence of progastrin-derived peptides. Hence, our data show an essential role for gastrin in glucose homeostasis; however, the gastrin gene is not an essential component of the genetic program directing islet development or beta-cell regeneration.

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