Enhanced Glucose-Dependent Insulinotropic Polypeptide Secretion and Insulinotropic Action in Glucagon-Like Peptide 1 Receptor –/– Mice

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Incretins are gastrointestinal hormones that act on the pancreas to potentiate glucose-stimulated insulin secretion. Despite the physiological importance of the enteroinsular axis, disruption of glucagon-like peptide (GLP)-1 action is associated with only modest glucose intolerance in GLP-1 receptor –/– (GLP-1R –/–) mice. We show here that GLP-1R –/– mice exhibit compensatory changes in the enteroinsular axis via increased glucose-dependent insulinotropic polypeptide (GIP) secretion and enhanced GIP action. Serum GIP levels in GLP-1R –/– mice were significantly elevated versus those in +/+ control mice after an oral glucose tolerance test (369 ± 40 vs. 236 ± 28 pmol/l; $P \leq 0.02$). Furthermore, GIP perfusion of mice pancreas and isolated islets in the presence of elevated glucose concentrations elicited a significantly greater insulin response in GLP-1R –/– than in +/+ mice ($P \leq 0.02$–0.05). In contrast, no significant perturbation in the insulin response to perfused glucagon was detected under conditions of low (4.4 mmol/l) or high (16.6 mmol/l) glucose in GLP-1R –/– mice. Total pancreatic insulin but not glucagon content was significantly reduced in GLP-1R –/– compared with in +/+ mice (77 ± 9 vs. 121 ± 10 pmol/mg protein; $P \leq 0.005$). These observations suggest that upregulation of the GIP component of the enteroinsular axis, at the levels of GIP secretion and action, modifies the phenotype resulting from interruption of the insulinotropic activity of GLP-1 in vivo. *Diabetes* 47:1046–1052, 1998

T he term "enteroinsular axis" refers to the signaling pathways between the gut and pancreatic islets that control nutrient-dependent insulin secretion. The increased insulin response resulting from oral glucose when compared with the response elicited by an isoglycemic intravenous infusion has been called the "incretin effect" (1). Unger and Eisentraut (1) demonstrated that ~50% of postprandial insulin release is triggered by the enteroinsular axis. Two peptides currently considered to be the most important incretin hormones are the glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide (GLP)-1 (2–4). Both GLP-1 and GIP bind to specific receptors on the islet β-cell and stimulate both insulin secretion and proinsulin gene transcription (5,6). In contrast, GLP-1 but not GIP lowers blood glucose via β-cell–independent mechanisms, including inhibition of gastric emptying (7,8) and glucagon secretion (9,10) in vivo.

The observation that both GIP and GLP-1 are synthesized in enteroendocrine cells of the small bowel and secreted after meal ingestion has stimulated considerable interest in the physiology of these peptides and their potential therapeutic use for glucose control. Although the incretin effect appears to be diminished in NIDDM patients, the precise mechanism underlying the diabetes-associated diminution of incretin activity remains unclear (11). Nevertheless, the insulinotropic activity of GLP-1, but not GIP, is preserved in NIDDM patients (12).

The relative importance and physiological potency of GIP and GLP-1 as candidate incretins remain controversial. Acute antagonism of GLP-1 action in rodents has been shown to diminish the incretin effect and increase blood glucose, results consistent with the concept that GLP-1 is a major component of the enteroinsular axis (13,14). In contrast, blockade of the GIP receptor in rats has been associated with a significant reduction in glucose-stimulated insulin secretion, suggesting that GIP may be the dominant incretin in vivo (15).

To understand the relative physiological importance of GLP-1 for long-term glucose control and food intake, we generated mice with a targeted disruption in the GLP-1 receptor (GLP-1R) gene. GLP-1R –/– mice are viable and do not exhibit disturbances in the regulation of feeding or body weight (16), suggesting that GLP-1 signaling is not required for central control of satiety; however, they do exhibit fasting hyperglycemia and defective glucose-stimulated insulin secretion (16), consistent with an essential role for GLP-1 in the control of glucose homeostasis. Because the GLP-1R –/– β-cell should still express functional receptors for glucagon and GIP coupled to stimulation of insulin secretion, we hypothesized that disruption of GLP-1 action might be associated with compensatory changes in the β-cell response to GIP and/or glucagon. We now report that GIP secretion and

GIP (but not glucagon) action at the β-cell level are upregulated in GLP-1R –/– mice.

RESEARCH DESIGN AND METHODS

Animals. The derivation of GLP-1R –/– mice studied in these experiments has been previously described (16). Mice were handled in accordance with ethical guidelines set forth by the University of British Columbia Animal Care Committee, Belgian regulations for animal welfare, and the Division of Comparative Medicine at the University of Toronto. The control age- and sex-matched CD1 mice (ages 5–16 weeks) used in this study were obtained from Charles River (Ontario, Canada). Approximately equal numbers of male and female control and GLP-1R –/– mice were studied using the oral glucose tolerance test (OGTT) and in perfused pancreas experiments, after which the data were pooled. Only male mice were used for analysis of pancreatic hormone content and RNA.

OGTT. Mice were administered glucose orally (1 g/kg via gavage) as a 10% glucose solution after an overnight fast. Groups of mice were rendered unconscious with CO_2 followed by exsanguination by heart puncture at $t = 0, 30$, and 60 min after oral glucose loading.

In vivo perfused pancreas studies. Control (CD1) and GLP-1R –/– mice (ages 8–12 weeks) were fasted for 5 h before pancreas isolation. Mice were then anesthetized with 80 mg/kg sodium pentobarbital administered intraperitoneally. The procedure for surgical isolation and vascular perfusion of the mouse pancreas was identical to that used for the rat (17), except smaller tubing was used for the arterial and portal venous cannulae (PE 50 tubing; Cole-Parmer, Chicago). The perfusate was a modified Krebs-Ringer buffer containing 3% dextran (Sigma, St. Louis, MO) and 0.2% bovine serum albumin (BSA; radioimmunoassay [RIA] grade), gassed with 95% O_2/5% CO_2 to achieve a pH of 7.4. Flow rate of the perfusate was 1 ml/min. Peptides (glucagon, GLP-1, or GIP) were delivered by side arm infusion with a Harvard infusion pump (Model 22; Harvard Apparatus, South Natick, MA) to achieve the appropriate perfusate concentration (17) in the presence of either 4.4 or 16.6 mmol/l glucose. Insulin was measured as previously described (17).

Isolation and culture of mouse pancreatic islets. Islets of Langerhans were isolated from female mice (ages 8–10 weeks) using collagenase P (GmbH Mannheim; Boehringer Mannheim, Indianapolis, IN). The pancreases of the decapitated animals were injected in situ with cold islet isolation medium (0.10 mol/l NaCl, 4.7 mmol/l KCl, 1.8 mmol/l $CaCl_2$, 20 mmol/l $NaHCO_3$, 1.2 mmol/l KH_2PO_4, 1.2 mmol/l $MgSO_4$, 16 mmol/l HEPES, 2.8 mmol/l glucose, and 1% BSA; pH 7.4) and their glands were removed. Pancreases were digested for 30 min at 37°C in a shaking incubator using islet isolation medium containing 2 mg/ml of collagenase, yielding approximately 200 islets per pancreas. After isolation, islets were cultured overnight in HAM F10 medium (Gibco, Strathclyde, U.K.) containing 0.075 mg/ml penicillin, 0.1 mg/ml streptomycin, 0.5% (wt/vol) BSA (fraction V, RIA grade; Sigma), 2 mmol/l glutamine, and 10 mmol/l glucose.

Insulin release from isolated islets. Cultured islets from one GLP-1R –/– pancreas and one GLP-1R +/+ animal were perifused in parallel on Biogel P2 columns (Bio-Rad, Richmond, CA) in HAM F10 medium supplemented with 0.5% BSA, 30 mg % glutamine, 2 mmol/l $CaCl_2$, and 1.4 mmol/l glucose medium equilibrated with 95% O_2/5% CO_2 (18). The flow rate was 0.5 ml/min, and samples were collected every minute and assayed for immunoreactive insulin. The dead space of perifusion was taken into account when expressing results as a function of time. Perifusion conditions were successively 1.4 mmol/l glucose (20 min), 20 mmol/l glucose (30 min), 20 mmol/l glucose together with 10 nmol/l GIP (50 min), and 1.4 mmol/l glucose (20 min). At the end of each perifusion, the islets were extracted in 2 mol/l acetic acid, 0.25% BSA, and assayed for immunoreactive insulin content.

Pancreatic RNA and hormone measurements. The pancreases were carefully dissected from their attachments to the spleen and intestine, homogenized in 3 ml of iced 2 mol/l acetic acid, and boiled for 10 min; the homogenate was then centrifuged. The supernatant was stored at –80° C. Total protein levels in extracts were measured using the Bradford method (19) with Bio-Rad dye reagent according to the manufacturer's instructions. BSA was used as the standard. Values for insulin, glucagon, and somatostatin immunoreactivity were normalized to total protein content in pancreatic extracts. Insulin was measured with an RIA kit (Linco, St. Charles, MO) using rat insulin as the standard. GIP was measured by RIA as previously described (20). The antibody used (LM 34) was supplied by Dr. Linda Morgan (University of Guildford, Surrey, U.K.) and is a specific rabbit anti-porcine GIP serum that cross-reacts completely with mouse GIP (21). Glucose was measured using a Beckman glucose analyzer (Beckman, Fullerton, CA). Glucagon was measured with an RIA kit (Inter Medico; Markham, Ontario, Canada). The sensitivity of the assay was 13 pg/ml; the antisera showed 0% cross-reactivity with GLP-1, GLP-2, and GIP, and 0.1% cross-reactivity for oxyntomodulin. An RIA for total somatostatin-like immunoreactivity was performed, with synthetic somatostatin-14 as a standard, using an antiserum that recognizes somatostatin-14 (pancreatic form) and somatostatin-28 (22,23) equally

(24). RNA and Northern blot analysis, using insulin and 18-s RNA-specific probes, were carried out as previously described (25,26).

Data analysis and statistics. An unpaired Student's t test was used to compare results obtained from control versus GLP-1R –/– mice. $P \le 0.05$ was considered significant.

RESULTS

Oral glucose tolerance test. Plasma glucose levels after oral glucose challenge (Fig. 1A) were significantly elevated at 30 min ($P < 0.05$) in GLP-1R –/– mice, consistent with observations from previous experiments (16). Although fasting GIP levels were comparable in GLP-1R –/– and +/+ mice, plasma GIP levels were significantly elevated in GLP-1R –/– mice at 30 and 60 min after oral glucose challenge ($P \le 0.01$ and $P \le 0.05$, respectively) (Fig. 1B). These observations clearly demonstrated that abrogation of GLP-1 signaling is associated with upregulation of the GIP response to enteral glucose challenge in vivo.

In vitro insulin release from the isolated perfused pancreas and perifused islets

Insulin response to GLP-1. To confirm that GLP-1 was not able to stimulate insulin secretion from the pancreas of GLP-1R –/– mice, thereby excluding effects mediated via a related but distinct receptor, we perfused pancreases with glucose, alone or in combination with GLP-1. Figure 2 indicates that the first-phase insulin release in response to 16.6 mmol/l glucose was similar in GLP-1R –/– and +/+ CD1 mice. However, GLP-

A

B
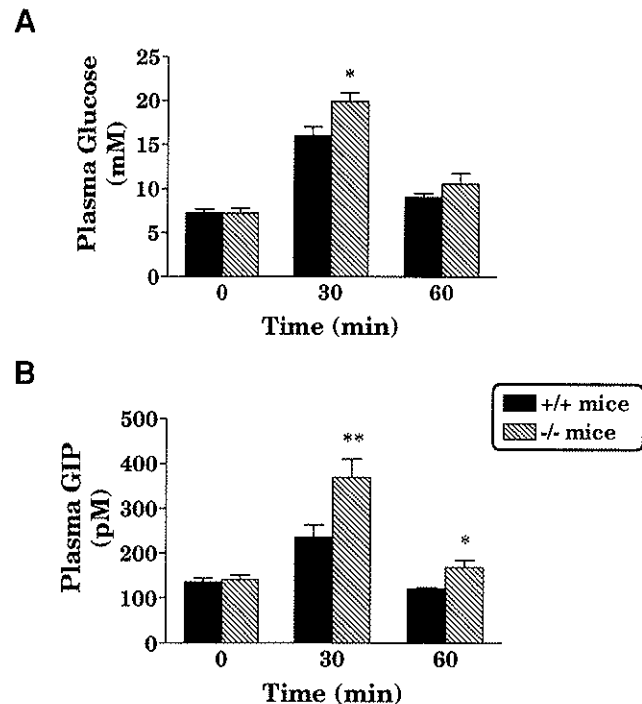
FIG. 1. Glucose tolerance in GLP-1R –/– and CD1 control mice. *A:* Plasma glucose levels after an OGTT in GLP-1R –/– (☒) and control CD1 (■) mice. At 30 min, plasma glucose was 16.0 ± 1.0 mmol/l for CD1 vs. 19.9 ± 1.0 mmol/l for GLP-1R –/– mice. Values are means ± SE. $n = 21$ measurements at 0 and 30 min, and $n = 10$ measurements at 60 min. *$P < 0.05$. *B:* Plasma GIP levels after an OGTT in GLP-1R –/– (☒) and control CD1 (■) mice. At 30 min, plasma GIP levels were 236 ± 28 pmol/l for CD1 ($n = 21$) vs. 369 ± 40 pmol/l for GLP-1R –/– mice ($n = 20$ mice, ages 8–12 weeks; **$P < 0.02$). At 60 min, GIP levels were 120 ± 3 pmol/l for CD1 ($n = 6$) vs. 168 ± 17 pmol/l for GLP-1R –/– mice ($n = 5$ mice, ages 8–12 weeks; *$P < 0.05$).
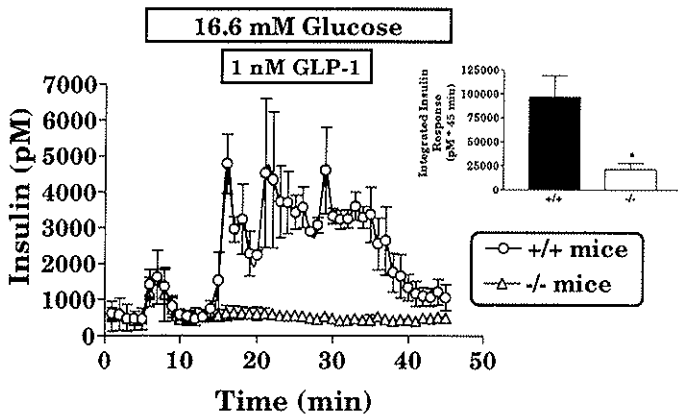
16.6 mM Glucose

1 nM GLP-1

FIG. 2. Insulin secretion from the isolated perfused pancreases in response to GLP-1. The mean integrated response (see inset) for GLP-1R −/− mice was $2.1 \times 10^4 \pm 0.6 \times 10^4$ pmol/l insulin over 45 min versus $9.7 \times 10^4 \pm 2.1 \times 10^4$ pmol/l insulin over 45 min for CD1 +/+ mice (*$P < 0.05$). Values are means ± SE of $n = 4$ for each group.

1 stimulated insulin secretion from the pancreas of control mice, but had no effect after perfusion of GLP-1R −/− animals ($P < 0.05$ for +/+ vs. −/− mice). These results verified the absence of functional GLP-1 receptors coupled to glucose-stimulated insulin secretion in the GLP-1R −/− mouse pancreases.

Insulin response to GIP. To ascertain whether the GLP-1R −/− pancreases exhibited a normal insulin response to GIP, pancreas perfusions were carried out in the presence of 16.6 mmol/l glucose with and without 1 nmol/l GIP. A significantly greater insulin response to GIP perfusion was observed in GLP-1R −/− mice (Fig. 3A). The mean integrated insulin response was 80% greater in GLP-1R −/− mice than in the wild-type controls ($P \leq 0.02$).

To assess whether the increased responsiveness of GIP-stimulated insulin release of perfused GLP-1R −/− pancreases was the result of adaptations that occurred in islet β-cells, we studied GIP-induced insulin release from isolated islets. As seen in Fig. 3B, the GLP-1R −/− islets responded more vigor-

ously to 10 nmol/l GIP than did the GLP-1R+/+ islets that were isolated and perifused in parallel. Consistent with the data from perfused pancreas experiments, the integrated insulin response of GLP-1R −/− islets to GIP and high glucose was 185 ± 34% of the corresponding values obtained from GLP-1R +/+ islets ($P < 0.05$).

Insulin response to glucagon. To clarify whether the insulinotropic effects of glucagon occur independently of the GLP-1 receptor, and to ascertain whether GLP-1R −/− mice also exhibit upregulation of the insulin secretory response to glucagon, pancreas perfusion experiments were carried out in the presence of 1 nmol/l glucagon. No difference in the insulin secretory response to glucagon was detected in the presence of 4.4 mmol/l glucose (Fig. 4A). A more sustained response with a greater amplitude of insulin secretion was observed in the presence of 16.6 mmol/l glucose (Fig. 4B). No significant differences were detected in the integrated insulin response to glucagon in the perfused pancreas of wild-type CD1 +/+ vs. GLP-1R −/− mice at either glucose concentration (Figs. 4A and 4B).

Pancreatic hormone content. The observation that GLP-1 stimulates proinsulin biosynthesis in vitro (5,27), taken together with the upregulation of proinsulin mRNA and insulin content in GLP-1-treated rats in vivo (28), raises the possibility that GLP-1R −/− mice might exhibit abnormalities in proinsulin gene expression and/or proinsulin biosynthesis. Fed GLP-1R −/− mice had significantly lower pancreatic insulin content compared with +/+ controls ($P \leq 0.005$) (Fig. 5A). In contrast, despite the known inhibitory effects of GLP-1 on glucagon secretion and biosynthesis (10,29), pancreatic glucagon content was comparable in GLP-1R −/− and +/+ mice (Fig. 5B). Furthermore, pancreatic somatostatin content was increased in GLP-1R −/− mice ($P \leq 0.001$) (Fig. 5C).

Proinsulin mRNA. To determine whether the decrease in pancreatic insulin content could be partially explained by a reduction in proinsulin gene expression, Northern blot analysis was performed on total cellular pancreatic RNA extracted from fed GLP-1R −/− and +/+ mice. Analysis of postprandial

A

16.6 mM Glucose

1 nM GIP

B

[Glucose] (mM) | 1.4 | 20 | 1.4
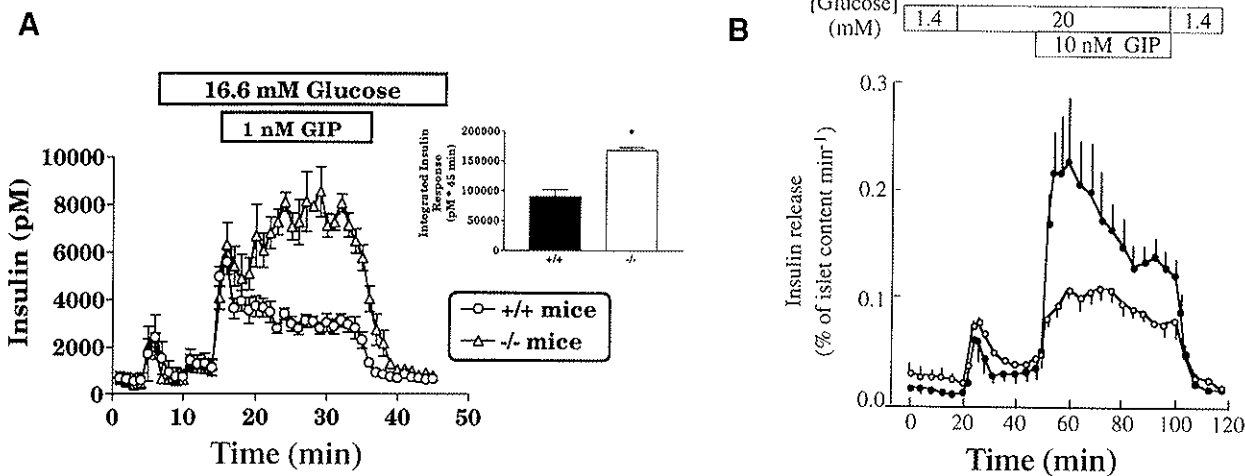
10 nM GIP

FIG. 3. *A:* Insulin secretion from the isolated perfused pancreases in response to GIP. The mean integrated response (see inset) for GLP-1R −/− mice was $1.7 \times 10^5 \pm 0.5 \times 10^4$ pmol/l insulin over 45 min vs. $9.1 \times 10^4 \pm 1.1 \times 10^4$ pmol/l for age- and sex-matched CD1 +/+ controls (*$P \leq 0.005$). Values are means ± SE of $n = 6$ mice for each group, ages 8–10 weeks. *B:* Effect of GIP on glucose-induced insulin release from isolated islets. Islets were stimulated with 20 mmol/l glucose alone or 20 mmol/l glucose plus 10 nmol/l GIP. Data are means ± SE of five experiments from GLP-1R +/+ mice (○) and GLP-1R −/− mice (●). For the sake of graphical clarity, mean symbols and error bars are shown once per four consecutive minute fractions.
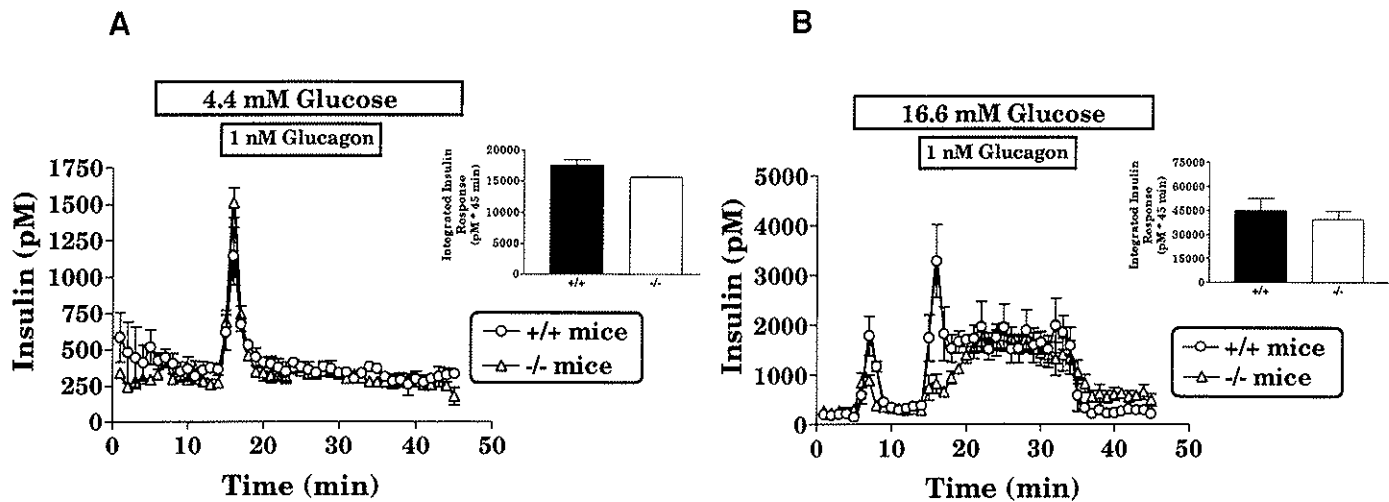
A

B

FIG. 4. Insulin secretion from the isolated perfused pancreas in response to glucagon. Insulin response to 1 nmol/l glucagon in the presence of low (*A*) and high (*B*) glucose. Values are means ± SE of *n* = 4 mice per group, ages 8–10 weeks. *A*: In the presence of 4.4 mmol/l glucose and 1 nmol/l glucagon, the mean integrated insulin response was $1.6 \times 10^4 \pm 0.1 \times 10^3$ pmol/l insulin over 45 min for GLP-1R −/− mice vs. $1.8 \times 10^4 \pm 0.9 \times 10^3$ pmol/l insulin for +/+ controls (*P* > 0.1). *B*: In the presence of 16.6 mmol/l glucose and 1 nmol/l glucagon, the mean integrated insulin response was $3.9 \times 10^4 \pm 0.5 \times 10^4$ pmol/l insulin over 45 min for GLP-1R −/− mice vs. $4.5 \times 10^4 \pm 0.7 \times 10^4$ pmol/l insulin for +/+ controls (*P* > 0.5).

proinsulin gene expression revealed a significant decrease in the levels of proinsulin mRNA transcripts in GLP-1R −/− mice (*P* < 0.002 for GLP-1R −/− vs. +/+ mice) (Fig. 6).

DISCUSSION

GLP-1 controls blood glucose via multiple effects on both the endocrine pancreas and peripheral tissues. The actions of GLP-1 on gastric emptying, inhibition of glucagon secretion, and central nervous system control of food intake are distinct yet complementary, in that the net effect of these biological activities is to lower blood glucose in vivo. At the present time, the only known receptor that transduces the actions of GLP-1 at physiologically relevant concentrations is the GLP-1 receptor, originally cloned from pancreatic islets (30,31). Although the GLP-1R is expressed in peripheral extrapancreatic sites, such as the lung, heart, gastrointestinal tract, and central nervous system (30–34), the expression of this receptor in muscle, liver, and adipocytes and the putative effects of GLP-1 on glucose uptake and glycogen synthesis in these organs (35) remain controversial.

The observation that GLP-1R −/− mice exhibited glucose intolerance after oral glucose challenge clearly demonstrated that GLP-1 is essential for blood glucose control in vivo (16). Furthermore, despite potential redundancy in the multiple molecular mechanisms important for control of glucose-stimulated insulin secretion, the β-cell in GLP-1R −/− mice could not sufficiently augment insulin secretion to control the glycemic excursion that ensued after enteral glucose loading. Nevertheless, glucose intolerance in GLP-1R −/− mice was moderate, suggesting that upregulation of one or more components of the enteroinsular axis may partially compensate for the lack of insulinotropic GLP-1 action in vivo. Analysis of GLP-1R −/− mice has indicated that fasting glucagon levels and glucagon secretion in response to oral glucose challenge are not perturbed in the absence of GLP-1 signaling in vivo (36). In contrast, the results presented here clearly demonstrate that GIP secretion and action are enhanced in GLP-1R −/− mice, changes that likely compensate in part for absence of GLP-1

action at the β-cell. Nevertheless, despite the enhanced release and action of GIP in the GLP-1R −/− mouse, the GIP compensation is not sufficient to restore glucose-stimulated insulin secretion to normal levels, thus further emphasizing the importance of GLP-1 as a physiologically essential incretin.

The finding that glucose-stimulated levels of GIP are elevated to a greater degree in GLP-1R −/− mice enhances our understanding of the interconnection between GIP and GLP-1 secretion pathways in the intestine. GLP-1 secretion and biosynthesis are both stimulated by GIP in rat intestinal cell cultures and perfused rat intestine (37,38). These observations have defined the existence of a proximal-distal endocrine loop, whereby nutrient stimulation of GIP release from the duodenum and proximal jejunum further enhances GLP-1 release from the ileum (39). The observation that glucose-induced GIP secretion is enhanced in GLP-1R −/− mice suggests that the activity of the intestinal GIP-secreting K-cell is upregulated after disruption of GLP-1 action; however, the mechanism underlying increased K-cell secretion remains unknown. Although GLP-1 receptor expression has been localized to the rodent intestine (32), including the crypt region of the duodenum (34), the specific intestinal cell types that express GLP-1 receptors have not yet been identified. Accordingly, whether GLP-1 acts directly on the intestinal K-cell to regulate GIP secretion remains unknown.

Alternatively, enhanced GIP release in GLP-1R −/− mice might be secondary to the reduced insulin response that follows enteric glucose challenge. We previously demonstrated that insulin reduces glucose-dependent GIP secretion in the rat (40). Furthermore, the results of several additional studies in rodents and humans have raised the possibility that GIP secretion may be regulated in part by insulin (41,42). Accordingly, the enhanced GIP secretion in our experiments may have resulted in part from defective feedback inhibition of insulin-regulated GIP release in the GLP-1R −/− mouse. A complementary explanation for enhanced GIP secretion derives from observations of GLP-1–mediated regulation of gastric emptying (7). It is interesting that the relative magni-
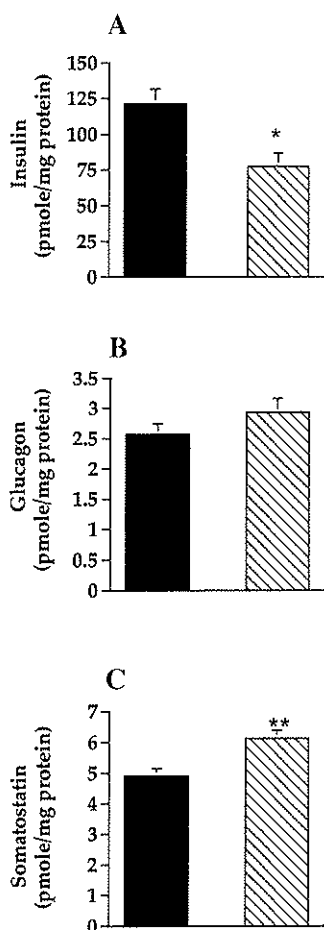
A

B

C

FIG. 5. Pancreatic hormone content: insulin (*A*), glucagon (*B*), and somatostatin (*C*) content of GLP-1R –/– (▨) and CD1 (■) mice. Hormone levels are expressed as picomoles per milligram protein. Values are means ± SE of n = 36 mice for each group, ages 12–14 weeks. *A*: Total pancreatic insulin content was significantly reduced in GLP-1R –/– mice compared with CD1 +/+ controls (77.4 ± 9.1 vs. 121.3 ± 10.3 pmol/mg protein, respectively; **P < 0.005). *B*: Total pancreatic glucagon was not significantly different between the two groups: 2.8 ± 0.2 vs. 2.6 ± 0.2 pmol/mg protein for GLP-1R –/– and CD1 mice, respectively (P = 0.4). *C*: Total pancreatic somatostatin content was slightly elevated in GLP-1R –/– mice: 4.9 ± 0.2 vs. 6.1 ± 0.3 pmol/mg protein in GLP-1R –/– vs. CD1 mice, respectively (*P < 0.001).
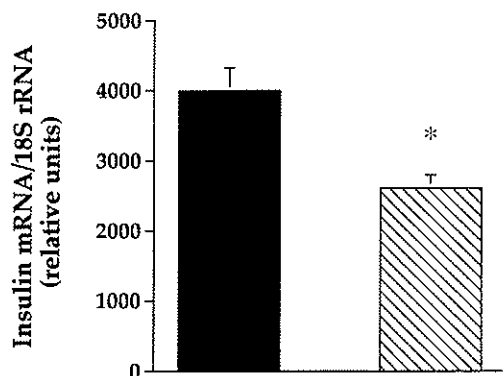

FIG. 6. Northern blot analysis of whole pancreatic insulin mRNA. Relative densitometric units for proinsulin and 18-s RNA signals from Northern blot analysis of pancreatic RNA isolated from fed GLP-1R –/– (▨) and age- and sex-matched control +/+ (■) mice; n = 12 per group. *P < 0.002.

tude of GIP, but not GLP-1, release appears to be greater after intraduodenal glucose perfusion compared with oral glucose loading in normal human subjects (43). It thus remains theoretically possible that increased transit of and more rapid exposure to glucose in the duodenum and proximal jejunum of GLP-1R –/– mice might augment the amplitude of the K-cell secretory response, leading to increased GIP secretion in vivo.

The insulin response to GIP in perfused pancreas studies of GLP-1R –/– mice was almost double that of +/+ controls (Fig. 3*A*). The fact that similar data were obtained from studies of isolated islets (Fig. 3*B*) indicates that the increased GIP-responsiveness of pancreases from GLP-1R –/– mice is likely due to changes in GIP signaling in pancreatic β-cells, rather than changes in pancreatic blood flow or innervation. It thus appears that the β-cell, located in its normal islet environment or dispersed in vitro, compensates in part for the disruption of GLP-1 signaling by upregulating insulin secretion in response to the second major component of the enteroinsular axis, GIP. It is intriguing that the total cumulative integrated amount of insulin secreted after glucose perfusion with GIP and GLP-1 in separate analyses of wild-type and GLP-1R –/– mice was similar. This observation raises the possibility that the maximal β-cell response to incretin stimulation may be regulated at a level distal to incretin receptor signaling. Because both the GLP-1 and GIP receptors are coupled to cAMP-dependent signaling pathways in the β-cell (44), disruption of GLP-1 receptor signaling may be associated with a new enhanced threshold for the glucose-dependent response to GIP receptor activation. The specific mechanisms that might account for enhanced β-cell GIP sensitivity remain unknown, but may include increased GIP receptor expression and/or signaling. These components of GIP receptor function in GLP-1R –/– mice clearly merit further investigation.

Although the insulinotropic actions of glucagon are well documented (45,46), there has been some controversy as to whether these actions are mediated via glucagon or GLP-1 receptors on the β-cell (47). The finding of specific glucagon receptors on the β-cell (44,48) and marked differences in the minimally effective insulinotropic concentrations of glucagon and GLP-1 (44) strongly suggest that these peptides stimulate insulin secretion by binding to their own structurally and functionally distinct receptors. This hypothesis is further strengthened by our finding (Fig. 4) that glucagon stimulation of insulin secretion is comparable in the perfused pancreases of GLP-1R –/– and +/+ mice.

Because GLP-1 stimulates proinsulin gene expression—likely via a direct effect on insulin gene transcription (5,27)—we hypothesized that disruption of β-cell GLP-1 signaling might be associated with reduced levels of pancreatic proinsulin gene expression. The reduced levels of postprandial proinsulin mRNA transcripts and insulin content in fed GLP-1R –/– mice were in keeping with a role for GLP-1 in the regulation of proinsulin biosynthesis in vivo. Consistent with these findings, administration of GLP-1 to Wistar rats for 48 h reversed the age-related deterioration in glucose tolerance and increased both islet insulin content and pancreatic proinsulin mRNA (28). The mechanism underlying the small but significant increase in pancreatic somatostatin in GLP-1R –/– mice is not clear. Nevertheless, insulin has been shown to suppress somatostatin release (49), whereas GIP has been shown to stimulate somatostatin release (50). Accord-

ingly, the decreased levels of glucose-stimulated circulating insulin and the increased levels of GIP may contribute to increased pancreatic somatostatin in GLP-1R –/– mice.

In summary, our experiments illustrated that disruption of the GLP-1 component of the enteroinsular axis in mice results in multiple β-cell abnormalities, including defective glucose-induced insulin secretion, reduced proinsulin gene expression, and enhanced sensitivity to GIP. Taken together with the finding of increased glucose-stimulated GIP secretion, our findings provide new evidence for plasticity in the enteroinsular axis, suggesting that in the mouse, the lack of GLP-1 action is compensated for, in part, by upregulation of the GIP-insulin axis. Future studies addressing the mechanisms underlying compensatory changes in GIP secretion and action may increase our understanding of the factors important for the physiological regulation of the enteroinsular axis in vivo.

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