

Glucagon-Like Peptide-1, But Not Glucose-Dependent Insulinotropic Peptide, Regulates Fasting Glycemia and Nonenteral Glucose Clearance in Mice*

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ABSTRACT

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) potentiate glucose-stimulated insulin secretion after enteral nutrient ingestion. We compared the relative incretin and nonincretin actions of GLP-1 and GIP in +/+ and GLP-1R^{-/-} mice using exendin(9–39) and immunopurified anti-GIP receptor antisera (GIPR Ab) to antagonize GLP-1 and GIP action, respectively. Both antagonists produced a significant increase in glycemic excursion after oral glucose loading of +/+ mice ($P < 0.05$ for antagonists vs. controls). Exendin(9–39) also increased blood glucose and decreased glucose-stimulated insulin in +/+ mice after ip glucose loading [0.58 ± 0.02 vs. 0.47 ± 0.02 ng/ml in saline- vs. exendin(9–39)-

treated mice, respectively, $P < 0.05$]. In contrast, GIPR Ab had no effect on glucose excursion or insulin secretion, after ip glucose challenge, in +/+ or GLP-1R^{-/-} mice. Repeated administration of exendin(9–39) significantly increased blood glucose and reduced circulating insulin levels but had no effect on levels of pancreatic insulin or insulin messenger RNA transcripts. In contrast, no changes in plasma glucose, circulating insulin, pancreatic insulin content, or insulin messenger RNA were observed in mice, 18 h after administration of GIPR Ab. These findings demonstrate that GLP-1, but not GIP, plays an essential role in regulating glycemia, independent of enteral nutrient ingestion in mice *in vivo*. (Endocrinology 141: 3703–3709, 2000)

THE OBSERVATION, that oral glucose administration stimulates a greater increase in insulin secretion from pancreatic β -cells than an isoglycemic iv infusion, has stimulated considerable interest in the identity of gut-derived molecules that enhance insulin secretion. The term incretin has been ascribed to factors released from the gut in response to nutrient ingestion that potentiate glucose-stimulated insulin secretion (1). To date, the two principal peptides that exhibit incretin-like activity are glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). Together these two peptides are thought to account for most, if not all, of the incretin effect (2–4).

GIP is a 42-amino acid peptide that is synthesized in intestinal K cells in the proximal jejunum and secreted primarily in response to the ingestion of glucose or fat (3, 4). In contrast, GLP-1 is a posttranslational product of the proglucagon gene (5) and is released from more distally located intestinal L cells in response to ingestion of glucose or a mixed meal (4, 6–8). Under conditions of elevated blood glucose concentrations, both GIP and GLP-1 stimulate insu-

lin secretion and proinsulin gene transcription via specific receptors expressed on islet β -cells (9–11).

The principle action of GIP seems to be the stimulation of glucose-dependent insulin secretion after enteral nutrient ingestion. Consistent with this hypothesis, GIP immuno-neutralizing antisera or a GIP peptide antagonist reduced insulin secretion after oral glucose challenge in rats (12, 13). The biological importance of GIP as an incretin is further illustrated by GIP receptor $-/-$ mice that exhibit defective glucose clearance after oral glucose loading; but normal fasting glucose and glycemic excursion, after ip glucose challenge (14). In contrast, GLP-1R^{-/-} mice exhibit fasting hyperglycemia and abnormal glycemic excursion in response to both oral and ip glucose challenge (15).

Although results of studies in knockout mice may be used to infer specific physiological actions of GIP and GLP-1 for control of glucose homeostasis, disruption of incretin receptor signaling from birth may be associated with subtle developmental and adaptive changes that could modify the interpretation of physiological studies. For example, GLP-1R^{-/-} mice exhibit abnormalities in the hypothalamic-pituitary-adrenal axis (16) and up-regulation of glucose-dependent GIP secretion and enhanced sensitivity to GIP action (17), complicating the interpretation of results ascribed simply to interruption of GLP-1R signaling *in vivo*. Accordingly, to control for potential confounding developmental or adaptive changes in incretin action observed in genetically modified mice, we have assessed the importance of GIP and GLP-1 for glycemic control in +/+ and GLP-1R^{-/-} mice using antagonists of GLP-1 and GIP action *in vivo*.

Received May 4, 2000.

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* This work was supported, in part, by operating grants from the Canadian Diabetes Association (to D.J.D. and T.J.K.) and Juvenile Diabetes Foundation International (to D.J.D.).

† Supported by a doctoral research award from the Medical Research Council of Canada.

‡ A CDA and Alberta Heritage Foundation for Medical Research Scholar.

§ A Senior Scientist of the Medical Research Council of Canada.

Materials and Methods

Animals

GLP-1 receptor $-/-$ (15) and age-matched (6- to 8-week-old males) wild-type CD1 mice (Charles River Laboratories, Inc., Montréal, Québec, Canada) were housed under a 12-h light, 12-h dark cycle in the Toronto General Hospital Animal facility, with free access to food (standard rodent chow) and water, except where noted. All $+/+$ mice used for these studies were acclimated to the animal facility for several weeks before analysis. All procedures were conducted according to protocols and guidelines approved by the Toronto General Hospital Animal Care Committee.

Glucose tolerance tests and measurement of plasma insulin levels

Oral glucose tolerance tests (OGTTs) or ip glucose tolerance tests (IPGTTs) were carried out after an overnight fast (16–18 h). The GLP-1 receptor antagonist exendin(9–39)-NH₂ (5 μ g; California Peptide Research Inc., Napa, CA) or PBS was administered ip 20 min before glucose administration, after a fasting blood glucose measurement had been obtained. Anti-GIPR Ab antiserum was raised in rabbits against a synthetic peptide containing an extracellular epitope of the GIP receptor GQTT-GELYQRWERYGWEC coupled to KLH. Immunopurified antibody (GIPR Ab) specifically displaces ¹²⁵I-GIP binding with half-maximal displacement at approximately 1 μ g/ml (data not shown). In the rat, plasma levels of GIPR Ab peak approximately 4 h after ip injection and remain at this level for 2 days. When delivered at a dose of 1 μ g/g body weight, the insulinotropic action of an exogenous bolus of GIP is completely abolished (data not shown). Immunopurified antisera [1 μ g/g body weight; GIPR Ab (18)] or a rabbit γ -globulin control (1 μ g/g body weight; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) was given ip at the onset of fasting, 16–18 h before the glucose tolerance tests. For glucose tolerance tests, mice were given 1.5 mg glucose/g body weight orally through a gavage tube (OGTT) or via injection into the peritoneal cavity (IPGTT). Blood was drawn from a tail vein at 0, 10, 20, 30, 60, 90, and 120 min after glucose administration; and blood glucose levels were measured by the glucose oxidase method using a One Touch Basic Glucometer (Lifescan Ltd., Burnaby, British Columbia, Canada). Blood samples (100 μ l) for measurement of insulin secretion were removed from tail veins during the 10- to 20-min time period after oral or ip glucose administration. Plasma was assayed for insulin content using a rat insulin enzyme-linked immunosorbent assay kit (Crystal Chem Inc., Chicago, IL) with mouse insulin as a standard.

Chronic exposure to incretin antagonists

For chronic studies, all mice were given free access to standard rodent chow and water. Wild-type CD1 and GLP-1 receptor $-/-$ mice were given ip injections of either PBS or 5 μ g of exendin(9–39) in 8% gelatin. Injections were commenced at 0500 h, and each animal was given an ip injection of the appropriate test substance every 4 h, with the last injection given 3 h before death, for a total of 15 h of treatment. Blood glucose levels were measured, animals were killed, and blood was obtained by cardiac puncture. Plasma was collected for analysis of insulin levels (as described above). The pancreas was removed from each animal. One portion was used for RNA isolation and Northern blot analysis. The remaining portion of the pancreas was homogenized twice in 5 ml of extraction medium [1 N HCl containing 5% (vol/vol) formic acid, 1% (vol/vol) trifluoroacetic acid, and 1% (wt/vol) NaCl] at 4 C. Peptides and small proteins were adsorbed from extracts by passage through a C18 silica cartridge (Waters Associates, Milford, MA). Adsorbed peptides were eluted with 4 ml of 80% (vol/vol) isopropanol containing 0.1% (vol/vol) trifluoroacetic acid. Pancreatic insulin levels were measured using a rat insulin enzyme-linked immunosorbent assay kit (Crystal Chem Inc.), with mouse insulin as a standard. Total protein levels in extracts were determined using the Bradford method (19) with dye reagent (Bio-Rad Laboratories, Inc., Hercules, CA). For chronic studies with GIPR Ab, wild-type CD1 and GLP-1 receptor $-/-$ mice were given ip injections of either rabbit γ -globulin or 1 μ g/g body weight of purified GIPR Ab. Only a single injection of GIPR Ab was required, because the antibody is stable in plasma for several days (18). At 18 h after administration of GIPR Ab or γ -globulin control, mice were

anesthetized with CO₂ and exsanguinated by cardiac puncture. Blood glucose, plasma insulin, and pancreatic insulin content were determined as described above.

RNA isolation and Northern blot analysis

After chronic exposure to PBS, exendin(9–39), rabbit γ -globulin, or GIPR Ab, mice were anesthetized with CO₂, and pancreases were removed immediately for RNA extraction by the acid-guanidinium isothiocyanate method (20). Total RNA (10 μ g) was electrophoresed in a 1% (wt/vol) formaldehyde-agarose gel and transferred to a nylon membrane (Nytran Plus; Schleicher & Schuell, Inc., Keene, NH). For Northern blot analysis, the blot was hybridized to ³²P-labeled random-primed complementary DNA probes corresponding to rat proglucagon, rat insulin, or 18S rRNA.

Statistics

Results are expressed as means \pm SEM. Statistical significance was calculated by ANOVA and Student's *t* test using INSTAT 1.12 (GraphPad Software, Inc., San Diego, CA). A *P* value < 0.05 was considered to be statistically significant.

Results

Effects of exendin(9–39)NH₂ on blood glucose and plasma insulin

To assess the effects of acute blockade of GLP-1R signaling *in vivo*, we used the GLP-1 receptor antagonist exendin(9–39)-NH₂, a truncated lizard GLP-1-related peptide that binds to and antagonizes mammalian GLP-1 receptors (21). Treatment of wild-type mice with exendin(9–39) immediately before oral glucose challenge produced a significant increase in blood glucose excursion during the 10- to 30-min time period after glucose administration [Fig. 1A; *P* < 0.05 for saline *vs.* exendin(9–39)-treated mice]. Surprisingly, plasma insulin levels were not significantly different, after oral glucose loading, in saline *vs.* exendin(9–39)-treated mice (Fig. 1B).

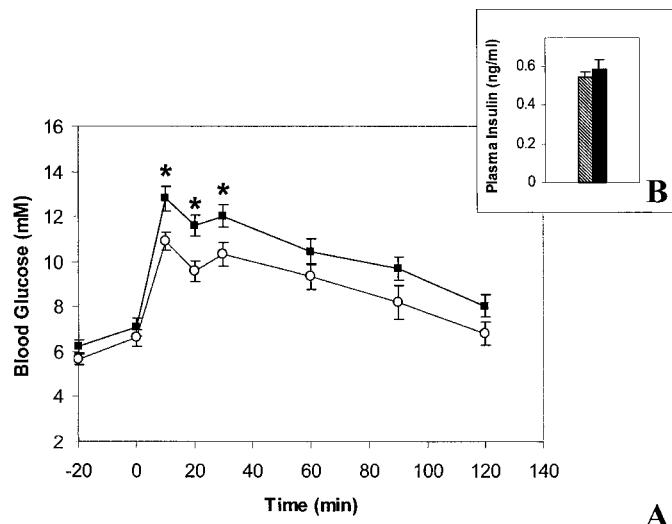


FIG. 1. Oral glucose tolerance and plasma insulin levels in wild-type mice receiving either saline or 5 μ g exendin(9–39)-NH₂. Values are expressed as means \pm SEM; $n = 11$ –12 mice/group; *, $P < 0.05$ *vs.* control (saline). A, OGTT in saline- (open circles) or exendin(9–39)-treated (solid squares) wild-type $+/+$ males; B, plasma insulin concentration at the 10- to 20-min time period, after oral glucose administration, in saline- (hatched bars) or exendin(9–39)-treated (solid bar) wild-type $+/+$ males.

Because GLP-1R^{-/-} mice exhibit abnormal glycemic excursion after both oral and ip glucose challenge, these findings suggest that GLP-1-mediated signaling events are important for β -cell function and glucose disposal independent of the site of glucose entry (15). Consistent with the importance of nonincretin actions of GLP-1 for glucoregulation, exendin(9–39) significantly increased glucose excursion after ip glucose challenge ($P < 0.05$, saline vs. exendin(9–39)-treated mice, from 30–120 min; Fig. 2A). Furthermore, the levels of glucose-stimulated circulating insulin were significantly reduced in exendin(9–39)-treated mice (Fig. 2B; $P < 0.05$; 0.58 ± 0.02 vs. 0.47 ± 0.02 ng/ml in saline- vs. exendin(9–39)-treated mice, respectively).

Although exendin(9–39) is generally viewed as a specific GLP-1 receptor antagonist, several reports suggest that exendin(9–39) may also bind to the GIP receptor and potentially antagonize the actions of GIP (22, 23). To verify that exendin(9–39) is a specific antagonist of murine GLP-1 receptor signaling *in vivo*, we assessed the effect of exendin(9–39) on glycemic excursion in mice with a targeted disruption of the gene encoding the GLP-1 receptor (15). Treatment of GLP-1R^{-/-} mice with exendin(9–39) had no effect on glycemic excursion after oral or ip glucose loading (Fig. 3, A and B), demonstrating the specificity of exendin(9–39) for GLP-1R receptor-mediated glucose clearance at the dose employed here *in vivo*.

To ascertain the contribution of GIP action to glucose disposal after glucose loading in mice, we used immunoneutralizing antisera directed against the GIP receptor (GIPR Ab). Administration of GIPR Ab to +/+ mice before oral glucose challenge led to no change in fasting glucose, but a significant increase in blood glucose was detected at the 10-min time point of an OGTT (Fig. 4A; 13.1 ± 0.6 vs. 10.8 ± 0.5 mM in GIPR Ab-treated vs. control +/+ mice, respectively, $P < 0.05$). The increase in blood glucose was associated with a small but nonsignificant increase in plasma insulin (Fig. 4B). In contrast, treatment of GLP-1R^{-/-} mice with GIPR Ab produced a significant increase in blood glucose (Fig. 5A; 10.4 ± 0.8 vs. 8.3 ± 0.5 mM in GIPR Ab vs. control-treated mice, $P < 0.05$) and a significant reduction in levels

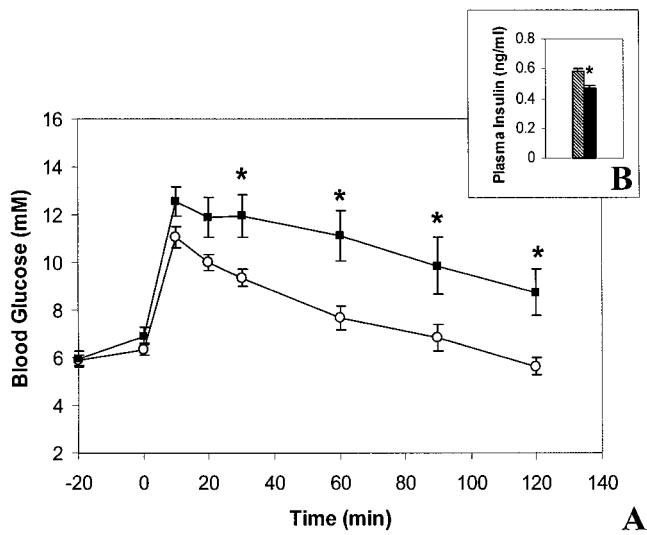


FIG. 2. Ip glucose tolerance and plasma insulin levels in wild-type mice receiving either saline or $5 \mu\text{g}$ exendin(9–39)-NH₂. Values are expressed as means \pm SEM; $n = 12$ –13 mice/group; *, $P < 0.05$ vs. control (saline). A, IPGTT in saline- (open circles) or exendin(9–39)-treated (solid squares) wild-type +/+ males; B, plasma insulin concentration at the 10- to 20-min time period, after ip glucose administration, in saline- (hatched bars) or exendin(9–39)-treated (solid bar) wild-type +/+ males.

± 0.5 mM in GIPR Ab-treated vs. control +/+ mice, respectively, $P < 0.05$). The increase in blood glucose was associated with a small but nonsignificant increase in plasma insulin (Fig. 4B). In contrast, treatment of GLP-1R^{-/-} mice with GIPR Ab produced a significant increase in blood glucose (Fig. 5A; 10.4 ± 0.8 vs. 8.3 ± 0.5 mM in GIPR Ab vs. control-treated mice, $P < 0.05$) and a significant reduction in levels

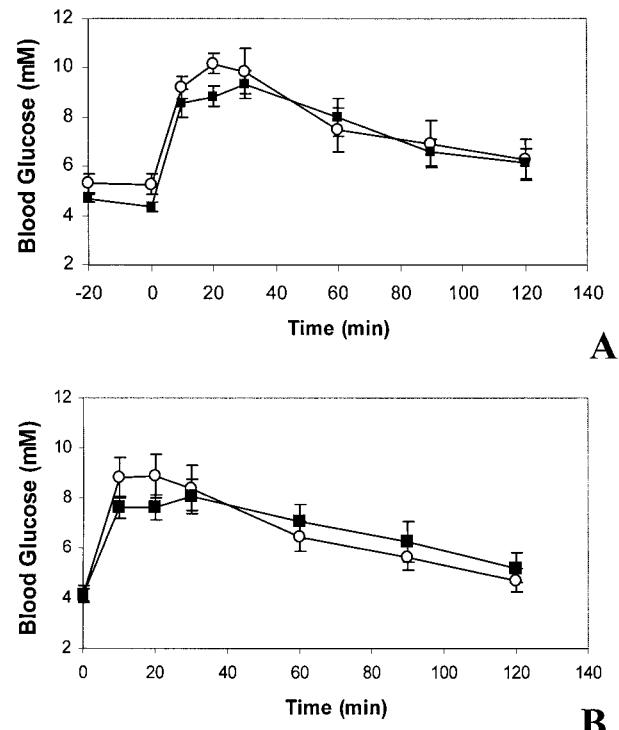


FIG. 3. OGTTs and IPGTTs in GLP-1R^{-/-} mice receiving either saline (open circles) or $5 \mu\text{g}$ exendin(9–39)-NH₂ (solid squares). Values are expressed as means \pm SEM; $n = 5$ –6 mice/group. A, OGTT in saline- or exendin(9–39)-treated GLP-1R^{-/-} males; B, IPGTT in saline- or exendin(9–39)-treated GLP-1R^{-/-} males.

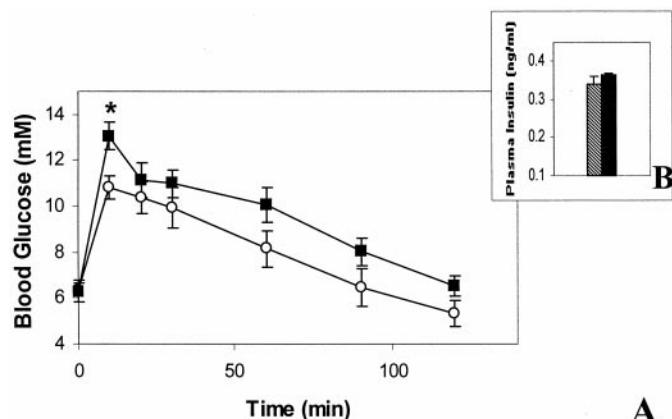


FIG. 4. Oral glucose tolerance and plasma insulin levels in wild-type mice receiving either $1 \mu\text{g/g}$ body wt rabbit γ -globulin or $1 \mu\text{g/g}$ body wt GIPR Ab. Values are expressed as means \pm SEM; $n = 8$ –10 mice/group. A, OGTT in rabbit γ -globulin- (open circles) or GIPR Ab-treated (solid squares) wild-type +/+ males. *, $P < 0.05$ vs. control (rabbit γ -globulin). B, Plasma insulin concentration at the 10- to 20-min time period, after oral glucose administration, in rabbit γ -globulin- (hatched bars) or GIPR Ab-treated (solid bar) wild-type +/+ males.

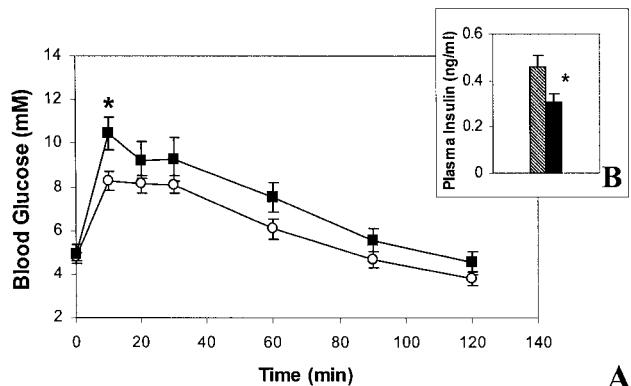


FIG. 5. Oral glucose tolerance and plasma insulin levels in GLP-1R^{-/-} mice receiving either 1 μ g/g body wt rabbit γ -globulin or 1 μ g/g body wt GIPR Ab. Values are expressed as means \pm SEM; n = 7–11 mice/group; *, P < 0.05 vs. control (rabbit γ -globulin). A, OGTT in rabbit γ -globulin- (open circles) or GIPR Ab-treated (solid squares) GLP-1 R^{-/-} males; B, plasma insulin concentration at the 10- to 20-min time period, after oral glucose administration, in rabbit γ -globulin- (hatched bars) or GIPR Ab-treated GLP-1R^{-/-} males (solid bar).

of glucose-stimulated insulin (Fig. 5B, $P < 0.05$; 0.31 ± 0.03 vs. 0.46 ± 0.05 ng/ml for mice receiving GIPR Ab vs. rabbit γ -globulin, respectively).

In contrast to the significant increase in blood glucose observed after ip glucose loading and treatment with exendin(9–39), administration of GIPR Ab had no effect on glucose clearance or plasma insulin after ip glucose loading in +/+ or GLP-1R^{-/-} mice (data not shown).

The results of these experiments demonstrated that acute antagonism of GLP-1 or GIP action produces differential effects on glycemic excursion after oral vs. ip glucose loading. Because both GLP-1 and GIP have been postulated to regulate glycemia, in part through effects on insulin biosynthesis at the level of insulin gene transcription (9–11), we examined the effects of administering either exendin(9–39) or GIPR Ab on glucose control, over a more prolonged 18-h time period. Repeated administration of exendin(9–39) to +/+ mice produced a significant elevation in blood glucose (Fig. 6A, $P < 0.05$; 7.1 ± 0.4 vs. 8.8 ± 0.4 mM for saline vs. exendin(9–39)-treated mice) and a significant reduction in the levels of plasma insulin (Fig. 6B, $P < 0.05$; 2.4 ± 0.1 vs. 1.7 ± 0.2 ng/ml for saline vs. exendin(9–39)-treated mice). Comparable treatment of GLP-1R^{-/-} mice with repeated injections of exendin(9–39) had no effect on either blood glucose or plasma insulin (Fig. 6, C and D). In contrast to changes in glucose and insulin in mice treated with repeated administration of exendin(9–39), no significant perturbation of blood glucose or plasma insulin levels was observed in +/+ or GLP-1R^{-/-} mice, 18 h after administration of GIPR Ab (Fig. 7, A–D).

Despite the postulated importance of GLP-1R signaling for insulin gene transcription, no significant alterations in the levels of insulin (or proglucagon) messenger RNA (mRNA) transcripts (Fig. 9, A and C) or pancreatic insulin content (Fig. 8) were detected, after repeated treatment with exendin(9–39), in either +/+ or GLP-1R^{-/-} mice. Similarly, no significant changes in the levels of proinsulin RNA or insulin

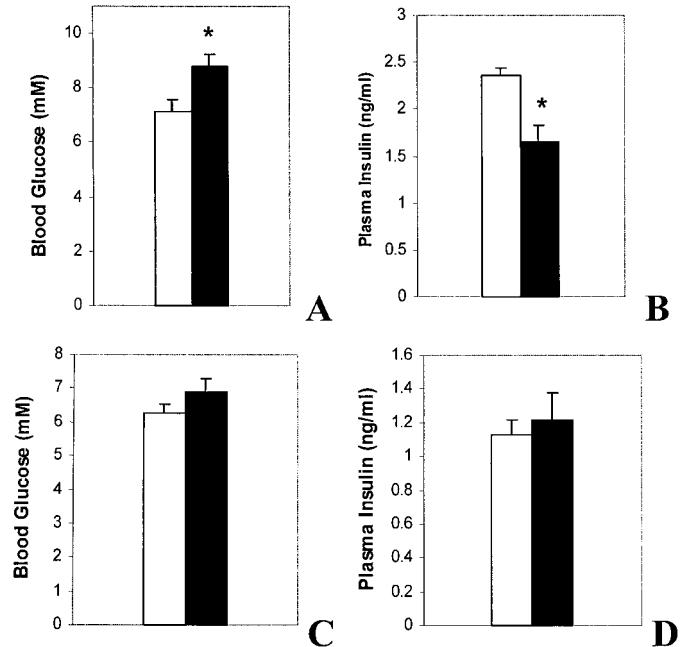


FIG. 6. Blood glucose and plasma insulin levels in wild-type +/+ and GLP-1 R^{-/-} mice treated chronically with saline or exendin(9–39). A, Blood glucose level in +/+ males treated with saline (open bar) or exendin (9–39; solid bar). Values are expressed as means \pm SEM; n = 8–9 mice/group; *, P < 0.05 vs. control (saline). B, Plasma insulin concentration in +/+ males treated with saline (open bar) or exendin (9–39; solid bar); *, P < 0.05 vs. control (saline). C, Blood glucose level in GLP-1 R^{-/-} males treated with saline (open bars) or exendin (9–39; solid bars). Values are expressed as means \pm SEM; n = 4 mice/group. D, Plasma insulin concentration in R^{-/-} males treated with saline (open bars) or exendin (9–39; solid bar). Mice treated with repeated injections were analyzed in the nonfasting state, as described in Materials and Methods.

content were detected in the pancreas of mice treated with GIPR Ab (Figs. 9, B and C; and 8B).

Discussion

Although originally identified as an incretin, GLP-1 has subsequently been shown to exhibit multiple nonincretin actions, including inhibition of glucagon secretion (24, 25) and gastric emptying (26, 27). GLP-1 also confers glucose sensitivity to glucose-resistant β -cells (28) and may also increase insulin-independent glucose disposal in peripheral tissues (29). Taken together with effects on reduction in food intake (30, 31), it seems that GLP-1 exerts both incretin and nonincretin mediated actions that contribute to glucose-lowering *in vivo*.

The importance of nonincretin effects of GLP-1 are further exemplified by experiments in mice with genetic disruption of GLP-1R signaling. Consistent with the concept of GLP-1 functioning as an incretin, GLP-1R^{-/-} mice exhibit defective glucose-stimulated insulin secretion and increased glycemic excursion after oral glucose challenge, even if only one GLP-1 receptor allele is disrupted (15, 32). Studies of islet function demonstrate defects in basal islet cAMP and glucose-stimulated calcium signaling in GLP-1R^{-/-} islets (33). The importance of basal GLP-1R signaling for β -cell function may partly explain why GLP-1R^{-/-} mice also exhibit mild

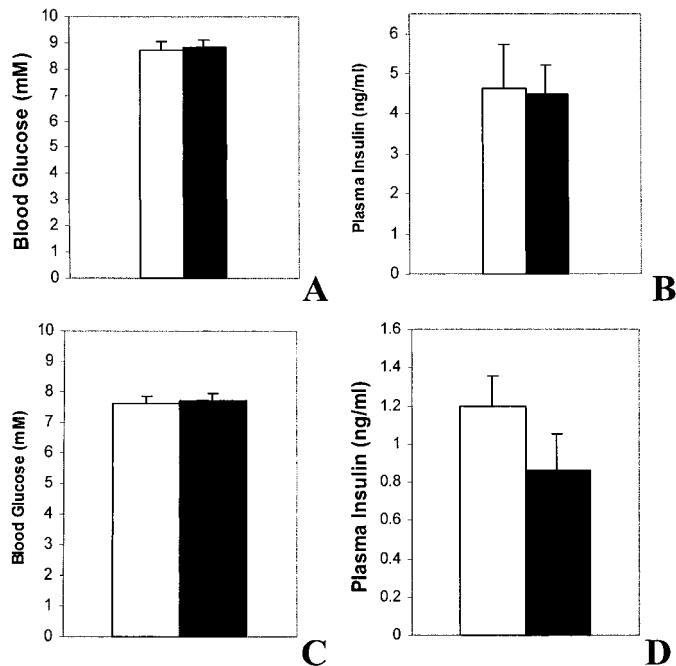


FIG. 7. Blood glucose and plasma insulin levels in wild-type +/+ and GLP-1 R-/- mice treated chronically with rabbit γ -globulin or anti-GIP R antiserum. A, Blood glucose level in +/+ males treated with rabbit γ -globulin (open bar) or anti-GIP R antiserum (solid bar). Values are expressed as means \pm SEM; n = 10 mice/group. B, Plasma insulin concentration in +/+ males treated with rabbit γ -globulin (open bar) or anti-GIP R antiserum (solid bar). C, Blood glucose level in GLP-1 R-/- males treated with rabbit γ -globulin (open bar) or anti-GIP R antiserum (solid bar). Values are expressed as means \pm SEM; n = 13 mice/group. D, Plasma insulin concentration in GLP-1 R-/- males treated with rabbit γ -globulin (open bar) or anti-GIP R antiserum (solid bar).

fasting hyperglycemia and abnormal glucose excursion after ip glucose challenge (15), conditions that would not be associated with increases in levels of circulating GLP-1. Although the phenotype of impaired glucose tolerance in GLP-1R-/- mice can be mild and variable, analysis of large numbers of knockout mice of different ages demonstrates statistically significant impairment of glucose homeostasis in the fasting state and after oral and ip glucose challenge (15, 32, 34).

Interpretation of the modest impairment of glucose tolerance after genetic GLP-1R disruption is complicated by the observation that GIP secretion and GIP-stimulated insulin secretion are up-regulated in GLP-1R-/- mice, suggesting that compensatory enhancement of GIP action partially modifies the phenotype of GLP-1 deficiency *in vivo* (17). Furthermore, it remains possible that potential developmental effects of GLP-1R deficiency might also modify islet and β -cell development and responsiveness, hence the abnormalities in β -cell function and glucoregulation detected in GLP-1R-/- mice may not necessarily be directly correlated with acute disruption of GLP-1 action *in vivo*.

Accordingly, to eliminate confounding variables introduced by the potential contribution of developmental and adaptive changes in physiological regulatory systems, we reexamined the importance of GLP-1 and GIP action for both incretin and nonincretin-mediated control of glycemia in

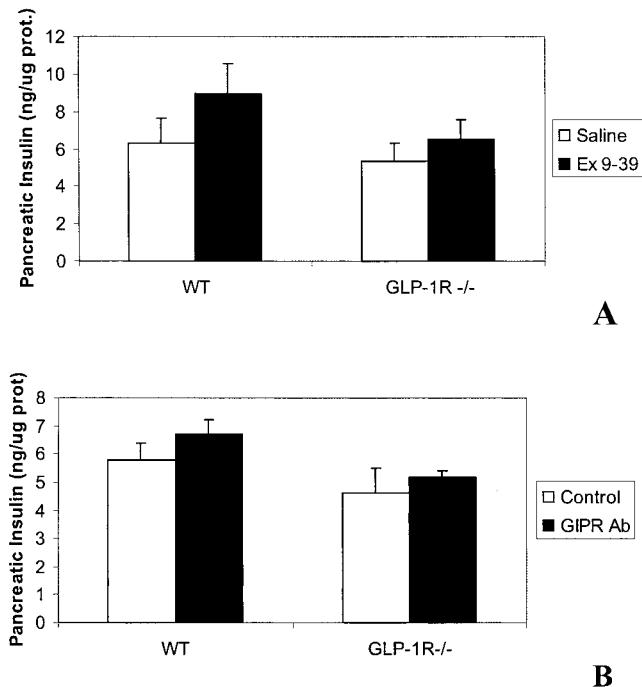


FIG. 8. Pancreatic insulin content in +/+ and GLP-1R-/- mice treated with exendin(9–39) (A) or GIP Ab (B). Values are expressed as mean \pm SEM; n = 8/group for experiments in A, and 4–9/group for experiments in B.

wild-type mice. Our data clearly show that inhibition of GLP-1 activity during ip glucose challenge produces abnormal glycemic excursion in +/+ mice, associated with a significant reduction in plasma insulin. Because nonenteral glucose challenge would not be expected to stimulate GLP-1 secretion, our findings strongly suggest that basal levels of circulating GLP-1 are essential for glycemic control, both in the fasting state and after glucose challenge, independent of the mode of glucose entry.

Further evidence supporting the importance of basal GLP-1 signaling for glucoregulation derives from studies using exendin(9–39) in both humans and baboons. Administration of exendin(9–39) produced significant elevations in fasting levels of both glucose and glucagon, suggesting that even basal GLP-1 signaling during the fasting state exerts a tonic inhibitory effect on glucagon secretion (35–37). The finding that glucagon secretion is under tonic inhibitory control by GLP-1 signaling is consistent with our observation that glucose levels rise in exendin(9–39)-treated mice, without an obligatory increase in the levels of circulating insulin. Additional evidence for the importance of basal GLP-1 signaling derives from studies demonstrating that exendin(9–39) is an inverse agonist of the β -cell GLP-1 receptor and that constitutive activity of the GLP-1 receptor, even in the absence of bound ligand, is important for maintaining basal levels of cAMP and for sustaining pancreatic β -cells in a glucose-competent state (33, 38).

In contrast to the importance of GLP-1 for glucose regulation and β -cell function in the fasting state, our current data strongly suggest that the role of GIP in glucose control is considerably more restricted, principally functioning as an

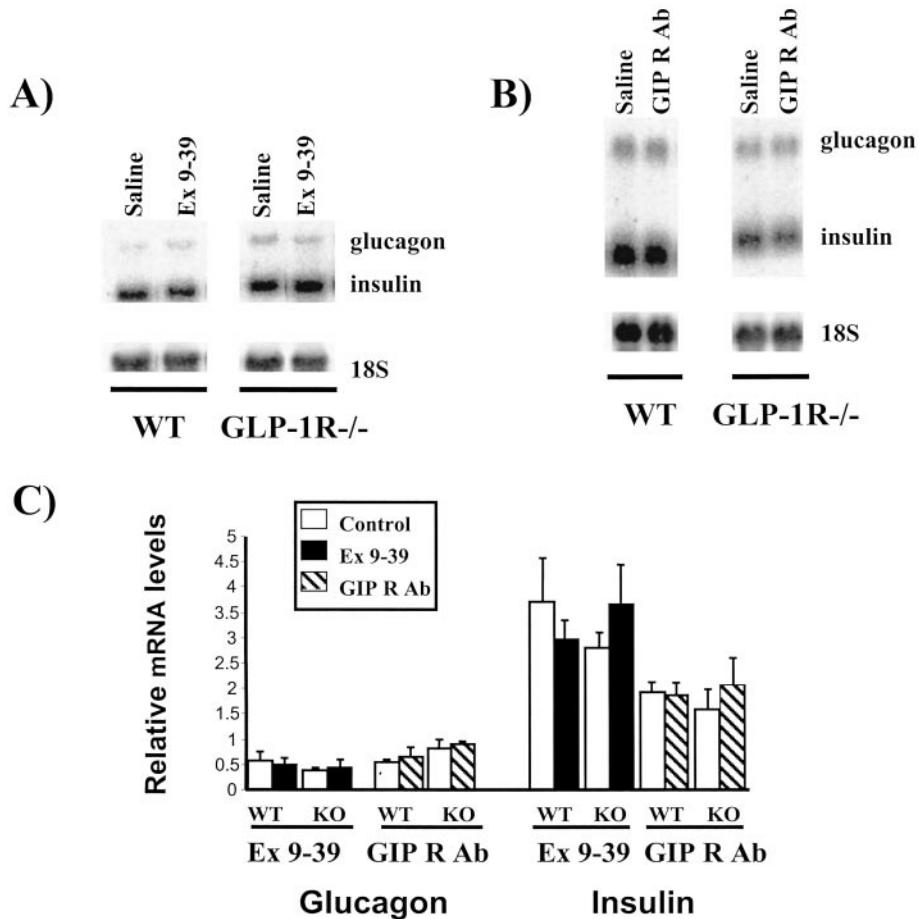


FIG. 9. Northern blot analysis of pancreatic insulin and glucagon mRNA transcripts in $+/+$ and GLP-1R $^{-/-}$ mice treated with exendin(9–39) [Ex 9–39(A)] or GIPR Ab (B). Relative mRNA levels (C) for insulin or glucagon and 18S rRNA. Values are expressed as means \pm SEM (all densitometry was quantified using the Molecular Dynamics, Inc., Sunnyvale, CA, PhosphorImager and ImageQuant). WT, Wild-type; KO, knockout.

incretin in the postabsorptive state. Disruption of GIP action during oral glucose challenge produced a significant increase in glycemic excursion in both $+/+$ and GLP-1R $^{-/-}$ mice, in association with a diminution of glucose-stimulated insulin secretion. In contrast, administration of GIPR Ab did not effect fasting glucose or glycemic excursion after ip glucose loading, even in mice with loss of GLP-1 function.

Although the incretin function of GIP is well established (3, 4, 12), a role for GIP in the control of β -cell function in the fasting state is less clearly defined. Infusion of GIP produced a dose-dependent increase in plasma insulin, in fasted rats, that was attenuated by coinfusion of a GIP peptide antagonist (39). Although the GIP peptide antagonist ANTGIP diminished glucose-stimulated insulin secretion in rats, the effect of ANTGIP on blood glucose or insulin secretion, in the fasting state or after ip glucose challenge, was not reported (13). Although we cannot be absolutely certain that the limitations of the immunoneutralizing GIPR Ab may affect our experimental results, the finding that GIP is primarily important for glucose clearance, after enteral (but not ip) glucose loading, is consistent with data from GIP receptor $-/-$ mice. These mice exhibit normal fasting glucose, and the glycemic response to ip glucose challenge is comparable and normal in the presence or absence of GIP receptor signaling (14). These findings are entirely consistent with our data showing no effect of GIPR Ab on fasting glucose or ip glucose clearance in mice *in vivo*. Taken together, the cumulative

evidence strongly suggests that the glucoregulatory actions of GIP on the β -cell are restricted to the potentiation of glucose-stimulated insulin secretion after enteral nutrient absorption.

The results of several studies have suggested an important role for GLP-1 in the regulation of insulin gene expression and insulin biosynthesis. Incubation of islet cell lines, with GLP-1 or exendin-4, increases proinsulin mRNA via activation of insulin gene transcription (9, 10). Similarly, GIP increases insulin mRNA and insulin content in islet cells, via induction of insulin promoter activity and insulin gene expression (11, 40). Despite the putative importance of GLP-1 and GIP for insulin gene expression and insulin biosynthesis, we did not detect changes in pancreatic insulin content or insulin RNA in $+/+$ mice treated with either exendin(9–39) or GIPR Ab. Furthermore, we found minimal to no changes in pancreatic insulin mRNA and insulin content in GLP-1R $^{-/-}$ mice (17, 32, 41). These findings, taken together with our data using exendin(9–39) or GIPR Ab, strongly suggest that GLP-1 and GIP are not essential for insulin gene expression and insulin biosynthesis in mice *in vivo*.

In summary, our studies demonstrate that GLP-1 is essential for glucose-stimulated insulin secretion in mice, independent of the mode of glucose entry. Although several investigators have suggested that the predominant effect of GLP-1 on glucose control resides at the level of gastric emptying (42, 43), our data clearly indicate an essential role for

GLP-1 in glucoregulation independent of nutrient entry via the gastrointestinal tract. In contrast, GIP plays a more restricted role in glucose homeostasis, with GIP actions restricted to the classical incretin function of potentiating nutrient-stimulated insulin secretion. The wider spectrum of GLP-1 actions on gastric emptying, β -cell function, glucagon secretion, food intake, and islet growth suggest that GLP-1 is likely to exhibit more potential, compared with GIP, as a therapeutic agent for the treatment of diabetes.

Acknowledgments

We thank J. F. Habener for his generous gift of GIPR Ab for these experiments.

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