Double Incretin Receptor Knockout (DIRKO) Mice Reveal an Essential Role for the Enteroinsular Axis in Transducing the Glucoregulatory Actions of DPP-IV Inhibitors

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Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) are gut-derived incretins that potentiate glucose clearance following nutrient ingestion. Elimination of incretin receptor action with GIPR−/− or GLP-1R−/− mice produces only modest impairment in glucose homeostasis, perhaps due to compensatory upregulation of the remaining incretin. We have now studied glucose homeostasis in double incretin receptor knockout (DIRKO) mice. DIRKO mice exhibit normal body weight and fail to exhibit an improved glycemic response after exogenous administration of GIP or the GLP-1R agonist exendin-4. Plasma glucagon and the hypoglycemic response to exogenous insulin were normal in DIRKO mice. Glycemic excursion was abnormally increased and levels of glucose-stimulated insulin secretion were decreased following oral but not intraperitoneal glucose challenge in DIRKO compared with GIPR−/− or GLP-1R−/− mice. Similarly, glucose-stimulated insulin secretion and the response to forskolin were well preserved in perfused DIRKO islets. Although the dipeptidyl peptidase-IV (DPP-IV) inhibitors valine pyrrolidide (Val-Pyr) and SYR106124 lowered glucose and increased plasma insulin in wild-type and single incretin receptor knockout mice, the glucose-lowering actions of DPP-IV inhibitors were eliminated in DIRKO mice. These findings demonstrate that glucose-stimulated insulin secretion is maintained despite complete absence of both incretin receptors, and they delineate a critical role for incretin receptors as essential downstream targets for the acute glucoregulatory actions of DPP-IV inhibitors. Diabetes 53: 1326–1335, 2004

The observation that a glycemic stimulus derived from enteral nutrients exerts a greater insulinotropic response than a comparable isoglycemic challenge achieved through parenteral glucose administration has been termed the incretin effect (1). The first enteroendocrine-derived incretin to be identified, glucose-dependent insulinotropic peptide (GIP), is secreted from duodenal K-cells and rapidly potentiates glucose-dependent insulin secretion (2,3). However, the finding that immunoneutralization of GIP could not completely eliminate the incretin response strongly suggested the existence of additional gut-derived incretins. A second peptide with incretin activity, glucagon-like peptide 1 (GLP-1), was subsequently identified following elucidation of the nucleotide sequence for preproglucagon in the 1980s (4–6).

Human subjects with type 2 diabetes exhibit significant defects in meal-stimulated insulin secretion, leading to the suggestion that diminished incretin action or subnormal incretin secretion may contribute to the pathogenesis of β-cell dysfunction in specific patients (1,7). This hypothesis is supported in part by observations demonstrating resistance to GIP action and reductions in meal-stimulated GLP-1 secretion in diabetic subjects (8,9). The physiological importance of incretin action for glucose control is further illustrated by results of experiments directed at eliminating incretin action in vivo. A combination of peptide antagonists and immunoneutralizing antisera directed against either GIP, GLP-1, or their respective receptors have demonstrated that both GIP and GLP-1 are independently essential for regulation of glucose-dependent insulin secretion (10–12).

A complementary approach for analysis of incretin biology involves the development of mouse models of disrupted incretin receptor action. GIP receptor null mice develop normally and exhibit only modest glucose intolerance following an oral glucose challenge (13,14). Similarly, mice with a null mutation in the GLP-1 receptor gene do not develop severe diabetes but exhibit defective glucose-stimulated insulin secretion and glucose intolerance (15,16). The unexpectedly modest phenotypes of both GIPR−/− and GLP-1R−/− mice have prompted suggestions that one or more compensatory mechanisms have evolved.
to supplant the role normally subserved by individual incretin receptors in control of glucose homeostasis.

Evidence supporting the upregulation of compensatory mechanisms derives from findings that GLP-1R−/− mice exhibit significantly enhanced β-cell sensitivity to the actions of GIP (17,18), whereas GIPR−/− mice exhibit an enhanced insulin secretory response to GLP-1 (14). Accordingly, we reasoned that the phenotype arising from disruption of single incretin receptor genes in mice may be partially modified as a result of complementary upregulation of the remaining intact incretin receptor axis. To identify the essential roles of GIP and GLP-1 for glucose homeostasis and to determine whether incretin receptors are key downstream targets essential for the action of dipeptidyl peptidase-IV (DPP-IV) inhibitors, we have now generated and characterized double incretin receptor knockout (DIRKO) mice with complete loss of both GIP and GLP-1 receptor action.

FIG. 1. Genotyping and body weight gain in DIRKO mice. A: Left panel: BamHI-digested genomic DNA from a GLP-1R+/−:GIPR+/− intercross was hybridized with a GLP-1R-specific probe (15). The sizes of the wild-type GLP-1R (6 kb) and targeted allele (4.3 kb) are indicated. Right panel: EcoRI-digested DNA from a GLP-1R+/−:GIPR+/− intercross was hybridized with a GIPR-specific probe. The sizes of the wild-type GIPR (7 kb) and targeted allele (3 kb) are indicated. B and C: Body weights in male (B) and female (C) wild-type (○), GIPR−/− (●), GLP-1R−/− (△), and DIRKO (■) mice. Body weight (g) was measured at 6, 8, 10, 12, and 16 weeks.

FIG. 2. GIP and exendin-4 lower glucose in wild-type mice but not in DIRKO mice. Blood glucose levels during oral glucose tolerance test in wild-type (A and C) and DIRKO (B and D) mice following administration of GIP (50 µg) or vehicle (saline). Administration of exendin-4 (100 ng) or saline to wild-type (C) and DIRKO (D) mice. Values are expressed as means ± SE; n = 3–7 mice/group. **P < 0.01 and ***P < 0.001, peptide vs. vehicle-treated mice.
RESEARCH DESIGN AND METHODS
Generation of GLP-1R−/−;GIPR−/− (DIRKO) mice. As GIP receptor mice were generated in the C57BL/6 background (13), whereas GLP-1R−/− mice were originally derived in the CD1 background (15), we first backcrossed GLP-1R−/− mice for five generations into the C57BL/6 background. The GIPR−/− mice were then crossed with the GLP-1R−/− mice on the same C57BL/6 background. The subsequent heterozygotes were crossed to generate GLP-1R−/−:GIPR−/− mice. All mice used in these studies were 9–15 weeks of age. Single and double incretin receptor knockout mice and age- and sex-matched control C57BL/6 wild-type mice (Charles River, Montreal, PQ) were housed under a light/dark cycle of 12 h in the Toronto General Hospital Animal facility with free access to food (standard rodent diet) and water, except where noted. All wild-type 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 and Vrije Universiteit Brussel animal care committees. For confirmation of genotypes, genomic DNA prepared from tail snips was analyzed by Southern blotting (16).

Glucose tolerance tests and measurement of plasma insulin. Oral or intraperitoneal glucose tolerance tests were carried out following an overnight fast (16–18 h) as described (11). Mice were given 1.5 mg glucose/g body wt through a gavage tube (oral glucose tolerance test) or via injection into the peritoneal cavity (intraperitoneal glucose tolerance test). Peptide administration was via the intraperitoneal route. GIP (California Peptide Research, Napa, CA) and exendin-4 (California Peptide Research) were injected immediately before glucose loading at a dose of 50 μg (GIP) or 100 ng (exendin-4) per mouse, respectively.

Studies with DPP-IV inhibitors. For analysis of the effects of DPP-IV inhibitors, mice were injected intraperitoneally with valine pyrrolidide (ValPyr; Merck Research Laboratories, Rahway, NJ) at a dose of 30 mg/kg body wt...
or the same volume of vehicle (H2O) 30 min before glucose administration. For studies with Syrrx 106124 (Syrrx, La Jolla, CA), the compound was administered orally 60 min before glucose challenge at a dose of 10 mg/kg body wt; the same volume of vehicle (H2O) alone was administered as a control. LAF237 was obtained from Novartis Pharmaceuticals (East Hanover, NJ) and was given orally 20 min before an oral glucose challenge at a dose of 5 mg/kg body wt. TP8211 was obtained from Dr. W. Bachovchin, Tufts University (Boston, MA) and was administered 60 min before an oral glucose challenge at a dose of 2.5 and 12.5 mg/kg body wt. Blood was drawn from a tail vein at 0, 10, 20, 30, 60, 90, and 120 min following glucose administration, and blood glucose levels were measured by the glucose oxidase method using a One Touch Basic Glucometer (LifeScan, Burnaby, BC, Canada). Blood samples (100 μl) for measurement of plasma insulin were removed from a tail vein during the 10- to 20-min time period following oral or intraperitoneal glucose administration. Plasma was assayed for insulin using a rat insulin enzyme-linked immunosorbent assay kit (Crystal Chem, Chicago, IL) with mouse insulin as a standard (11).

**Islet isolation and perfusion.** The pancreatic islets of Langerhans were isolated from overnight fasted 11- to 15-week old male and female C57BL/6 mice, as previously described (19,23). Islets from wild-type and knockout mice were immediately perfused in parallel (100 islets/chamber), using pH 7.4 Ham’s F10 media (Gibco) supplemented with 15 mmol/l HEPES (Sigma), 0.5% BSA (Boehringer Mannheim), 2 mmol/l glutamine (Gibco), and 2 mmol/l Ca2+ with varied glucose concentrations. Perfusion media was bubbled with carbogen (95% O2/5% CO2) to ensure adequate oxygen tension and pH stability. Chambers were perfused at a rate of 0.5 ml/min, and samples were taken each minute. The perfusion protocol consisted of 30 min of basal 1 mmol/l glucose perfusate followed by stepwise increases in glucose concentration (5, 8, 11, and 20 mmol/l; 10 min/step). Following this protocol, islets were perfused for 10 min each with 20 mmol/l glucose + 10 mmol/l GLP-1 + 10 mmol/l GIP and then 20 mmol/l glucose + 3 μmol/l forskolin + 0.1 mmol/l IBMX (peptides and compounds from Sigma), allowing islets to return to baseline secretory levels (by 15 min) between each test. At the end of the protocol, islet insulin content was determined by sonicating islets in 0.25% BSA with 2 mol/l acetic acid, followed by radioimmunoassays as described (16,18). Insulin secretion was measured in each fraction by radioimmunoassay and expressed as a percent of total cell content (16,18). Pancreatic insulin content was measured after an overnight fast as described previously (19).

**Measurement of plasma glucagon, GLP-1, and GIP.** Mice were killed after an overnight fast or 20 min following administration of oral glucose (1.5 mg glucose/g body wt). Mice were anesthetized with CO2, and blood samples were collected by cardiac puncture and mixed with 10% (vol/vol) TED (500,000 KIU/ml Trasylol, 1.2 mg/ml EDTA, and 0.1 mmol/l Diprotin A). The plasma was separated by centrifugation at 4°C and stored at −80°C. Plasma glucagon was measured using a glucagon radioimmunoassay kit (Linco Research, St. Louis, MO). GIP and GLP-1 concentrations in plasma were measured after extraction of plasma with 70% ethanol (vol/vol, final concentration). For the GIP radioimmunoassay (20,21), we used the COOH-terminally directed antiserum R 65, which cross-reacts fully with murine GIP and with GIP (3-42), but not with larger forms of GIP. Human GIP and [125I]human GIP (70 MBq/nmol) were used for standards and tracer. The plasma concentrations of GLP-1 were measured (22) against standards of synthetic GLP-1(7-36amide) using antiserum code no. 80880, which is specific for the amidated COOH-terminus of GLP-1 and does not react with GLP-1–containing peptides from the pancreas. The assay measures the sum of intact GLP-1 and the primary metabolite, GLP-1(9-36amide). For both assays, sensitivity was <1 pmol/l, intraassay coefficient of variation was <6% at 20 pmol/l, and recovery of standard, added to plasma before extraction, was almost 100% when corrected for losses inherent in the plasma extraction procedure.

**Insulin tolerance test.** Wild-type and DIRKO mice were fasted for 5 h and given 1.7 units/kg insulin (Humulin R, 100 units/ml; Lilly, Toronto, ON) by intraperitoneal injection. Blood glucose was monitored for 4 h after the insulin challenge.

**Histology and immunohistochemistry.** The pancreas was removed, fixed overnight in 5% glacial acetic acid/25% formaldehyde (vol/vol), and embedded in paraffin. Sections were obtained and stained with hematoxylin and eosin using standard protocols. Immunostaining for insulin was carried out as previously described (19,23).

**Statistics.** Results are expressed as means ± SE. Statistical significance was assessed by one-way and two-way ANOVA and, where appropriate, a Student’s t test using GraphPad Prism 3 (GraphPad Software, San Diego, CA). A P value of <0.05 was considered to be statistically significant.

**RESULTS**

GIPR−/− and GLP-1R−/− mice were crossed (Fig. 1A) to generate DIRKO mice. Transmission of the mutant alleles was confirmed by Southern blotting (Fig. 1A). DIRKO mice were viable and fertile, appeared normal, and exhibited no disturbances in growth (Fig. 1B and C). Analysis of food intake over a 24-h time period revealed small but significant increases in food intake at some time points in DIRKO and GLP-1R−/− mice but not in GIPR−/− mice, consistent with our previous studies of food intake in GLP-1R−/− mice in the CD1 background (15,24,25). In view of reports...
continuing to suggest evidence for a second functional GLP-1R and to verify the functional absence of responses to exogenous incretins, we administered either the GLP-1R agonist exendin-4 or GIP to glucose-loaded wild-type and DIRKO mice. GIP and exendin-4 significantly reduced glycemic excursion following glucose challenge in wild-type mice; however, these peptides had no glucose-lowering effect in DIRKO mice (Fig. 2). Hence, combined targeted disruption of the two established incretin receptors completely eliminates the glucose-lowering effects of GIP and GLP-1 receptor agonists in mice.

In agreement with the presumed physiological importance of GIP and GLP-1, GIPR−/− and GLP-1R−/− mice exhibited glucose intolerance following oral glucose challenge. In contrast, fasting glucose was significantly elevated in GLP-1R−/− in the CD1 background, but not in GIPR−/− mice (13,15). Fasting glucose was not significantly elevated in DIRKO mice (Figs. 2 and 3). Nevertheless, glycemic excursion was abnormally increased following oral glucose administration to DIRKO relative to wild-type mice or to mice lacking a single incretin receptor (Fig. 3A and B). Furthermore, the levels of glucose-stimulated plasma insulin were lower in GIPR−/− and GLP-1R−/− mice following oral glucose challenge and were significantly reduced in DIRKO mice relative to levels in wild-type mice (Fig. 3E). In contrast, glycemic excursion following intraperitoneal glucose challenge was abnormally increased in DIRKO relative to wild-type mice but was not significantly different compared with excursion profiles seen with GLP-1R−/− mice (Fig. 3C and D). A trend toward sexual dimorphism was observed, with GLP-1R−/− and DIRKO females being comparatively more glucose intolerant to an intraperitoneal glucose load than their male counterparts (Fig. 3C and D). Furthermore, no significant difference was detected in levels of glucose-stimulated plasma insulin in single incretin receptor knockout versus DIRKO mice following intraperitoneal glucose loading (Fig. 3E). Moreover, total pancreatic insulin content measured in the fasted state was normal in DIRKO mice (Fig. 3F), and no apparent difference in the number and size of DIRKO versus wild-type islets was observed (Fig. 3G). Mean levels of GIP were similar in fasted wild-type and DIRKO mice (8.3 ± 0.8 vs. 6.2 ± 0.8 pmol/l, respectively). In contrast, following oral glucose challenge, the levels of circulating GIP were significantly higher in wild-type compared with DIRKO mice (34.4 ± 5.6 vs. 12.6 ± 2.0 pmol/l, respectively, P < 0.05). In contrast, circulating levels of GLP-1 were lower in DIRKO mice in both the fasted state and following glucose challenge (17.2 ± 1.0 vs. 12.1 ± 1.7 pmol/l and 28.5 ± 0.5 vs. 11.5 ± 2.5 pmol/l, wild-type vs. DIRKO mice, respectively).

To determine whether the perturbations in glycemic excursion detected in DIRKO mice reflected compensatory changes in sensitivity to insulin, we compared the acute glycemic response to exogenous insulin in wild-type versus DIRKO mice. No differences were observed in the magnitude of glucose reduction or the recovery from insulin-induced hypoglycemia in wild-type versus DIRKO mice (data not shown). Similarly, although both GLP-1 and GIP are known regulators of plasma glucagon secretion (26,27), no significant differences (wild-type versus single incretin receptor knockout versus DIRKO mice) were observed in the levels of plasma glucagon in the fasting state or following oral glucose administration (Fig. 3H).

The modest impairment in glucose-stimulated insulin secretion observed in DIRKO mice prompted us to assess whether potential compensatory factors upregulating β-cell secretory function might be revealed by analysis of glucose-stimulated insulin secretion in isolated DIRKO islets. The dynamic secretion of insulin by freshly isolated islets is shown in Fig. 4. By stepwise increases in glucose concentration in the perifusate, it was possible to plot concentration response curves for assessment of islet sensitivity to glucose. The perifusion profiles (Fig. 4A and B), comparing wild-type with DIRKO mice and separating results by sex, indicated that islets from either sex respond similarly to glucose. A trend toward sexual dimorphism was observed between male and female DIRKO mice, with males being more responsive to glucose than females (Fig. 4A and B). Comparison of the integrated insulin responses showed that the male DIRKO mice were more responsive than both male wild-type and female DIRKO animals at the step from 11–20 mmol/l glucose (P < 0.05). However, calculation of the initial secretion rate (average of the peak two to three tubes immediately following the change in glucose concentration) or the “plateau” secretion rate (average of the last three tubes before changing perifusate glucose concentration) indicated that the male DIRKO islets showed normal secretion kinetics; in contrast, although the sensitivity of female DIRKO islets to glucose was preserved, their secretion kinetics showed a trend (not statistically significant) toward impairment.

While freshly isolated islets are commonly used for study of responsiveness to glucose and direct activators of signal transduction cascades (28), the acute secretory response to hormones may be blunted, likely due to receptor damage by collagenase (29). Nevertheless, both GIP and GLP-1 acutely stimulated insulin secretion immediately following islet isolation (20% increase) from wild-type but not DIRKO islets, and perifusion following overnight islet culture confirmed a complete lack of responsiveness to each incretin alone in islets from DIRKO mice (data not shown). Comparing the integrated insulin secretory responses obtained with either GIP or GLP-1, either incretin was able to account for a difference of 80–90% of the insulin response between wild-type and DIRKO islets (data not shown).

To confirm retention of islet secretory capacity at the conclusion of the perifusion studies, islets were perifused for 10 min with the phosphodiesterase inhibitor (IBMX; 0.1 mmol/l) and forskolin (3 μmol/l), a direct activator of adenylyl cyclase. Figure 4E shows the integrated insulin responses to 20 mmol/l glucose alone or with forskolin/IBMX for both male and female wild-type and DIRKO islets. Activation of the cAMP pathway resulted in similar amplification of insulin secretion for all mice (P > 0.05). These findings confirmed the viability of the islets for the duration of the perifusion protocol and demonstrate that genetic removal of both incretin receptors does not impair responsiveness to second messenger systems coupled to incretin receptor activation. It is also noteworthy that another sexual dimorphism was observed, independent of the genetic disruption of the incretin receptors. Both
wild-type and DIRKO female mice contained ~50% of the islet insulin content of their male counterparts (Fig. 4F; \( P < 0.05 \)). The underlying reason for this finding remains unclear; however, the amount of insulin released as a percentage of total cell content was not altered in wild-type mice, regardless of sex.

Activation of incretin receptor pathways by enhancing endogenous levels of GLP-1 and GIP contributes to the glucose-lowering activity of DPP-IV inhibitors (30). DPP-IV is the key enzyme responsible for inactivating both GLP-1 and GIP, and genetic disruption or pharmacological inhibition of DPP-IV lowers blood glucose in rodent and human studies (31,32). DPP-IV inhibitors modify the activity of multiple peptide substrates with glucose-lowering effects in addition to GIP and GLP-1 (33–35). Accordingly, we examined the activity of DPP-IV inhibitors in mice with single and combined genetic disruption of incretin receptor action. The DPP-IV inhibitor Val-Pyr significantly reduced glycemic excursion following oral glucose challenge in association with increased levels of plasma insulin not only in wild-type mice (Fig. 5A), but also in GLP-1R\(^{-/-}\) and GIPR\(^{-/-}\) mice (Fig. 5B and C). In contrast, Val-Pyr had no effect on plasma insulin or glucose in DIRKO mice (Fig. 5D). To ascertain that the elimination of glucose lowering with Val-Pyr did not represent a compound-specific effect, we assessed glucose excursion following administration of SYR106124, a structurally different DPP-IV inhibitor. Although SYR106124 reduced glycemia and increased levels of plasma insulin following glucose loading in wild-type mice (Fig. 6A and B), SYR106124 had no effect on blood glucose or insulin in DIRKO mice (Fig. 6C and D). Similarly, two additional structurally distinct DPP-IV inhibitors, TP8211 and LAP237, significantly lowered glucose in wild-type but not in DIRKO mice (data not shown). Taken together, these findings demonstrate that functional incretin receptors represent essential downstream targets for transducing the acute glucose-lowering effects of DPP-IV inhibitors.
DISCUSSION

The enteroinsular axis, a term coined by Unger and Eisentraut (36), describes the phenomena of greater insulin release when nutrients are taken orally versus intravenously, even if glycemic conditions are matched (37,38). Consistent with the importance of GIP and GLP-1 for function of the enteroinsular axis, mice lacking either GLP-1 or GIP receptors exhibit defective glucose-stimu-

![Image](120x453 to 492x734)

FIG. 5. The DPP-IV inhibitor Val-Pyr fails to lower blood glucose in DIRKO mice. Male mice were given intraperitoneal injections of vehicle (H2O) or 30 mg/kg Val-Pyr 30 min before oral glucose loading. Oral glucose tolerance following administration of either vehicle (open symbols) or Val-Pyr (solid symbols) to wild-type (A), GIPR−/− (B), GLP-1R−/− (C), and DIRKO (D) mice (n = 6–14 mice/group). Plasma insulin levels (ng/ml) were determined in samples obtained 10 min after oral glucose challenge are shown as insets (n = 4–11 mice/group). Values are expressed as means ± SE. *P < 0.05, **P < 0.01, and ***P < 0.001 vehicle vs. Val-Pyr–treated mice.

![Image](46x46 to 342x328)

FIG. 6. Administration of Syrrx106124 improves glucose tolerance in wild-type but not in DIRKO mice. Vehicle (H2O) or 10 mg/kg Syrrx106124 was administered 60 min before oral glucose loading. Oral glucose tolerance testing was carried out following oral administration of vehicle or Syrrx106124 to wild-type (A) and DIRKO (C) mice (n = 10–13 mice/group). Plasma insulin levels were determined in samples obtained 10 min after oral glucose administration in wild-type (B) and DIRKO (D) mice (n = 5–8 mice/group). Values are expressed as means ± SE. *P < 0.05 and ***P < 0.001 vehicle vs. Syrrx101624-treated mice.
related insulin secretion (13–15,39). Surprisingly, however, glucose intolerance in mice with disruption of single incretin receptor genes is relatively modest and thought to be due in part to compensatory upregulation of the remaining functionally intact incretin receptor axis (14,17). Accordingly, we reasoned that inactivation of both incretin receptor genes in a single mouse would substantially impair glucose tolerance, further illustrating the essential roles of incretin receptor action in glucose homeostasis.

DIRKO mice exhibit significantly greater glucose intolerance following oral glucose challenge than mice with genetic inactivation of a single incretin receptor gene. Furthermore, despite the potential for multiple complementary actions (effects on glucagon and gastric emptying) of GLP-1 to complicate interpretation of the effects of incretin receptor disruption on the β-cell, the increased glycemic excursion following oral glucose challenge in DIRKO mice was associated with inappropriately reduced levels of plasma insulin. These data strongly affirm the importance of incretin receptor signaling for β-cell function after enteral nutrient administration. Surprisingly, however, fasting glucose was normal and levels of glucose-stimulated insulin were not significantly different in wild-type versus DIRKO mice following IPGTT, consistent with the absence of a severe generalized β-cell defect in DIRKO islets. The preservation of β-cell function in DIRKO mice was not likely due to enhanced GIP or GLP-1 action through novel incretin receptors, as no changes in plasma glucose were observed following pharmacological GIP or exendin-4 administration.

Our previous studies of GLP-1R−/− mice revealed evidence for mild fasting hyperglycemia (15), whereas fasting glucose was normal in GLP-1R−/− mice studied here, as well as in the DIRKO mouse. Furthermore, we previously described a modest but significant reduction in pancreatic insulin content in GLP-1R−/− mice (17), whereas pancreatic insulin content was normal in both the DIRKO and GLP-1R−/− pancreas in the present studies. One important potential explanation for the variations in phenotypes observed in GLP-1R−/− mice is the different genetic backgrounds of these mice. Our previous studies describing characterization of the GLP-1R−/− mouse were obtained in experiments analyzing the GLP-1R null mutation in the CD1 background (15–17,39). In contrast, the data obtained in our current study represent the first analysis of the phenotype of the GLP-1R null mutation in the C57BL/6 background. Given the potential importance of even minor strain variation on phenotypes related to glucose homeostasis (40), we cannot exclude the possibility that one or more subtle differences in phenotypic characterization of the current GLP-1R−/− mouse is related to the new genetic background.

The classic definition of the enteroinsular axis invokes a comparatively greater release of insulin following oral versus parenteral glucose challenge (1). Indeed, wild-type mice clearly exhibit greater levels of plasma insulin following oral versus intraperitoneal glucose loading (Fig. 3). In contrast, the incretin effect is eliminated in mice with a single disrupted incretin receptor gene, and levels of plasma insulin are paradoxically lower in DIRKO mice after oral versus intraperitoneal glucose administration. Surprisingly, however, DIRKO islets maintain relatively normal glucose-induced insulin secretion in vitro. Previous studies have shown that exposure of murine β-cells to the GLP-1R antagonist exendin (9–39) reduces levels of cAMP and glucose-stimulated insulin secretion, implying that constitutive GLP-1 receptor signaling is essential for stimulus secretion coupling in the β-cell (18,41). Nevertheless, the relative preservation of glucose-stimulated insulin secretion in perfused islets, the normal fasting glucose, taken together with the comparatively well-preserved response to intraperitoneal glucose, suggests that DIRKO islets may have evolved as yet unidentified compensatory mechanisms that mask the functional absence of the GIP and GLP-1 receptors. Furthermore, it appears that additional defects in the secretory response of DIRKO islets may be unmasked following incubation of islets for varying periods of time in nutrient-depleted medium (B. Thorens, unpublished observations).

Although the GLP-1 and GIP receptors are viewed as important modulators of β-cell signal transduction and glucose-stimulated insulin secretion, the DIRKO β-cell seems likely to have adapted to the genetic absence of incretin receptors via upregulation of related compensatory signaling systems. For example, PACAP receptors are expressed on islet β-cells, and genetic disruption of the PAC1 receptor in mice results in reduction of glucose-stimulated insulin secretion in mice in vivo and in intact islets in vitro (42). Similarly, glucagon is a potent activator of cyclic AMP formation and insulin secretion (43), and studies using a specific glucagon receptor antagonist (44) reveal an essential role for the β-cell glucagon receptor as an important regulator of glucose-stimulated insulin secretion. Hence, it remains possible that the comparatively intact β-cell function observed in the DIRKO mouse reflects enhanced sensitivity to functionally related peptides with insulinotropic activity and/or upregulation of downstream signaling molecules that contribute to the maintenance of appropriate glucose-stimulated insulin secretion.

The increasing interest in the evaluation of DPP-IV inhibitors for the treatment of subjects with type 2 diabetes (45,46) prompted us to assess whether incretin receptors are essential for DPP-IV inhibitor action. Although multiple regulatory peptides, including GIP, are known substrates for DPP-IV (47), current concepts invoke GLP-1 as the principal mediator of DPP-IV inhibitor–associated reduction in blood glucose (46,48). Nevertheless, some studies examining the glucose-lowering properties of DPP-IV inhibitors demonstrate more pronounced effects on stabilization of plasma GIP compared with GLP-1. Moreover, previous studies have shown that the GLP-1R is not essential for DPP-IV inhibitor action, as Val-Pyr lowered blood glucose and stimulated insulin secretion in mice with complete absence of GLP-1 activity (31). Accordingly, the relative importance of GIP and GLP-1 as targets for the actions of DPP-IV inhibitors remains uncertain.

We demonstrate here that Val-Pyr not only reduces glycemic excursion in GLP-1R−/− mice, but also lowers blood glucose in GIP−/− mice. One possible explanation for these findings lies in the broad substrate specificity of DPP-IV (47). For example, pituitary adenylate cyclase–activating peptide, bradykinin, gastrin-releasing peptide, and GIP are known DPP-IV substrates and exert glucose-lowering effects in vivo (33–35,49). In preliminary studies
using smaller numbers of mice, we initially detected a small reduction in blood glucose using Val-Pyr in DIRKO mice (50). In contrast, our studies described here using larger numbers of mice and a panel of different DPP-IV inhibitors demonstrate that the acute glucose-lowering effects of these compounds are absent in DIRKO mice. Our findings that multiple structurally distinct DPP-IV inhibitors have no acute effect on blood glucose or plasma insulin in DIRKO mice strongly suggest that the GLP-1 and GIP receptors are the principal targets essential for the acute glucose-lowering actions of DPP-IV inhibitors.

Nevertheless, our experiments examining the acute effects of single-dose DPP-IV inhibitor administration on glucose tolerance may not necessarily predict the results obtained in studies with these inhibitors in different experimental paradigms. For example, DPP-IV inhibitors may exert differential effects on substrate activity in diabetic versus normoglycemic settings. Furthermore, chronic treatment with DPP-IV inhibitors has been observed to exert progressive changes in metabolic parameters beyond those detected in single-dose administration studies (51). Accordingly, analysis of the activity of DPP-IV inhibitors following prolonged administration to DIRKO mice following induction of experimental diabetes may be required to fully elucidate the complete spectrum of DPP-IV–dependent substrates contributing to improvement in glucose homeostasis.

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REFERENCES


