Defective Glucose-Dependent Insulinotropic Polypeptide Receptor Expression in Diabetic Fatty Zucker Rats

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Glucose-dependent insulinotropic polypeptide (GIP) is a peptide hormone that is released postprandially from the small intestine and acts in concert with glucagon-like peptide (GLP)-1 to potentiate glucose-induced insulin secretion from the pancreatic β-cell. In type 2 diabetes, there is a decreased responsiveness of the pancreas to GIP; however, the insulin response to GLP-1 remains intact. The literature suggests that the ineffectiveness of GIP in type 2 diabetes may be a result of chronic homologous desensitization of the GIP receptor. Yet, there has been no conclusive evidence suggesting that GIP levels are elevated in diabetes. The hypothesis of the present study is that one cause of decreased responsiveness to GIP in type 2 diabetes is an inappropriate expression of the GIP receptor in the pancreatic islet. This hypothesis was tested using a strain of diabetic fatty Zucker rats. The obese rats displayed basal GIP levels similar to the control animals; however, they were unresponsive to a GIP infusion (4 pmol·min⁻¹·kg⁻¹), whereas the lean animals displayed a significant reduction in blood glucose (GIP levels, 50% control after 60 min, P < 0.05) as well as a significant increase in circulating insulin. GIP also potently stimulated first-phase insulin secretion from isolated perfused islets (10.3 ± 3.0 × basal), and GIP and GLP-1 potentiated insulin secretion from the perfused pancreas (6 × control area under the curve [AUC]) from lean animals. GIP yielded no significant effect in the Vancouver diabetic fatty Zucker (VDF) rat pancreases, whereas GLP-1 elicited an eightfold increase of insulin secretion from the perfused VDF pancreas. Islets from lean animals subjected to static incubations with GIP showed a 2.2-fold increase in cAMP, whereas GIP failed to increase islet cAMP in the VDF islets. Finally, the expression of both GIP receptor mRNA and protein was decreased in islets from VDF rats. These data suggest that the decreased effectiveness of GIP in the VDF rat and in type 2 diabetes may be a result of a decreased receptor expression in the islet. Diabetes 50:1004–1011, 2001

A large proportion of postprandial insulin secretion is stimulated by hormones secreted from the small intestine. Glucose-dependent insulinotropic polypeptide (GIP) (gastric inhibitory polypeptide), the proglucagon gene–derived glucagon-like peptide (GLP)-1(7-37), and the COOH-terminal truncated form GLP-1(7-36) amide are the major incretins that act via this endocrine system to potentiate glucose-induced insulin secretion. GIP and GLP-1 both signal via serpentine seven-transmembrane G-protein–coupled receptors of the secretin/VIP superfamily. Binding of the incretins to their respective receptors on the β-cell surface activates adenyl cyclase, increases cAMP, and stimulates insulin secretion. Recent studies have demonstrated that the GIP receptor displays characteristics similar to other G-protein–coupled receptors in terms of ligand binding, desensitization, and subsequent internalization.

A wide range of experimental techniques have been used to demonstrate the physiological importance of GIP and GLP-1. In vivo administration of exendin(9-39) and GIP(7-30), GLP-1, and GIP receptor antagonists resulted in decreased insulin responses to oral glucose (9–11). Furthermore, both GIP and GLP-1 receptor knockout mice display compromised insulin release and, therefore, altered glucose tolerance to an oral load (12,13). From these studies, it has been concluded that secretion of the incretins could account for up to 70% of the postprandial insulin response to glucose (14). GIP and GLP-1 both require elevated levels of ambient glucose to stimulate pancreatic β-cell insulin secretion; hence, there is considerable interest in using analogs of these incretin peptides in the treatment of type 2 diabetes (15–20).

One shortfall of using GIP in therapy is the controversy over its effectiveness as an incretin in type 2 diabetes (21,22). Human studies have shown that there is a decreased incretin effect in type 2 diabetes, and this has been attributed mainly to an attenuation of GIP-stimulated insulin secretion either via a change in GIP receptor expression or a change in circulating GIP levels, although altered signal transduction pathways could play a role (23). Currently, there is no consensus regarding the circulating levels of GIP in type 2 diabetic subjects; studies have demonstrated increased, decreased, and unchanged GIP levels (24–27). Thus, it cannot be concluded that chronic homologous desensitization of the GIP receptor in type 2 diabetes causes an ineffective incretin response (28,29). Furthermore, studies have shown point mutations in the

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AUC, area under the curve; BSA, borine serum albumin; DAPI, dipyridyl peptidase IV; DTT, dithiothreitol; GIP, glucose-dependent insulinotropic polypeptide; GLP, glucagon-like peptide; HBSS+, Hank’s balanced salt solution supplemented with 10 mmol/l HEPES, 2 mmol/l t-glutamine, and 0.2% BSA; IBMX, 3-isobutyl-1-methylxanthine; IP, intraperitoneal; KRBH, HEPES-buffered Krebs-Ringer bicarbonate buffer; PCR, polymerase chain reaction; Rf, relative mobility; RIP A, radioimmunoprecipitation assay; RT, reverse transcription; TBST, Tris-buffered saline with 0.5% Tween 20.
GIP receptor gene in human populations that affect GIP signaling in cell models; however, it has not been possible to associate these mutations with type 2 diabetes (30,31).

In the current study, we set out to test the hypothesis that the ineffective GIP responses observed in type 2 diabetes can result from long-term downregulation of GIP receptor expression in the β-cell plasma membrane. To test this hypothesis, we used the Vancouver diabetic fatty Zucker (VDF) rat as a model of type 2 diabetes. We demonstrate that there is a marked decrease in the GIP receptor mRNA expression in the pancreatic islets of these animals, which results in a decrease in GIP-stimulated insulin release from the pancreatic β-cells despite normal sensitivity to GLP-1. This provides evidence that defective GIP receptor expression may be a cause of the inappropriate incretin response observed in many type 2 diabetic subjects.

RESEARCH DESIGN AND METHODS

Chemicals. Synthetic human GIP and GLP-1 were purchased from Bachem California (Torrance, CA), and 3-isobutyl-1-methylxanthine (IBMX) was purchased from Research Biochemicals International (Natick, MA). All chemicals of reagent or molecular biology grade were from Sigma (Oakville, ON, Canada) or Fisher Scientific International (Pittsburgh, PA).

Animals. VDF rats spontaneously developed from a Zucker strain kept in our laboratory. These diabetic rats were homozygous recessive for a mutation in the leptin receptor gene, fa (Gln269Pro). Rats carrying one normal Fa allele were phenotypically lean and displayed normal glucose tolerance. Only male animals aged 14–16 weeks were used in this study. All animals tested displayed severe glucose intolerance and decreased first-phase insulin response characteristic of VDF rats. Lean littermates were used as control animals in these experiments.

Intraportal glucose tolerance test. Zucker rats were anesthetized with an intraperitoneal (IP) injection of sodium pentobarbital (65 mg/kg) (Somnotol; MTC Pharmaceuticals, Cambridge, ON, Canada). The right jugular vein was then exposed and cannulated with heparinized polyethylene tubing (PE50; Becton Dickinson, Sparks, MD). GIP (4 pmol/kg min) and insulin (10,000 pg/kg) using a handheld blood glucose meter (SureStep; LifeScan, Burnaby, BC, Canada) were administered at baseline (0 min) and 10, 20, 30, and 60 min after glucose injection. Blood glucose levels were measured by a handheld glucose meter (SureStep; LifeScan, Burnaby, BC, Canada). Plasma was then separated from red cells by centrifugation at 10,000g for 4°C and then stored at −20°C until GIP and insulin radioimmunoassays could be carried out (32).

In vitro pancreatic perfusion. Anesthesia was established using Somnotol, and pancreases were isolated as previously described (16). The perfusate consisted of a modified Krebs-Ringer bicarbonate buffer containing 3% dextrose and 0.2% bovine serum albumin, 0.0025% human apotransferrin, 25 pmol/l sodium selenite, and 20 pmol/l ethanolamine hydrochloride for 20–24 h in 10-cm plastic culture dishes (Falcon; Becton Dickinson) in a humidified 5% CO2 environment.

Perfusion of pancreatic islets. After the culture period, 40 healthy islets were selected and sandwiched between two layers of Collagen-3 beads (Sigma, Lavington, PQ, Canada). Antibiotics (50 U/ml each penicillin G and streptomycin), 1 μmol/l L-glutamine, and 0.2% BSA (Fraction V, RIA grade; Sigma) were included in the perfusion chambers (Endotronics, Coon Rapids, MN). The chambers were then perfused in an Acusyst-s perfusion apparatus (Endotronics) under a humid 37°C 5% CO2 environment at a flow rate of 0.5 ml/min with 10 mmol/l HEPES-buffered Krebs-Ringer bicarbonate buffer (KRBB) supplemented with 0.2% BSA. Perfusion experiments were carried out for 80 min after a 60-min equilibration period in 2.8 mmol/l glucose (low glucose) perfusate. After 20 min, the perfusate glucose concentration was switched to 16 mmol/l with or without GIP. Samples were collected every 2 min, and insulin levels were determined by radioimmunoassay as previously described (32).

Measurement of cAMP production. After an overnight culture, 40 healthy islets were selected, washed twice with 0.5 ml of KRBB supplemented with 0.2% BSA, and allowed to equilibrate for 30 min in a humidified 5% CO2 environment. The islets were then stimulated with either vehicle, 10 pmol/l forskolin, or 10 nmol/l GIP for 30 min in the presence of 0.5 mmol/l IBMX. The perifusate glucose concentration was switched to 16 mmol/l glucose for the remainder of the experiment. GIP (10 pmol/l), GLP-1 (50 pmol/l), or forskolin was included in the perfusate buffer at concentrations of 1 mmol/l phenylmethylsulfonyl fluoride, 1 mmol/l dithiothreitol (DTT), and 10 μmol/l sodium selenite. The perifusate glucose concentration was switched to 16 mmol/l with or without GIP. Samples were collected every 2 min, and insulin levels were determined by radioimmunoassay as previously described (32).

Isolation and measurement of islet GIP receptor mRNA by real-time reverse transcriptase-polymerase chain reaction. Rat islet mRNA was isolated immediately after islet isolation using Trizol and the standard protocol provided by the manufacturer (Life Technologies). Specifically, 1 ml Trizol reagent was used per 100 islets, and all Amax/Amin ratios of isolated RNA were ≥1.80. After RNA isolation, 1 μg islet RNA was subjected to reverse transcription (RT). Total RNA was reverse transcribed in a volume of 10 μl containing 0.5 mmol/l 5′-deoxynucleotide triphosphate, 15 pmol gene-specific primer targeted at the COOH-terminus of the rat GIP receptor (GGT CTG GAG TAG AGC TGG TA), 75 pmol random hexamers (Amersham Pharmacien), 100 U Superscript II RNA H− Reverse Transcriptase (Life Technologies), 10 U RNase inhibitor (RNA Guard; Amersham Pharmacien), 1 mmol/l diithio-ri-itol, 50 mmol/l Tris-HCl, pH 8.3, 75 mmol/l KCl, and 3 mmol/l MgCl2. After RT, 100 ng rat islet tissue cDNA was used in the real-time polymerase chain reaction (PCR) to measure GIP receptor expression, whereas 10 ng cDNA was used in the GPR3 gene control PCR. The PCR mix consisted of 1× TaqMan Buffer A (PE Applied Biosystems, Foster City, CA), 10 mmol/l MgCl2, 200 μmol/l dATP, dCTP, and dGTP, and 400 μmol/l dUTP; 200 nmol/l rat GIP receptor 5′ forward primer (5′-CGG GGC TTT TCG TCA TCA TCC-3′), 200 nmol/l rat GIP receptor 3′ reverse primer (5′-CCA CCA AAT GGC TTT GAC TTC-3′), 200 nmol/l GIP receptor probe colabeled with the fluorescent dyes FAM and TAMRA, 5′-CCC AGC ACT GCG TGT TCT CGT ACA GG-3′, 6.01 μM AmpliErase uracil-DNA glycosylase (UNG) (PE Applied Biosystems), and 0.025 μM of AmpliTaq Gold (PE Applied Biosystems). The GADPH reactions included the above reaction conditions with the exception of the primers and probes, which were purchased from PE Applied Biosystems and were directed toward rodent GADPH. PCRs were carried out in triplicate in the PE Applied Biosystems 7700 sequence detection system. The reaction profile included a 10-min preincubation at 50°C to allow the UNG to degrade any uracil-containing nucleic acids and a further 10 min incubation at 94°C to activate the AmpliTaq Gold. After these preincubations, a two-step PCR protocol was carried out, which included a denaturation step at 94°C for 15 s followed by a 1-min annealing/extension step at 60°C. Fluorescence was measured during the annealing/extension steps over 40 cycles and used to calculate a cycle threshold. The cycle threshold of the reaction is not detectable by the hardware. All reactions followed the typical sigmoidal reaction profile, and cycle threshold was used as a measure of amplicon abundance (34).

Western analysis of islet GIP receptor protein. Islets were isolated as previously described. After isolation, islet GIP receptor protein was analyzed as previously described (35). Briefly, islets were lysed in ice-cold radioimmunoassay buffer (20 mmol/l NaCl, 20 mmol/l Tris-HCl, pH 7.5, 0.1% Triton X-100, 0.1% SDS, 5 mmol/l NaF, 1 mmol/l phenylmethylsulfonyl fluoride, 1 mmol/l dithiothreitol [DTT], 10 μg/ml leupeptin, 10 μg/ml pepstatin A, 10 μg/ml bestatin, and 1% Trasylol [Bayer Pharmaceuticals, Etobicoke, ON, Canada]) for 30 min on ice. Protein concentra-
tation was determined using a Bicinchoninic Acid kit (Pierce, Rockford, IL). Then, 50 μg total islet protein was denatured under reducing conditions (100 mmol/l DTT) at 100°C for 5 min and run by SDS-PAGE. Proteins were then transferred to nitrocellulose membrane, blocked with 5% skim milk (in Tris-buffered saline with 0.5% Tween 20 [TBST]), and incubated with a well-characterized polyclonal anti-GIP receptor antibody (35). Membranes were then washed three times in TBST and incubated with horseradish peroxidase–conjugated goat anti-rabbit IgG secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA). After further washing, the immunoreactive bands were visualized using enhanced chemiluminescence (Amersham Pharmacia). Finally, bands were subjected to densitometry using Eagle Eye II software (Stratagene, La Jolla, CA), and molecular weight was determined using relative mobility (Rv) analysis.

**Data analysis.** All data are expressed as mean ± SE, with the sample size indicated in the appropriate figure legend. Unpaired two-tailed t tests were carried out to compare groups of animals. P values ≤0.05 were considered statistically significant. Area under the curve was determined using curve analysis software (Graphpad; Prism, San Diego, CA).

**RESULTS**

**Effect of GIP on glucose tolerance in the VDF rat.** The optimal GIP dose was determined by carrying out the bioassay with various GIP concentrations ranging from 2 pmol/min · kg⁻¹ to 200 pmol/min · kg⁻¹ (data not shown). The optimum dose determined from this study (4 pmol/min · kg⁻¹) produced a submaximal glucose-lowering response in the lean animals. Thus, this dose was used for the remainder of the experiments. Figure 1A shows the blood glucose response to an IP glucose tolerance test with and without GIP in the lean control animals. This figure clearly shows that GIP was able to significantly improve glucose tolerance in the lean animals as early as 30 min after IP glucose injection. Furthermore, this improvement in glucose tolerance was maintained as long as the GIP infusion was continued. Figure 1B shows that the integrated glucose response (over 60 min) for the lean animals receiving GIP was significantly smaller than that in animals receiving saline.

The fat animals displayed basal hyperglycemia, with an average of 8.3 ± 0.4 mmol/l, compared with 5.5 ± 0.2 mmol/l for the lean animals (Fig. 1A and C). The fat animals also had much higher peak glucose levels in response to the glucose challenge, with the control (saline) values peaking at 15 mmol/l (compared with 11 mmol/l in lean animals); thus, the fatty animals were glucose intolerant and hyperglycemic. The GIP infusion did not result in a decrease in circulating glucose levels in the fat animals, as no difference (P > 0.05) was observed between GIP and saline infusions at any time after IP glucose (Fig. 1C). Furthermore, the integrated glucose response for the fatty animals that received GIP was not different from the integrated response of saline-infused animals (Fig. 1D). Thus, GIP yielded no improvement in glucose tolerance in the diabetic fatty animals.

**Effect of GIP on insulin secretion in the VDF rat.** Plasma was collected at −5, 10, 20, 30, and 60 min after IP glucose and assayed for insulin and GIP content. There was no difference observed in basal circulating GIP levels between the fat (16.7 ± 1.8 pmol/l) and lean (15.5 ± 1.6 pmol/l) animals. GIP yielded a significant increase in circulating insulin levels in the lean animals with a peak of 400 pmol/l at 20 min, which was before the glucose peak, in the same test group (Figs. 1A and 2A). Additionally, the integrated insulin response was significantly greater in the lean animals that received GIP (Fig. 2B). Because of the insulin-resistant state of the fatty animals, the insulin levels were much higher at all times during the infusion protocol in this group (Fig. 2C). Furthermore, there was neither a significant increase in insulin secretion elicited by GIP infusion (Fig. 2C) nor an increase in the integrated insulin response in these animals (Fig. 2D).

**Effect of GIP on insulin release from the pancreas of the VDF rat.** Pancreatic perfusions were carried out to determine if the release of insulin was a direct response to GIP in the intraperitoneal glucose tolerance test. As indicated in Fig. 3A and C, both 10 pmol/l GIP and 50 pmol/l GLP-1 in the presence of 8.8 mmol/l glucose evoked approximately equal peak 2.8-fold (6.1 AUC) and 3.4-fold (5.3 AUC) increases, respectively, in insulin secretion from the lean pancreas compared with control conditions. However, this augmentation in insulin secretion was not observed in the fatty Zucker pancreas when stimulated with GIP (Fig. 3B and D) where insulin secretion decreased from ~8.5 nmol/l to around 3.7 nmol/l during the high glucose and GIP infusion period. In contrast, GLP-1 caused
a significant insulin secretion from the pancreas of the fat animals that reached a plateau at 10 times the control perfusion level of secretion (Fig. 3B and D).

Pancreatic islet perifusion was carried out to determine whether the defect in GIP stimulation of insulin secretion was confined to the islet. As seen in Fig. 4A, 10 nmol/l GIP in the presence of 16 mmol/l glucose was able to stimulate insulin secretion from the lean Zucker islet, with a peak level 10 times the basal level of 62 pmol/l. High glucose (16 mmol/l) alone only produced a fourfold increase in insulin release from the islets. Figure 4B illustrates the effects of GIP on the islets from the fatty Zucker rat. As illustrated, 10 nmol/l GIP had little effect on insulin release from these islets in the presence of 16 mmol/l glucose. The peak GIP-stimulated insulin release from the fat islets in the perfusion system was 4.3 ± 1.5 × basal, whereas glucose alone produced about a 3.2 ± 1.5-fold increase from a basal level of 95 pmol/l; this response was similar to the response to glucose alone seen in the lean rat islets (Fig. 4A and B).

**Effect of GIP on cAMP production in VDF rat islets.**

Islet cAMP studies were carried out to elucidate where the defect in the GIP signaling pathway in the fatty animals occurred. Figure 5 illustrates the effect of GIP and forskolin on cAMP production in islets from fat and lean rats. GIP (10 nmol/l) produced a significant (2.2-fold basal) response in the lean control islets; however, there was no observable cAMP response to GIP in the islets from obese animals. The basal values of cAMP production did not differ significantly between the two phenotypes. Additionally, the forskolin responses of the fat and lean animals did not differ significantly, indicating that the cAMP production machinery was not defective in the VDF rats.

**FIG. 2.** Insulin responses to infused GIP (△) and saline (■) in control *Fa*/*^r^* (A and B) and VDF *fa*/*fa* (C and D) rats. Basal blood glucose samples were taken, and then a 4 pmol ⋅ min⁻¹ ⋅ kg⁻¹ dose of GIP was infused into the jugular vein. After 5 min of infusion, glucose (1 g/kg) was administered via an IP injection. Blood samples (500 μl) were collected from the tip of the nicked tail, and plasma insulin was assayed using radioimmunoassay. B and D were obtained by taking the AUC from the time course studies in A and C. *Statistical significance (n = 4, P < 0.05). Values are expressed as means ± SE.

**FIG. 3.** Insulin responses from perfused pancreas of control (A and C) and VDF (B and D) rats. Pancreases were perfused at a rate of 4 ml/min with Krebs buffer. At 4 min (t = 4) after equilibration, the preparations were subjected to 8.8 mmol/l glucose (○, glucose alone). GIP (●, 10 pmol/l) or GLP-1 (△, 50 pmol/l) was then added via an infusion pump and a side arm at 20 min. Samples were collected every minute and assayed for insulin content using radioimmunoassay. The AUCs (C and D) were determined using Graphpad software. Values are expressed as means ± SE. n = 3–6.
GIP receptor mRNA expression in the VDF rat islets.
The cAMP data suggested that a decrease of GIP receptor expression could be responsible for the decreased effectiveness of GIP in the fatty Zucker rat. This hypothesis was tested by carrying out real-time RT-PCR on RNA isolated from islets of lean and fat animals. We observed a significant (75% ± 5%) decrease in GIP receptor mRNA in the islets from the fatty Zucker rats, as seen in Fig. 6A. Additionally, the reduction of GIP receptor mRNA was obtained when measured with RT-competitive PCR—an alternate means of measuring RNA expression (data not shown).

GIP receptor protein expression in the Zucker rat islets. The Western blot in Fig. 6B is representative of those obtained when comparing fat and lean islet GIP receptor protein content. The posttranslationally modified GIP receptor appears to run at ~65 kDa, which is in agreement with previous work (35). Figure 6B illustrates a marked decrease in GIP receptor protein level in islets from VDF rats. This decrease is in accordance with that seen with mRNA levels as well as insulin release and cAMP stimulation of islets with GIP.

DISCUSSION
Human type 2 diabetic subjects have been characterized by a decreased incretin response (21,26). This response has been attributed to dysfunction in the GIP portion of the enteroinsular axis because GLP-1 continues to have both insulinotropic and blood glucose–lowering actions in these individuals (22,26,36). The current study examined the expression and function of the GIP receptor on the pancreatic β-cell of the VDF rat—an animal model for type 2 diabetes. It was shown for the first time that there is a decreased level of GIP receptor mRNA and protein in the pancreatic islets of animals exhibiting characteristics of type 2 diabetes: hyperinsulinemia and insulin resistance. This decrease in GIP receptor resulted in a decreased insulin response to physiological levels of GIP.

The VDF rats used in this study were both glucose intolerant (Fig. 1) and hyperinsulinemic (Figs. 2 and 3). Additionally, these animals did not exhibit a second-phase insulin response to glucose (Fig. 3) and, therefore, have developed a syndrome similar to that observed in human type 2 diabetes. The pancreas perfusions (Fig. 3) demonstrated that there is a blunted first-phase insulin secretion in the fat animals: 125% increase compared with a 600% increase in lean animals in response to the introduction of 8.8 mmol/l glucose. Furthermore, in the normal lean animal, 10 pmol/l GIP stimulated a characteristic biphasic insulin response, whereas this hormone had no effect on second-phase insulin secretion in the fat animal (Fig. 3A and B). Interestingly, 10 pmol/l GIP and 50 pmol/l GLP-1 had similar insulinotropic effects in the perfused pancreas from the lean animals (Fig. 3C); however, only GLP-1 was able to potentiate glucose-induced insulin secretion from the VDF pancreas (Fig. 3D). The perfusion data demonstrate that GIP was able to significantly increase first-
Recent studies have shown that the incretin system plays a crucial role in the regulation of insulin secretion. In islets from lean animals, increased exposure to 10 nmol/l GIP may have resulted in desensitization of the β-cell surface GIP receptors, and no further significant effect of GIP on insulin secretion was observed (29). Recently, there has been considerable interest in using dipeptidylpeptidase IV (DPIV) inhibitors in type 2 diabetes therapy (37,38). Circulating DPIV inactivates both GLP-1 and GIP by cleaving the NH2-terminal dipeptide from the parent incretin polypeptide, rendering both peptides biologically inactive and thereby decreasing the circulating half-life of the incretins (39). Currently, there is controversy as to whether GIP levels are elevated, normal, or lowered in type 2 diabetes and animal models for the disease (24–27,40). One possible explanation for the ineffectiveness of GIP in the VDF rat is an increase in DPIV in these animals, which could inactivate GIP prior to its actions on the β-cell. Because it was impossible to measure differences in intact versus NH2-terminally truncated GIP with our radioimmunoassay, it was not possible to determine the role of DPIV in our findings, nor was it possible to determine the concentration of bioactive GIP in these animals. However, it has been demonstrated by Pederson et al. (41) that the levels of circulating DPIV in VDF rats and their lean littermates are similar; therefore, this explanation for GIP ineffectiveness can be ruled out.

Exposure of the rat islet to GIP leads to desensitization of the islet to GIP (29). Currently, it is not clear whether this is due to a reversible phosphorylation of the GIP receptor, a decrease in GIP receptor mRNA expression, or both. However, it was demonstrated that there was a rapid homologous desensitization of GIP-stimulated insulin secretion in mouse β-cells (βTC3) that occurred at the receptor level as well as further downstream in the signaling cascade. In contrast, in the current study, the cAMP response to GIP in the islets of the VDF animals suggests there is a defect in the GIP receptor–adenylyl cyclase segment of the GIP receptor signaling pathway. Furthermore, we were unable to measure elevated ambient GIP levels in the VDF rat, indicating that GIP is not causing a loss of functional cell surface receptors, either by desensitization or downregulation, in these animals at this level of glucose intolerance. Expression of other G-protein–coupled receptors (such as the glucagon receptor) in the superfamily are regulated by glucose as well as the adenylyl cyclase activator forskolin (42). Thus, it is likely that GIP receptor downregulation and dysfunction are a result of inappropriate stimulation of the β-cell by abnormal levels of metabolites and hormones in this animal model.

The current study demonstrates for the first time that GIP receptor expression is downregulated at the mRNA and protein levels in the pancreatic β-cell of the diabetic Zucker rat (Fig. 6A and B). These observations suggest that there is a decrease in receptor expression on the pancreatic β-cell and that this decrease in expression leads to the loss of islet sensitivity to GIP. Furthermore, we have recently demonstrated in clonal β-cells that changes in mRNA expression also produce changes in cell surface GIP receptor expression using radioligand saturation binding curves (F.C.L., unpublished data). Therefore, we believe that there is a decrease in cell surface GIP receptor concomitant to the decrease in total GIP receptor mRNA and protein in these islets.

Recently, Béguin et al. (43) demonstrated that stimulation of the β-cell by GIP caused phosphorylation of the Kir6.2 (KATP) channel via protein kinase A on serine 372. Phosphorylation of this serine residue led to an increased open probability of the channel. This article first demonstrates that GIP stimulation of the β-cell leads to protein phosphorylation. However, the physiological basis for this phosphorylation event is still unclear since Béguin et al. believe that Kir6.2 is maximally phosphorylated in the basal state. It is tempting to speculate that decreased

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**FIG. 6.** A: GIP receptor mRNA levels in the islets of control (■, Fa/?), and VDF (□, fa/fa) rats. Islets were isolated as described in Research Design and Methods. After isolation, RNA was extracted from the islets, and 1 μg pancreatic islet RNA was subjected to real-time RT-PCR. GIP receptor mRNA was normalized to GAPDH mRNA content and expressed as a fraction of control islet content. Data are expressed as means ± SE (n = 4). *Statistical significance (P ≤ 0.05). B: GIP receptor protein expression in the islets of control (Fa/?) and VDF (fa/fa) rats. Islets were isolated as described in Research Design and Methods. After isolation, islets were lysed in ice-cold RIPA buffer. Then, 50 μg total cellular protein was run on a 13% polyacrylamide gel. The gel was transferred to a nitrocellulose membrane and blotted with GIP receptor antibody, followed by horseradish peroxidase–conjugated goat anti-rabbit secondary antibody. The immunoreactive bands were visualized using enhanced chemiluminescence, and GIP receptor molecular weight (65 kDa) was determined using Kt analysis. n = 3.
levels of GIP receptor on the β-cell surface result in a decrease in the phosphorylation state of the K<sub>ATP</sub> channel and decreased open probability. This in turn would lead to membrane depolarization and insulin secretion. If this receptor deficit was great enough, there could be uncoupling of glucose-stimulated insulin secretion and concomitant β-cell decompensation, as observed in fatty rats.

In conclusion, glucose tolerance and insulin responses were studied after GIP infusion in the Vancouver Zucker diabetic fatty rat. In these animals, GIP did not potentiate glucose-induced insulin secretion, either in vivo or from the perfused rat pancreas and isolated perfused rat islets, whereas the GLP-1 response remained intact. Moreover, GIP failed to stimulate cAMP production in isolated fat/α fat islet static incubations. Finally, GIP receptor mRNA and protein levels were shown to be downregulated in the islets of these animals, and this was hypothesized to be the basis for their insensitivity to GIP. This study suggests that the decreased GIP response observed in this animal model and in type 2 diabetes may be a result of a downregulation of the pancreatic GIP receptor.

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