Original Article

Dominant-Negative α-Subunit of Farnesyl- and Geranyltransferase Inhibits Glucose-Stimulated, but Not KCl-Stimulated, Insulin Secretion in INS 832/13 Cells

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The majority of small G-proteins undergo posttranslational modifications (e.g., isoprenylation) at their C-terminal cysteine residues. Such modifications increase their hydrophobicity, culminating in translocation of the modified proteins to their relevant membranous sites for interaction with their respective effectors. Previously, we reported glucose-dependent activation and membrane association of Rac1 in INS 832/13 cells. We also demonstrated modulatory roles for Rac1/GDP dissociation inhibitor in glucose-stimulated insulin secretion (GSIS) in INS 832/13 cells, further affirming roles for Rac1 in GSIS. Herein, we demonstrate that geranylgeranyltransferase inhibitor-2147 (GGTI-2147), an inhibitor of protein prenylation, markedly increased cytosolic accumulation of Rac1 and elicited significant inhibition of GSIS from INS 832/13 cells. In the current study, we also examined the localization of protein prenyltransferases (PPTases) and regulation of GSIS by PPTases in INS 832/13 cells. Western blot analyses indicated that the regulatory α-subunit and the structural β-subunit of PPTase holoenzyme are predominantly cytosolic in their distribution. Overexpression of an inactive mutant of the regulatory α-subunit of PPTase markedly attenuated glucose- but not KCl-induced insulin secretion from INS 832/13 cells. Together, our findings provide the first evidence for the regulation of GSIS by PPTase in INS 832/13 cells. Furthermore, they support our original hypothesis that prenylation of specific G-proteins may be necessary for GSIS. *Diabetes* 56:204–210, 2007

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ELISA, enzyme-linked immunosorbent assay; FTase, farnesyltransferase; GDI, GDP dissociation inhibitor; GGTase, geranylgeranyltransferase; GGTI, GGTase inhibitor; GSIS, glucose-stimulated insulin secretion; PPTase, protein prenyltransferase.

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Insulin secretion from pancreatic β-cells is regulated principally by the ambient concentration of glucose. However, the molecular and cellular mechanisms underlying the stimulus-secretion coupling of glucose-stimulated insulin secretion (GSIS) from the pancreatic β-cell remain only partially understood. In this context, it is well established that the signaling steps involved in GSIS from the β-cell involve well-regulated trafficking of insulin-laden secretory granules for their docking and fusion with the plasma membrane (1–3). Emerging evidence also suggests that such cellular events are under the fine control of small G-proteins, which have been implicated in cytoskeletal remodeling to facilitate granule movement (3,4). Original observations from multiple laboratories, including our own, demonstrated critical involvement of small G-proteins, such as Rac1, Cdc42, Rap1, and ARF6 (ADP-ribosylation factor 6) in GSIS from normal rat islets, human islets, and clonal β-cell preparations (3,5–23). Such conclusions were primarily based on data from experiments using *1*) inhibitors of requisite posttranslational modifications of these G-proteins; *2*) clostridial toxins, which monoglucosylate and inactivate specific G-proteins; and *3*) gene depletion (5–23). Despite this compelling evidence, the precise biochemical mechanisms underlying glucose-mediated activation of these signaling proteins leading to GSIS remain only partially understood.

The majority of small G-proteins and the γ-subunits of trimeric G-proteins undergo posttranslational modification steps (e.g., isoprenylation) at their C-terminal cysteine residues. Such modifications are felt to be responsible in targeting the modified proteins to specific membranous compartments for optimal interaction with their effector proteins (3,4). The prenylating enzymes farnesyltransferase (FTase) and geranylgeranyltransferase (GGTase) catalyze the incorporation of either a 15-carbon (farnesyl moiety) or a 20-carbon (geranylgeranyl moiety) derivative of mevalonic acid into the C-terminal cysteine residues of the candidate proteins (3,4). Examples of farnesylated proteins include Ras, nuclear lamin B, certain proteins involved in visual signal transduction, and fungal mating factors. Small G-proteins, such as Cdc42, Rac and Rho, as well as most γ-subunits of trimeric G-proteins (other than γ-subunit of transducin, which is farnesylated), represent some examples of geranylgeranylated proteins (3,4). As mentioned above, several earlier studies have verified putative roles for G-protein prenylation in GSIS; this was accomplished primarily via the use of inhibitors of protein

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prenyltransferases (PPTases). For example, original studies from our laboratory and by others have demonstrated significant reduction in GSIS by lovastatin, a generic inhibitor of protein prenylation, in normal rat islets and clonal β-cells (12,13). More recently, using specific inhibitors of FTase and GGTases (e.g., allyl- or vinylfarnesols and geranylgeraniols), we have been able to further confirm potential roles for prenylation steps in GSIS (14). Along these lines, it has been reported that at least two members of the Rho subfamily of G-proteins (e.g., Cdc42 and Rac1), both of which require isoprenylation for their membrane targeting, have been implicated in GSIS (3,12,13,24). Despite this convincing evidence to suggest key functional roles for protein prenylation in GSIS, virtually no information is available with regard to the identity and regulatory roles for PPTases in islet β-cell function.

To date, three distinct PPTases have been described in the literature (25–28). FTase and GGTase-I are also referred to as CAAX PPTases because they share the CAAX motif around the C-terminal cysteine region of their substrate proteins. GGTase-II (also referred to as Rab GGTase) prenylates the Rab subfamily of proteins at a different motif, and hence this group of PPTases is often referred to as non-CAAX PPTases (25–28). FTase and GGTase-I and -II are heterodimeric (i.e., consisting of α- and β-subunits) in nature. Interestingly, both FTase and GGTase-I share a common α-subunit (48 kDa) and different β-subunits with apparent molecular weights of 46 and 43 kDa, respectively, for FTase-β and GGTase-1β. The α-subunit is the regulatory subunit, whereas the β-subunit confers substrate specificity. The molecular sizes of GGTase-II α- and β-subunits are reported to be 60 and 38 kDa, respectively (25–28). Despite the aforementioned evidence for regulatory roles for protein prenylation in GSIS, very little is known to date on the localization, identity, and potential regulation of PPTases in the β-cell. Therefore, in the current study, we determined: *1*) the immunological localization, subcellular distribution, and identification of regulatory and structural subunits of PPTases in INS 832/13 cells, and *2*) the potential consequences of overexpression of a dominant-negative mutant of the α-subunit of FTase/GGTase (dn FTase/GGTase) on glucose- and KCl-induced insulin secretion in insulin-secreting INS 832/13 cells.

RESEARCH DESIGN AND METHODS

Antisera directed against Rac1, Cdc42, and GGTase-β were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antisera raised against FTase/GGTase α-subunit and FTase β-subunit were from Transduction Laboratories (San Diego, CA). The rat insulin enzyme-linked immunosorbent assay (ELISA) kit was from American Laboratory Products (Windham, NH).

Normal rat islets and INS 832/13 cells. Islets were isolated from normal Sprague-Dawley rats using the collagenase digestion method (7,23). INS 832/13 cells were provided by Dr. Chris Newgard (Duke University School of Medicine, Durham, NC) (8,23).

Expression plasmid for the dn FTase/GGTase α-subunit. The pUC18/FTase α-subunit and pCMV-Tag 2A plasmids were separately digested with *Eco*RI restriction enzyme at 37°C for 2 h. The DNA from each of these reactions was ethanol-precipitated and resuspended in 100 μl of Tris-EDTA (10 mmol/l Tris and 1 mmol/l EDTA). In a reaction volume of 20 μl, a 3:1 (wt/wt) mixture of pUC18FTase α-subunit cDNA and pCMV-Tag 2A cDNA was incubated with T4 ligase at room temperature for 1 h. Competent cells were plated onto LB (Luria-Bertani broth)-kanamycin (50 μg/ml) plus agar and incubated at 37°C overnight, and colonies were isolated for plasmid preparation. Diagnostic restriction digest and gel analyses were performed to determine the presence and orientation of the FTase α-subunit gene in the pCMV-Tag 2A expression vector (29–31).

Transfection of dn FTase/GGTase α-subunit into INS 832/13 cells. INS 832/13 cells were subcultured at 70–80% confluence and transfected using Effectene (Qiagen, Valencia, CA), with a maximum 0.2 μg of expression vector only (pCMV-Tag 2A) or the expression vector containing the mutated FTase/GGTase α-subunit (pCMV-Tag2A/FTase α-subunit). After transfection, cells were cultured overnight in the presence of 5 mmol/l glucose and 2.5% fetal bovine serum. After preincubation in the presence of 2 mmol/l glucose for an additional 60 min, cells were incubated in the presence of low glucose (5 mmol/l), high glucose (25 mmol/l), or KCl (40 mmol/l) for 30 min at 37°C. The supernatant was then removed and centrifuged at 300*g* for 10 min, and the amount of insulin released was quantitated by ELISA (23).

Insulin release studies involving GGTase inhibitor-2147. INS 832/13 cells were treated with either diluent alone or GGTase inhibitor-2147 (GGTI-2147; 10 μmol/l) and cultured overnight in low-glucose media. Cells were further incubated in the presence of either low (5 mmol/l) or high (25 mmol/l) glucose for 30 min at 37°C in the continuous presence of either GGTI-2147 or diluent as indicated. Insulin released into the medium was quantitated by ELISA (23).

Subcellular distribution of FTase/GGTase in INS 832/13 cells. Total membrane and soluble fractions were isolated according to a previously described method (29,30). In brief, INS 832/13 cell lysates were subjected to single-step centrifugation at 105,000*g* for 60 min. Total particulate and soluble fractions were separated and used for the determination of relative abundance of FTase/GGTase α-subunit by Western blotting. The secretory granule fraction was isolated as previously described (6,8,16–18). In brief, INS 832/13 cells were homogenized in 250 mmol/l mannitol, 70 mmol/l sucrose, and 5 mmol/l HEPES, pH 7.4, containing 1 mmol/l EGTA, 1 mmol/l dithiothreitol, and protease inhibitor cocktail. The homogenates were spun at 600*g* for 5 min to remove the nuclear and cell debris pellet. The resulting supernatant (postnuclear supernatant) was centrifuged at 5,500*g* for 10 min to yield a pellet enriched in mitochondria. The resulting postmitochondrial supernatant was further centrifuged at 25,000*g* for 20 min to yield a secretory granule–enriched pellet. The purified plasma membrane fraction was obtained according to Hubbard and Ma (32) and Nevins and Thurmond (33). Briefly, the postnuclear pellet from the initial 600*g* centrifugation (as described above) was mixed with 1 ml of buffer A consisting of 250 mmol/l sucrose, 1 mmol/l $MgCl_2$, and 10 mmol/l Tris-HCl, pH 7.4, and 2 volumes of buffer B consisting of 2 mol/l sucrose, 1 mmol/l $MgCl_2$, and 10 mmol/l Tris-HCl, pH 7.4. The mixture was then overlaid with buffer A and centrifuged at 113,000*g* for 1 h to obtain an interface primarily containing the plasma membrane. The interface was then diluted with homogenization buffer for centrifugation at 3,000*g* for 10 min, and the resulting pellet was collected as the plasma membrane fraction, the purity of which was assessed by the relative enrichment of E-cadherin, a plasma membrane marker previously described (23). The relative abundance of FTase/GGTase α-subunit in different fractions was determined by Western blotting (as above).

Translocation and membrane association of Rac1 in INS 832/13 cells. This was determined according to a previously described method (29,30). In brief, lysates derived from low- or high-glucose–treated INS 832/13 cells transfected with either the expression vector or the inactive mutant of FTase/GGTase α-subunit were subjected to single-step centrifugation at 105,000*g* for 60 min. Total membranous and cytosolic fractions were separated and used for the determination of relative abundance of Rac1 in these fractions by Western blotting (23).

Rac1 and FTase/GGTase α-subunit coimmunoprecipitation. INS 832/13 cell lysate proteins (500 μg protein) were precleared with respective preimmune serum and Protein G–Agarose, and the supernatants were then incubated overnight with either anti-FTase/GGTase α-subunit or with preimmune serum at 4°C. After this, Protein G–Agarose (20 μl) was added, and the mixture was further incubated for an additional 4 h at 4°C. The beads were briefly washed (3×) with 0.5 ml PBS and reconstituted in Laemmli buffer, and solubilized proteins were resolved by SDS-PAGE. Relative abundance of Rac1 in the immunoprecipitates was determined by Western blotting.

Other assays. Protein concentration was quantitated according to Bradford using BSA as the standard.

Statistical analyses. The statistical significance of the differences between the experimental conditions was determined by Student's *t* test. *P* values <0.05 were considered significant.

RESULTS

As stated above, several earlier studies from our laboratory have implicated the Rho subfamily of G-proteins (e.g., Rac1) in physiological insulin secretion (23). We and others have demonstrated that insulin secretion induced by glucose or mitochondrial fuels (e.g., succinic acid methyl ester) requires the intermediacy of protein prenylation (3,10,12,13,22). Therefore, as a logical extension to those findings, we determined whether GGTI-2147, a spe-

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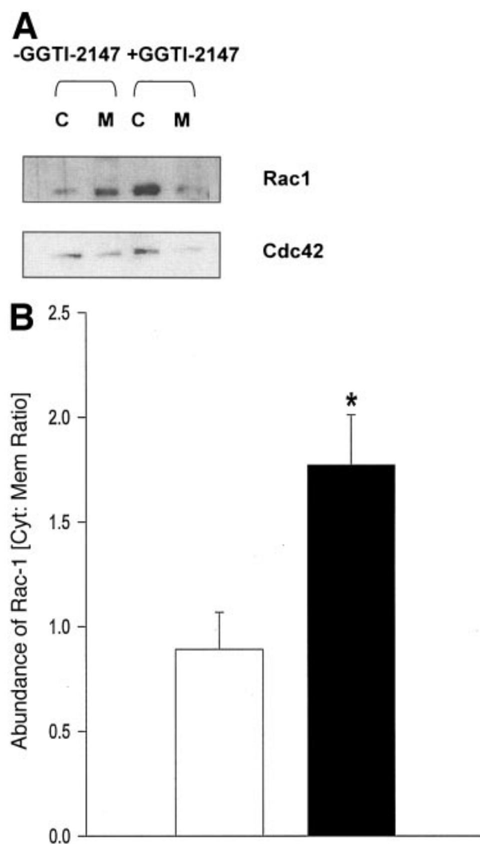

FIG. 1. Incubation of INS 832/13 cells with GGTI-2147 results in cytosolic accumulation of Rac1 and Cdc42. *A*: Lysates from INS 832/13 cells treated with either diluent alone or GGTI-2147 (10 μmol/l, 24 h) were separated into cytosolic (C) and membranous (M) fractions by a single-step centrifugation method (see RESEARCH DESIGN AND METHODS). Proteins from each fraction were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The membranes were then blocked and incubated with anti-Rac1 or anti-Cdc42 (1:1,000 dilutions, 1 h) followed by incubation with horseradish peroxidase–conjugated secondary antibody. Immune complexes were detected using an enhanced chemiluminescence kit. A representative blot of three independent experiments yielding similar results is shown here. *B*: Relative intensities of the Rac1 bands were quantified by densitometry and plotted as the cytosol (Cyt) versus membrane (Mem) ratio. Data are the means ± SE from three independent experiments. □, −GGTI-2147; ■, +GGTI-2147. $^{*}P < 0.05$.

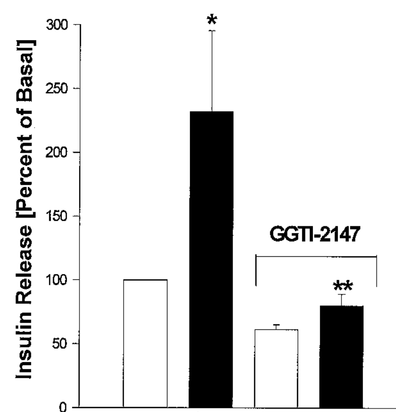

FIG. 2. GGTI-2147 inhibits GSIS from INS 832/13 cells under conditions promoting cytosolic accumulation of Rac1. INS 832/13 cells were treated with either diluent alone or GGTI-2147 (10 μmol/l) and cultured overnight in low-glucose media. Cells were further incubated in the presence of either low (5 mmol/l) or high (25 mmol/l) glucose for 30 min at 37°C in the continuous presence of either GGTI-2147 or diluent as indicated in the figure. Insulin released into the medium was quantitated by ELISA. Data are the means ± SE from three independent experiments. $^{*}P < 0.05$ vs. 5 mmol/l glucose; $^{**}P < 0.05$ vs. 25 mmol/l glucose alone. □, 5 mmol/l glucose; ■, 25 mmol/l glucose.

cific inhibitor of prenylation, inhibits the prenylation of Rac1, thereby promoting its accumulation in the cytosolic compartment. To determine this, we first examined the relative abundance of Rac1 in cytosolic and membranous fractions derived from INS 832/13 cells treated in the absence (diluent alone) or presence of GGTI-2147 (10 μmol/l, 24 h). Data in Fig. 1*A* indicate that incubation of these cells with GGTI-2147 results in a significant accumulation of Rac1 in the cytosolic fraction. The cytosolic-to-membranous ratio of Rac1 represented 0.891 ± 0.18 versus 1.77 ± 0.24 in control versus GGTI-treated cells ($n = 3$ independent experiments) (Fig. 1*B*). It must be added that Cdc42, another geranylgeranylated protein, which we and others have implicated in GSIS (3,6,7,9,10,22), also accumulated in the cytosolic fraction in INS 832/13 cells after incubation with GGTI-2147 (Fig. 1*A*). Together, these data further support our original hypothesis that inhibition of prenylation leads to cytosolic accumulation of specific G-proteins (e.g., Rac1 and Cdc42) in insulin-secreting cells.

We next determined the effects of GGTI-2147 on GSIS in INS 832/13 cells under conditions promoting cytosolic accumulation of specific G-proteins (10 μmol/l, 24 h). Data in Fig. 2 demonstrate that incubation of INS 832/13 cells with GGTI-2147 results in complete inhibition of GSIS. These data further support our original hypothesis, as well as data accrued using pharmacological inhibitors (12–14,22), that inhibition of geranylgeranylation of candidate G-proteins (e.g., Rac1) results in their accumulation in the cytosolic fraction with a concomitant inhibition of GSIS.

To directly demonstrate that PPTases are necessary for GSIS, we next determined, by Western blotting, the localization of the regulatory and structural subunits of PPTases in lysates derived from normal rat islets and INS 832/13 cells. Data in Fig. 3*A* indicate that both the regulatory α-subunit of FTase/GGTase (common to both; see above), and the structural β-subunits of FTase or GGTase are detectable in lysates from normal rat islets and INS 832/13 cells. The next series of studies was directed at determining the subcellular localization of PPTases in insulin-secreting cells. We determined, by Western blotting, the relative abundance of PPTase α-subunit in the postnuclear supernatant, secretory granules, and purified plasma membrane fractions isolated from INS 832/13 cells (see RESEARCH DESIGN AND METHODS for isolation procedures). Data in Fig. 3*B* indicate that FTase/GGTase α-subunit is predominantly localized in the postnuclear supernatant fraction. We observed little or no localization of this protein in the plasma membrane or the secretory granule fractions. To further determine the precise localization of this protein, we subjected the postnuclear supernatant to single-step centrifugation (105,000*g* for 60 min) to isolate the total particulate or soluble fractions, and we then determined the relative abundance of FTase/GGTase α-subunit by Western blotting. Data in Fig. 3*C* demonstrate that the FTase/GGTase α-subunit is predominantly cytosolic in its distribution in INS 832/13 cells.

We next determined potential consequences of overexpression of dn FTase/GGTase on GSIS from INS 832/13 cells. To examine this, INS 832/13 cells were transfected with either the empty vector or with dn FTase/GGTase (see RESEARCH DESIGN AND METHODS for additional details). Insulin secretion was quantitated in these cells in the

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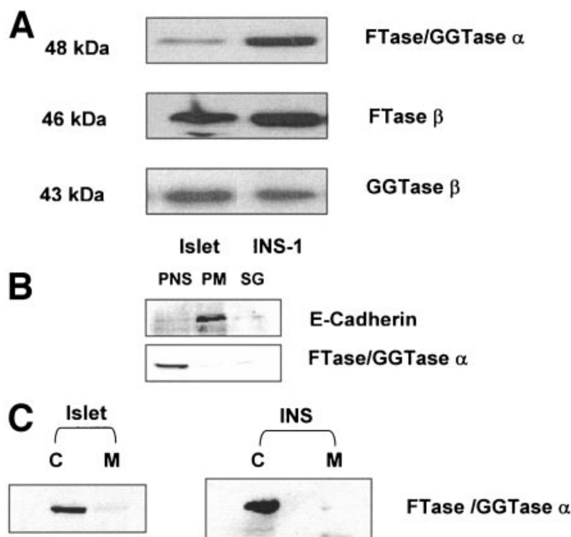

FIG. 3. Immunological detection of regulatory and structural subunits of prenyltransferases in normal rat islets and INS 832/13 cells. *A*: INS 832/13 or islet protein lysates (as indicated in the figure) were separated by SDS-PAGE followed by transfer to a nitrocellulose membrane. The membranes were then blocked and incubated with anti–FTase/GGTase-α, –FTase-β, or –GGTase-β antibodies (1:1,000 dilutions, 1 h) followed by incubation with horseradish peroxidase–conjugated secondary antibody. Immune complexes were detected using an enhanced chemiluminescence kit. A representative blot from three experiments yielding similar results is shown. *B*: INS 832/13 cell protein from the different fractions isolated were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The membranes were then blocked and incubated with anti–FTase/GGTase-α or –E-cadherin (1:1,000 dilutions, 1 h) followed by incubation with horseradish peroxidase–conjugated secondary antibody. Immune complexes were detected using an enhanced chemiluminescence kit. A representative blot of three independent experiments yielding similar results is shown here. *C*: INS 832/13 cell or islet protein lysates were separated into cytosolic (C) and membraneous (M) fractions by a single-step centrifugation method (see RESEARCH DESIGN AND METHODS). Relative abundance of FTase/GGTase-α was determined by Western blotting as in *A* and *B*. A representative blot of three independent experiments yielding similar results is shown here. PM, plasma membrane; PNS, postnuclear supernatant; SG, secretory granules.

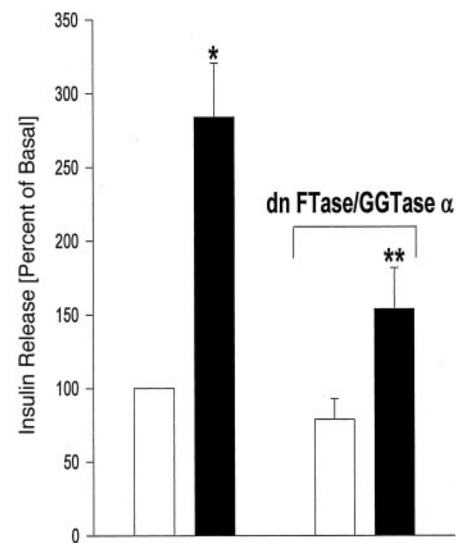

FIG. 4. Overexpression of the α-subunit of dn FTase/GGTase inhibits GSIS in INS 832/13 cells. INS 832/13 cells were transfected with either expression vector or dn FTase/GGTase α-subunit (see RESEARCH DESIGN AND METHODS), and cells were cultured overnight in the presence of 5 mmol/l glucose and 2.5% fetal bovine serum. After preincubation in the presence of 2 mmol/l glucose for an additional 60 min, cells were incubated in the presence of low (5 mmol/l) or high (25 mmol/l) glucose for 30 min at 37°C. Insulin released into the medium was quantitated by ELISA. Data are the means ± SE from three independent experiments. *$P < 0.05$ vs. 5 mmol/l glucose; **$P < 0.05$ vs. 25 mmol/l glucose alone. □, 5 mmol/l glucose; ■, 25 mmol/l glucose.

presence of either 5 or 25 mmol/l glucose. Data in Fig. 4 demonstrate that although the basal secretion remained unchanged, the degree of GSIS was markedly reduced (~50%) in cells expressing the dn FTase/GGTase mutant.

We previously reported a significant degree of translocation of Rac1 in isolated β-cells after stimulation by glucose; based on this, we hypothesized that this might represent a key signaling step in GSIS (8,23). In the next set of experiments, we determined the ability of glucose to promote membrane association of candidate G-proteins (e.g., Rac1) in INS 832/13 cells in which protein prenylation is compromised via overexpression of dn FTase/GGTase. To determine this, INS 832/13 cells were transfected with either vector alone or dn FTase/GGTase, and they were then incubated in the presence of either 5 or 25 mmol/l glucose. Relative abundance of Rac1 in the cytosolic and membrane fractions isolated from these cells was determined by Western blotting. Data in Fig. 5 indicate a significant degree of translocation of Rac1 to the membrane fraction, by high glucose, in cells expressing the empty vector alone. However, such a stimulatory effect by glucose on Rac1 translocation to the membrane fraction could not be observed in cells expressing dn FTase/GGTase (Fig. 5). Instead, Rac1 was found to accumulate in the cytosolic fraction in these cells, thus affirming our original hypothesis that inhibition of prenylation of specific G-proteins (e.g., Rac1) leads to their accumulation in the cytosolic fraction, culminating in impaired GSIS (see below).

Finally, we determined whether insulin secretion elicited by a membrane-depolarizing concentration of KCl (40 mmol/l) also requires the intermediacy of protein prenylation. To achieve this, INS 832/13 cells were transfected with vector alone or dn FTase/GGTase, and insulin secretion in the presence of either basal glucose or 40 mmol/l KCl was quantitated. Data in Fig. 6 demonstrate that unlike GSIS (Fig. 4), insulin secretion elicited by KCl remained resistant to inhibition of protein prenylation achieved after overexpression of dn FTase/GGTase. These findings raise an interesting possibility that G-proteins, which are prenylated specifically by FTase/GGTase-I (see above), might

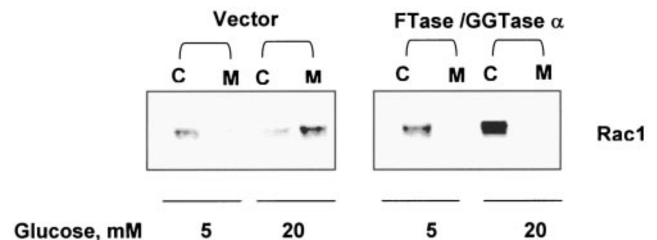

FIG. 5. The ability of glucose to promote translocation of Rac1 to the membranous fraction is significantly reduced in INS 832/13 cells overexpressing the α-subunit of dn FTase/GGTase. INS 832/13 cells were transfected with vector alone or dn FTase/GGTase as described in RESEARCH DESIGN AND METHODS, and cells were cultured overnight in the presence of 5 mmol/l glucose and 2.5% fetal bovine serum. After preincubation in the presence of 2 mmol/l glucose for an additional 60 min, cells were incubated in the presence of low (5 mmol/l) or high (25 mmol/l) glucose for 30 min at 37°C. Protein lysates were separated into cytosolic (C) and membraneous (M) fractions by a single-step centrifugation method (see RESEARCH DESIGN AND METHODS). Relative abundance of Rac1 was determined by Western blotting as described under RESEARCH DESIGN AND METHODS. A representative blot of three independent experiments yielding similar results is shown here.

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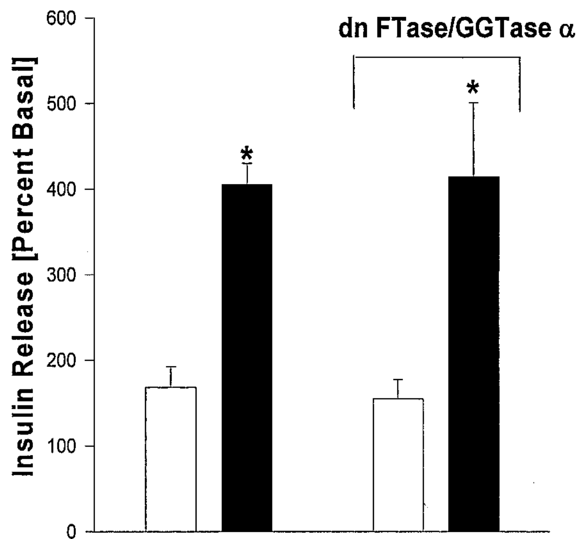

FIG. 6. KCl-induced insulin secretion is resistant to inhibition of protein prenylation accomplished via overexpression of the α-subunit of dn FTase/GGTase in INS 832/13 cells. INS 832/13 cells were transfected with either vector alone or with dn FTase/GGTase α-subunit as described in RESEARCH DESIGN AND METHODS. Cells were cultured overnight in the presence of 5 mmol/l glucose and 2.5% fetal bovine serum. After preincubation in the presence of 2 mmol/l glucose for an additional 60 min, cells were incubated in the presence of low (5 mmol/l) glucose or KCl (40 mmol/l) for 30 min at 37°C. Insulin released into the medium was quantitated by ELISA. Data are the means ± SE from three independent experiments. *P < 0.05 vs. 5 mmol/l glucose. □, 5 mmol/l glucose; ■, 25 mmol/l KCl.

play a significant regulatory role(s) in GSIS, but not KCl-induced insulin secretion (see below).

DISCUSSION

Several lines of evidence implicate regulatory roles for the Rho subfamily of small G-proteins (e.g., Cdc42 and Rac1) in GSIS. Such conclusions were drawn primarily via the use of pharmacological probes (e.g., inhibitors of post-translational prenylation, carboxylmethylation, or fatty acylation) or clostridial toxins, which selectively monoglucosylate and inactivate specific GTPases, such as Rac1 (3). Also, molecular biological approaches (e.g., the use of dominant-negative mutants of candidate G-proteins) provided compelling evidence to further substantiate a role for these signaling proteins in GSIS (3). However, very little is known with regard to localization and/or regulation of PPTases, which catalyze the first committed step in a series of modification steps necessary for Rho G-protein activation. The current study represents the first step in the direction of identification of such enzymes and their regulatory roles in GSIS from the β-cell. Salient findings of our current study are: *1*) GGTI-2147, a known inhibitor of protein prenylation, promotes cytosolic accumulation of geranylgeranylated proteins (e.g., Rac1 and Cdc42) and inhibition of GSIS in INS 832/13 cells; *2*) individual regulatory and catalytic subunits of PPTases are immunologically identifiable in INS 832/13 cells and normal rat islet lysates and are predominantly cytosolic in their distribution; and *3*) overexpression of dn FTase/GGTase α-subunit significantly attenuates GSIS, but not KCl-induced insulin secretion, in INS 832/13 cells.

Previous studies from our laboratory (3,12) and by Li et al. (13) used pharmacological inhibitors of isoprenoid biosynthesis, such as lovastatin, in the demonstration of regulatory roles for protein prenylation in GSIS. It has also been demonstrated that the effects of lovastatin on GSIS are primarily attributable to its ability to deplete endogenous mevalonic acid; coprovision of exogenous mevalonic acid (either as a sodium salt or as its lactone form) completely reversed inhibitory effects of lovastatin on GSIS (3,12,13). The above studies also demonstrated that unlike GSIS, insulin secretion seen in the presence of KCl was resistant to lovastatin (3,12,13). From these findings, it was concluded that mevalonic acid–derived isoprenoid biosynthesis is critical for GSIS, but not KCl-induced insulin secretion. As a logical extension, in subsequent studies, we examined potential roles of PPTases, which transfer isoprenoids into candidate G-proteins endogenous to the islet β-cell. This was accomplished via the use of specific inhibitors of PPTases synthesized in collaboration with Gibbs et al. (14). These studies demonstrated that the chemical inhibitors 3-allyl- and vinylfarnesols and their geranylgeraniol counterparts significantly attenuated GSIS and KCl-induced insulin secretion from βTC3 cells. Findings from the current investigation indicate, however, that overexpression of an inactive mutant of FTase/GGTase α-subunit results in inhibition of only glucose-induced, but not KCl-induced, insulin secretion. Reasons for differences between the pharmacological inhibitors (e.g., allyl- or vinylfarnesols or geranylgeraniols) and the inactive PPTase mutant in their ability to elicit effects on KCl-induced insulin secretion remain unknown at the current time. It is possible that although these findings conclusively demonstrate that the requirement for prenylation of candidate G-proteins (e.g., Rac1) mediated by GGTase-I is relevant for GSIS, it is likely that activation of additional (and yet unidentified) G-proteins, which might be subjected to prenylation by GGTase II (e.g., Rab subfamily of G-proteins; see above), might be relevant for calcium-dependent functions, as demonstrated in other cell types (34–36). In this context it may also be germane to point out that potential inhibitory properties of allyl or vinyl fanresols/geranyl geraniols against GGTase II remain to be determined (Gibbs R., personal communication). Together, our findings clearly imply that prenylation of specific G-proteins catalyzed by FTase/GGTase I is relevant for GSIS.

Our current findings indicate that incubation of INS 832/13 cells with GGTI-2147 results in selective accumulation of G-proteins in the cytosolic fraction and that the ability of glucose to translocate Rac1 to the membrane is significantly reduced in INS 832/13 cells overexpressing the inactive mutant of PPTase (Fig. 5). Several earlier studies in multiple cell types, including the islet β-cell, have suggested that inhibition of prenylation leads to cytosolic accumulation of G-proteins, which is, in part, caused by decreased hydrophobicity of the unmodified proteins (rev. in 3–5). Furthermore, recent studies by Nevins and Thurmond (33) demonstrated significant decreases in the interaction of Cdc42 with caveolin-1, its GDP dissociation inhibitor (GDI), after inhibition of protein prenylation with GGTI-2147 in MIN6 cells. These data suggest that prenylation of G-proteins is critical for optimal interaction with their regulatory proteins. Finally, it is also likely that inhibition of protein prenylation of specific G-proteins (e.g., Rac1) might prevent their degradation, resulting in increased abundance of these proteins in the cytosolic fraction. The current studies do not address the issue of potential effects of inhibition of prenylation on G-protein degradation, as reported earlier (37–39). Additional studies are needed to conclusively demonstrate the

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consequences of inhibition of islet endogenous GGTase I, including inhibition of G-protein hydrophobicity and/or their degradation.

A growing body of evidence also suggests that the essential role for protein prenylation is for optimal interaction of specific G-proteins, such as Rac1, with their regulatory proteins, such as Rho GDI (33,40,41). It is well established that GDI plays a regulatory role in membrane targeting of the active GTP-bound Rac1, as well as sequestration of the GDP-bound (inactive form) of Rac1 (4). Indeed, we recently provided evidence for the localization of Rho GDI in normal rat islets and clonal β-cell preparations and implicated a direct regulatory role(s) for this protein in glucose-induced, but not KCl- or mastoparan-induced, insulin secretion (23). Our data from these studies also provided evidence for possible colocalization and complexation of Rac1 with GDI in the resting β-cell. However, in a limited number of experiments, we failed to detect any colocalization of Rac1 with PPTase α-subunit in resting INS 832/13 cells (additional data not shown). Studies are in progress to determine potential alterations in the subcellular localization and/or association of the PPTase holoenzyme with candidate G-proteins under conditions of GSIS.

How then are these PPTases regulated by glucose in the islet? It is likely that one such regulatory mechanism might involve phosphorylation of the regulatory α-subunit after stimulation by glucose, thereby facilitating PPTase activation and prenylation of candidate G-proteins. This, in turn, leads to association of prenylated G-proteins, such as Rac1, with their regulatory protein GDI. On receipt of appropriate signals, the Rac1/GDI complex then moves to the membrane, where the GDI dissociates from the complex, enabling Rac1 to attain its GTP-bound active conformation for regulation of its effector proteins. Indeed, emerging evidence from M.G.'s laboratory (29–31) demonstrated that insulin stimulates the phosphorylation and activation of the α-subunit of PPTases, thereby providing increased amounts of prenylated p21Ras (farnesylated) and Rho A (geranylgeranylated) necessary for its mitogenic effects (42). They also reported that substitution of alanine for two serine residues (i.e., S60A and S62A) of the α-subunit of FTase/GGTase creates a dominant-negative mutant for both PPTases (the one used herein). Finally, these investigators also demonstrated that overexpression of the dn FTase/GGTase α-mutant markedly attenuated the ability of insulin to increase the activities of FTase/GGTase and the abundance of prenylated p21Ras and Rho A (29,30). Taken together, and at least based on the transfection experiments involving a mutant lacking serine phosphorylation sites, these results have led us to conclude that glucose-mediated effects on insulin secretion require phosphorylation of serine-60 and -62 residues of the PPTase α-subunits. Studies are in progress to precisely determine the effects of glucose on PPTase phosphorylation and functional activation in the events necessary for GSIS.

Finally, it is important to note that the PPTase inactive mutant we used herein is against the common regulatory subunit for FTase/GGTase I. Although our current investigation demonstrated roles for GGTase I activation in GSIS, it is likely that farnesylation of key proteins is also necessary for GSIS. Some of these include the γ-subunits of trimeric G-proteins and nuclear lamins. Along these lines, we have provided evidence (43) for the direct activation, by glucose, of the carboxylmethylation of γ-subunits of trimeric G-proteins in islet β-cells. It should be noted that the carboxylmethylation is not expected to take place until the candidate γ-subunit is prenylated. Together, we believe that the current findings are important because they provide the first evidence to suggest that prenylation of specific G-proteins is necessary for GSIS.

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