Regulatory roles for small G proteins in the pancreatic β-cell: lessons from models of impaired insulin secretion

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Kowluru, Anjaneyulu. Regulatory roles for small G proteins in the pancreatic β-cell: lessons from models of impaired insulin secretion. Am J Physiol Endocrinol Metab 285: E669–E684, 2003;10.1152/ajpendo.00196.2003.—Emerging evidence suggests that GTP-binding proteins (G proteins) play important regulatory roles in physiological insulin secretion from the islet β-cell. Such conclusions were drawn primarily from experimental data derived through the use of specific inhibitors of G protein function. Data from gene depletion experiments appear to further substantiate key roles for these signaling proteins in the islet metabolism. The first part of this review will focus on findings supporting the hypothesis that activation of specific G proteins is essential for insulin secretion, including regulation of their function by posttranslational modifications at their COOH-terminal cysteines (e.g., isoprenylation). The second part will overview novel, non-receptor-dependent mechanism(s) whereby glucose might activate specific G proteins via protein histidine phosphorylation. The third section will review findings that appear to link abnormalities in the expression and/or functional activation of these key signaling proteins to impaired insulin secretion. It is hoped that this review will establish a basis for future research in this area of islet signal transduction, which presents a significant potential, not only in identifying key signaling proteins that are involved in physiological insulin secretion, but also in examining potential abnormalities in this signaling cascade that lead to islet dysfunction and onset of diabetes.

cytokines; posttranslational modifications; histidine phosphorylation; diabetes mellitus; pancreatic islet

GLUCOSE-INDUCED INSULIN SECRETION from pancreatic β-cells is mediated largely via the generation of soluble second messengers, such as cyclic nucleotides, hydrolytic products of phospholipases (A2, C, and D), and adenine nucleotides (44, 48, 59, 60, 73, 81). However, the exact molecular and cellular mechanisms underlying glucose-stimulated insulin secretion remain only partially understood. It is widely accepted that, after its entry into the β-cell (facilitated via the glucose-transporter protein GLUT2), glucose is metabolized with a resultant increase in the ATP/ADP ratio. Such an increase in the intracellular ATP results in the closure of ATP-sensitive K⁺ channels localized on the plasma membrane, as a consequence of which membrane depolarization occurs. This facilitates the influx of extracellular calcium through the voltage-sensitive calcium channels. Increase in intracellular calcium is known to be critical for the transport of insulin-containing secretory granules to the plasma membrane for fusion and release of insulin into circulation (73, 81).

GTP-BINDING PROTEINS IN THE PANCREATIC β-CELL AND THEIR REGULATION BY POSTTRANSLATIONAL MODIFICATIONS

In addition to regulation by adenine nucleotides of glucose-stimulated insulin secretion, earlier studies (59, 73, 81) have examined the contributory roles for guanine nucleotides (i.e., GTP) in physiological insulin secretion. For example, using selective inhibitors of the GTP biosynthetic pathway (e.g., mycophenolic acid), several studies have documented a permissive role for GTP in insulin secretion elicited by glucose (33, 68, 66). Although the precise mechanisms underlying the regulatory role(s) of GTP remain elusive, available evidence indicates that they might involve activation of one (or more) G proteins (44, 48, 85). Two major groups

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of G proteins have been identified in β-cells (44, 48, 85). The first group consists of trimeric G proteins comprised of α (39–43 kDa), β (35–37 kDa), and γ (6–8 kDa)-subunits. These are involved in the coupling of various receptors to their intracellular effectors, such as adenylate cyclase, phosphodiesterase, or phospholipases (6, 17, 85). The second group of G proteins (which is the main focus of this review) is comprised of small-molecular-mass (20–25 kDa) monomeric G proteins, which are involved in protein sorting as well as trafficking of secretory vesicles (see Refs. 39, 44, 48 for reviews). A large body of evidence indicates that this family of G proteins undergoes posttranslational modifications, such as isoprenylation and carboxyl methylation, at their COOH-terminal cysteine residues (often referred to as the CAAX motif; 39, 44, 48).

The first of a four-step modification sequence (Fig. 1) includes incorporation of a 15-carbon (farnesyl) or 20-carbon (geranylgeranyl) isoprenoid moiety, which is derived from mevalonic acid (MVA), onto a cysteine residue toward the carboxyl terminus of the candidate G proteins. This is followed by proteolysis of several amino acids (up to a maximum of three). A carboxyl methylation step then modifies the newly exposed carboxylate anion of the cysteine. In some cases, the covalent addition of a long-chain fatty acid, typically palmitate, at cysteine residues, which are upstream to the CAAX motif, completes the cascade. Such modification(s) are thought to render the modified G proteins more hydrophobic and enable them to associate with membranes for interaction with their respective effectors (39, 44, 48, 92). Because the isoprenylation of G proteins occurs shortly after their synthesis, and because “half-lives” of prenylated proteins are rather long, this is not likely to be an acute regulatory step; however, in many cases, prenylation is necessary to allow candidate G proteins to intercalate into the relevant membrane compartment. In contrast, the methylation and acylation steps (Fig. 1) are subject to acute regulation at the level of the “on” steps (i.e., addition of methyl or acyl groups) as well as the “off” steps (i.e., deletion of methyl or acyl groups). The addition and removal of methyl groups are catalyzed by carboxyl methyl transferase and esterase, respectively. Like-

![Fig. 1. Posttranslational modifications of small G proteins.](http://ajpendo.physiology.org/)

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**Fig. 1.** Posttranslational modifications of small G proteins. The first of the four-step reaction is incorporation of either a 15-carbon (farnesyl) or 20-carbon (geranylgeranyl) carbon derivative of mevalonic acid (MVA) into the COOH-terminal cysteine via a thioether linkage. This reaction is catalyzed by either the farnesyl or geranylgeranyl transferases, respectively. After this, the three amino acids after the prenylated cysteine are removed by a protease of microsomal origin, thereby exposing the carboxylate anion. This site is then methylated by a carboxyl methyl transferase, which transfers a methyl group onto the carboxylate group using S-adenosyl methionine (SAM) as the methyl donor. We have shown that the carboxyl methylation of specific G proteins (e.g., Cdc42) increases their hydrophobicity and translocation to the membrane fraction (see text for additional details). In addition to these, certain G proteins (e.g., H-Ras) have also been shown to undergo palmitoylation at a cysteine residue, which is upstream to the prenylated cysteine. It is thought that palmitoylation provides a “firm” anchoring for the modified protein into the cell membrane for optimal interaction with its respective effector proteins. FPP, farnesyl pyrophosphate; FTase, farnesyl transferase; CMT, carboxyl methyl transferase; PMT, palmitoyl transferase.
wise, addition and deletion of palmitoyl groups are facilitated by palmitoyl transferase and esterase, respectively (58). Studies from our laboratory (2, 35–37, 39, 41–53, 55, 65, 69) and those of others (34, 54, 56, 83) have demonstrated the requisite nature and roles of posttranslational modifications of these proteins in physiological insulin secretion. They are discussed in the following sections.

Islet G Protein Prenylation and Insulin Secretion

Using generic as well as more specific inhibitors (see Table 1), numerous earlier studies have demonstrated critical regulatory roles for protein prenylation in physiological insulin secretion and identified some of these proteins as Cdc42, H-Ras, γ-subunits of trimeric G proteins, and the nuclear lamin-B (2, and see Ref. 48 for a review). Needless to say, this list is only partial. Initial studies that examined possible roles of protein prenylation in islet function utilized statins (56, 69, 106), as they inhibit the synthesis of MVA, a precursor for the biosynthesis of isoprenoid derivatives (e.g., farnesy1 or geranylgeranyl pyrophosphates), which are incorporated into respective proteins to complete the isoprenylation step (Fig. 1). Preincubation of isolated normal rat islets or clonal β-cells with lovastatin has been shown to result in selective accumulation of non-prenylated proteins in the soluble fraction, with a concomitant decrease in their abundance in the membrane fraction. Under these conditions, lovastatin significantly inhibited glucose-stimulated insulin secretion from normal rat islets (69) (Fig. 2) and from bombesin- and vasopressin-mediated insulin secretion HIT-T15 cells (56). Even though the identity of all of the G proteins critical for this process has not been determined, indirect evidence suggests that Cdc42 might represent one such protein. For example, in transformed β-cells, lovastatin reduces prenylation of Cdc42 and thereby impedes its complexing with a GDP-dissociation inhibitor (83). This, in turn, leads to its redistribution from membranes to cytosol, effects not seen with some other monomeric G proteins (e.g., Rho or ADP ribosylation factor (ARF)). Together, data from these studies indicate that inhibition of protein prenylation in β-cells results in selective accumulation of unprenylated G proteins in the soluble compartment, possibly interfering with the interaction of these proteins with their respective effector proteins, which may be required for nutrient-induced insulin secretion. Data from studies using more generic inhibitors of protein isoprenylation (e.g., limonene, perilic acid; see Table 1) were not very conclusive because of their nonspecific and cytotoxic effects on islet function (39, 56, 69).

Recently, we synthesized a novel class of prodrug inhibitors, such as 3-allyl and vinyl-farnesols and 3-allyl and 3-vinyl geranylgeraniols, which inhibited (with a greater specificity) the protein farnesyl and geranylgeranyl transferases, respectively. These two classes of inhibitors significantly reduced glucose- and calcium-stimulated insulin secretion from β-TC3 cells (2). The degree of inhibition was much greater than what was demonstrable in the presence of lovastatin in isolated rat islets, suggesting that they are much more site specific than the classical hydroxymethyl glutaryl-CoA reductase blockers (Table 1). Akin to lovastatin, allyl and vinyl farnesols and geranylgeraniols significantly influenced the subcellular distribution of small G proteins, as evidenced by a considerable degree of accumulation of the unprenylated proteins in the cytosolic fraction, with a concomitant decrease in their abundance in the membrane fraction (2). Together, these cited studies indicate that protein prenylation plays a significant regulatory role in physiological insulin secretion. It is also apparent that a substantial amount of work is still needed, especially in the area of identification of these prenylated proteins, as well as the prenylating enzymes (e.g., isoprenyl transferases). Recent evidence from our laboratory suggests immunological localization of farnesyl and geranylgeranyl transferases in insulin-secreting cells (40). As we have pointed out, even though protein prenylation is not acutely regulable, it seems to dictate the subsequent modification steps (e.g., carboxyl methylation) that are acutely regulated and to determine the functional status of a given G protein.

Islet G Protein Methylation and Insulin Secretion

Unlike protein prenylation, the carboxyl methylation of prenylated cysteine is acutely regulable, and both the methylating and demethylating enzymes have been characterized in mammalian cells, including the
pancreatic β-cell (39, 41, 55). The carboxyl methyl transferase catalyzes the incorporation of a methyl group onto the carboxylate anion of the prenylated cysteine via an ester linkage. It utilizes intracellular S-adenosyl methionine (SAM) as the methyl donor. Several studies, including our own, have identified carboxyl-methylated proteins in the pancreatic β-cell. These include Cdc42, Rap1, Rac1, H-Ras, the γ-subunits of trimeric G proteins, and the nuclear lamin-B (35, 41, 49, 54, 95).

A previous study characterized the prenyl cysteine methyl transferase activity in insulin-secreting cells and normal rat islets (55). This activity was monitored by quantitating the degree of methylation of an artificial substrate [e.g., acetyl farnesyl cysteine (AFC)] with [3H]SAM as methyl donor. Subcellular fractionation studies revealed that this enzyme is localized in the plasma membrane and the endoplasmic reticular fractions. Even though several lines of experimental evidence indicate that the carboxyl methylation of specific G proteins (e.g., Cdc42 and Rap1) is stimulated by exogenous GTP (49, 54), we observed that exogenous GTP had no demonstrable effect on this enzyme, suggesting that this enzyme may be constitutively active within the β-cell, and that the methylation of target proteins in vivo is regulated by the access of these proteins to the methyl transferase, as well as their active GTP-bound conformation (55). It may be germane to point out that, in addition to the carboxyl methylation at COOH-terminal cysteine, we reported methylation of COOH-terminal leucine, especially of the catalytic subunit of protein phosphatase 2A (PP2Ac) (51). Inhibitors of protein phosphatases, such as okadaic acid, inhibited the carboxyl methylation of PP2Ac. Data derived from the inhibitor experiments provide useful insights into the applicability of inhibitors of protein carboxyl methylation for study of putative roles of different proteins in cellular regulation. For example, AFC inhibits the methylation at a COOH-terminal cysteine, whereas okadaic acid specifically inhibits the carboxyl methylation of COOH-terminal leucine (41, 49, 51, 54).

Several earlier investigations have examined the relevance of prenyl cysteine carboxyl methylation in glucose-induced insulin secretion (49, 69) (see Fig. 2). For example, by use of rat islets and cloning β-cells, glucose has been shown to stimulate the carboxyl methylation of Cdc42 and Rap1 in a transient manner. Stimulation of carboxyl methylation of these proteins was demonstrable within 15–30 s after exposure of cells to glucose (49). It was also shown that such an increase in the carboxyl methylation of these proteins was specifically blocked by AFC, because a structurally similar inactive analog of AFC, namely, acetyl geranylgeranyl cysteine (AGGC), was without any effect. Studies from Fleischer’s group (Leiser et al., Ref. 54) have also utilized these specific probes to determine the relative contribution of Rap1, another monomeric G protein, in glucose- and calcium-mediated insulin secretion. Follow-up studies from our laboratory have utilized similar experimental approaches and probes to decipher the roles of the carboxyl methylation of the γ-subunits of trimeric G proteins in glucose-mediated insulin secretion (41).

Finally, by use of specific inhibitors of GTP biosynthesis [e.g., mycophenolic acid (MPA)], it was possible to establish a critical requirement for endogenous GTP in glucose-stimulated carboxyl methylation of specific G proteins and concomitant stimulation of insulin secretion from isolated rat islets (39, 41, 49). Depletion of endogenous GTP markedly reduced the ability of glucose to stimulate the carboxyl methylation of specific islet proteins (e.g., Cdc42, Gγ-subunits of trimeric G proteins) as well as insulin secretion, suggesting that endogenous GTP is essential for these signaling steps leading to insulin secretion (39, 41, 49). Such a formulation was further supported by additional observations indicating that provision of guanosine exogenously to GTP-depleted cells completely reversed the ability of glucose to activate the carboxyl methylation of these two proteins, as well as insulin secretion. The reversal effects appear to be specific for guanosine, since exogenous adenosine failed to reverse the inhibitory effects demonstrable after GTP depletion (39, 41, 49).

These data indicate a clear dependence of endogenous GTP in physiological insulin secretion, presumably mediated by the activation of trimeric as well as monomeric G proteins. The reader is referred to Table 1 for a summary of findings from various laboratories on the effects of inhibitors of protein carboxyl methylation on insulin secretion from isolated β-cells.

Islet G Protein Palmitoylation and Insulin Secretion

As indicated in Fig. 1, fatty acids (typically, palmitate) are incorporated posttranslationally into specific G proteins via a thioester linkage at cysteine residues upstream of the prenylated and methylated cysteine (48, 92, 103). This modification is thought to further facilitate the interaction of G proteins with their membrane-bound effectors. Several previous studies indicated that the α-subunits of trimeric G proteins may be acylated; this is regulated acutely in response to receptor activation, thereby controlling the subcellular distribution of these α-subunits (i.e., membrane vs. cytosolic). Receptor activation has also been shown to regulate protein deacylation (103). Cerulolin, a selective blocker of protein acylation, has been shown to reduce nutrient-induced insulin secretion from isolated rat islets (69) (Fig. 2); these data were further confirmed also in normal rat islets by Yajima et al. (109). Interestingly, cerulolin failed to inhibit insulin secretion facilitated by nonnutrient secretagogues, such as a membrane-depolarizing concentration of potassium, activators of protein kinase A, or mastoparan. Together, these data support a critical regulatory role for protein acylation steps in β-cell function. It may be mentioned that the inhibitory effects of cerulolin (specifically, at higher concentrations and over longer periods of incubation) on protein acylation are rather
nonspecific, because this probe can inhibit fatty acid, sterol, and protein synthesis. 2-Bromopalmitate has also been used to study the roles of protein acylation in cellular function (102). More specific cerulein analogs have been reported recently (13) and await further investigations. Interestingly, experimental and structural data indicate that certain proteins, which undergo prenylation as well as carboxyl methylation (e.g., Cdc42 or \( \gamma \)-subunits of trimeric G proteins), are not subject to fatty acylation (see Ref. 48 for a review). Therefore, it is likely that acylation of \( \alpha \)-subunits of trimeric G proteins and/or other low-molecular-weight G proteins (e.g., Ras) may also be necessary for insulin secretion. Alternatively, other proteins involved in the exocytotic process, such as SNAP-25 (18, 97), may be critically acylated. Additional studies are needed to demonstrate conclusively a putative role(s) for fatty acylation, as well as the identity of candidate G proteins in physiological insulin secretion.

**Use of Clostridial Toxins To Examine the Role of G Proteins in Insulin Secretion**

Several lines of evidence suggest that clostridial toxins serve as extremely useful tools to study putative regulatory roles of the Rho subfamily of G proteins in cellular function (39, 42, 49, 86). These toxins specifically monoglucosylate and inactivate G proteins with reliable specificity (Table 2). For example, *Clostridium difficile* toxins A or B monoglucosylate (at threonine residues) Rho, Rac, and Cdc42 (but not Ras, Rab, or ARF) proteins; this modification impairs the function of these small G proteins. *Clostridium sordellii* lethal toxin monoglucosylates Rac, Rap, and Ras specifically,

### Table 1. Known effects of inhibitors of posttranslational modifications of G proteins on insulin secretion

<table>
<thead>
<tr>
<th>Type of Modification</th>
<th>Observation</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenylation</td>
<td>Significantly inhibited (–46 to 57%) glucose-stimulated insulin secretion from normal rat islets. No significant to minimal effects on phorbol ester-, high potassium- or ( \alpha )-oxo-4-methyl-pentanoic acid-induced insulin secretion</td>
<td>69</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>Inhibited potentiating effects by bombesin and vasopressin of nutrient-induced insulin secretion from HIT-T15 cells. However, potentiating effects by phorbol ester or forskolin were unaffected</td>
<td>55</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>Significantly inhibited glucose-stimulated insulin secretion from single ( \beta )-cells and normal rat islets via inhibition of L-type calcium channels. Simvastatin acid, a less lipophilic inhibitor, was less potent</td>
<td>106</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>No effect on L-type calcium channels and glucose-induced insulin secretion, probably due to its hydrophilicity</td>
<td>106</td>
</tr>
<tr>
<td>Allyl and vinyl farnesols and geranylgeraniols</td>
<td>Significantly inhibited glucose- and calcium-induced secretion from ( \beta )TC3 cells</td>
<td>2</td>
</tr>
<tr>
<td>GGTT-2147</td>
<td>Significantly inhibited glucose- and calcium-induced secretion from ( \beta )TC3 cells</td>
<td>2</td>
</tr>
<tr>
<td>Manumycin</td>
<td>Significantly inhibited glucose- and calcium-induced secretion from ( \beta )TC3 cells</td>
<td>2</td>
</tr>
<tr>
<td>Methylation</td>
<td>Acute exposure to rat islets attenuated glucose-stimulated (–40 to 60%) and ( \alpha )-oxo-4-methyl-pentanoate-induced (–68 to 85%) insulin secretion. Acetyl geranyl cysteine (AGC), an inactive analog of AFC, had no effect on glucose-stimulated insulin release. AFC had no effect on mastoparan- or high-potassium-stimulated insulin secretion</td>
<td>69</td>
</tr>
<tr>
<td>Acetyl farnesyl cysteine</td>
<td>Stimulated basal, calcium- or GDPγs-induced insulin secretion from streptolysin-O permeabilized HIT-T15 cells. AFC was less potent and AGC was inactive</td>
<td>84</td>
</tr>
<tr>
<td>Acetyl geranylgeranyl cysteine</td>
<td>More global inhibitors of methylation. Potently inhibited glucose-stimulated (–35%) or amino acid-induced (–62%) insulin secretion</td>
<td>69</td>
</tr>
<tr>
<td>Homocysteine plus deazaadenosine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acylation</td>
<td>Significantly reduced fractional rates of insulin secretion stimulated by glucose (–63 to 88%), amino acid-induced (–73 to 100%), but not mastoparan-induced insulin secretion from isolated rat islets</td>
<td>69</td>
</tr>
<tr>
<td>Cerulenin</td>
<td>Significantly inhibited both phases of glucose-induced, but not potassium-induced, insulin secretion from isolated rat islets</td>
<td>91</td>
</tr>
</tbody>
</table>

### Table 2. Specificity of bacterial toxins used for addressing the roles of small G proteins in stimulus-secretion coupling of the islet \( \beta \)-cell

<table>
<thead>
<tr>
<th>Toxin Used</th>
<th>Type of Modification</th>
<th>Target G Protein(s)</th>
<th>Effect on Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium difficile</em></td>
<td>Glucosylation</td>
<td>Rho, Rac, and Cdc42</td>
<td>Inactivation</td>
</tr>
<tr>
<td><em>Clostridium sordellii</em></td>
<td>Glucosylation</td>
<td>Rac, Rap, and Ras</td>
<td>Inactivation</td>
</tr>
<tr>
<td><em>Clostridium novyi</em></td>
<td>Glucosaminylation</td>
<td>Rho, Rac, and Cdc42</td>
<td>Inactivation</td>
</tr>
<tr>
<td><em>Clostridium C3-exoenzyme</em></td>
<td>Ribosylation</td>
<td>Rho</td>
<td>Inactivation</td>
</tr>
<tr>
<td>Cytotoxic necrotizing factor</td>
<td>Deamidation</td>
<td>Rho</td>
<td>Activation</td>
</tr>
</tbody>
</table>
but not Cdc42, Rho, or Rab. In recent years, clotridial toxins have been used to seek further support for the above formulation that Rho proteins (e.g., Cdc42 and Rac) are involved in β-cell signal transduction. Exposure of normal rat islets or clonal β-cells to C. difficile toxin A or B significantly reduced glucose-induced insulin secretion. These data indicated that Rac, Cdc42, and Rho G proteins are involved in this phenomenon (42). Interestingly, C. sordellii toxin also reduced glucose-induced insulin secretion from these cells under similar experimental conditions, suggesting that Ras, Rap, and Rac are also involved in this phenomenon. C3 exoenzyme, which ADP ribosylates and inactivates Rho, failed to inhibit glucose-induced insulin secretion from these cells, suggesting that Rho may not be involved in this process (42). Together, these findings have led to the conclusion that Cdc42, Rap, Rac (all geranylgeranylated proteins), and Ras (a farnesylated protein) might be involved in physiological insulin secretion. These findings are compatible with our observations using allyl farnesols and geranylgeraniols (2).

Use of Mastoparan to Examine the Role of G Proteins in Insulin Secretion

Mastoparan (Mas), a tetradecapeptide from wasp venom, has been shown to activate a wide variety of heterotrimeric as well as small G proteins, presumably by facilitating GTP/GDP exchange (21, 22). Several earlier studies have demonstrated that Mas stimulates insulin secretion from normal rat islets, human islets, and clonal β-cells (see Table 3 for a summary of these studies). However, the precise loci for Mas regulation of insulin secretion remain less understood. Recent evidence from our laboratory suggested that Mas-induced insulin secretion from isolated β-cells involves activation of Rac (3). Further experiments indicated that Mas activates Rac via GTP/GDP exchange but not

Table 3. Summary of data from earlier studies that used mastoparan to study stimulus-secretion coupling in the islet β-cell

<table>
<thead>
<tr>
<th>Cell Type Studied</th>
<th>Observation</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat pancreatic islet</td>
<td>Ptx- or bromophenacyl bromide, a PLA₂ inhibitor, abolished Mas-stimulated insulin secretion</td>
<td>110</td>
</tr>
<tr>
<td>Rat pancreatic islet</td>
<td>Ptx or neomycin, an inhibitor of PLC, blocked Mas-stimulated insulin secretion. Nifedipine, somatostatin, inhibitors of PKA or PKC, had no demonstrable effects</td>
<td>31</td>
</tr>
<tr>
<td>RINm5F cells</td>
<td>Ptx or Ctx treatment had no demonstrable effects on Mas-induced insulin secretion</td>
<td>23</td>
</tr>
<tr>
<td>Intact or permeabilized rat islets</td>
<td>Mas caused temperature-dependent insulin secretion. Extracellular calcium was not necessary. PKC or cAMP antagonists had no effects. Inhibited by GDPβS</td>
<td>26</td>
</tr>
<tr>
<td>Rat pancreatic islets</td>
<td>Mas-induced insulin secretion was unaffected by inhibitors of posttranslational modifications of G proteins, including lovastatin and acetyl farnesyl cytochrome C</td>
<td>69</td>
</tr>
<tr>
<td>RINm5F cells</td>
<td>Ptx pretreatment enhanced insulin secretion induced by Mas</td>
<td>32</td>
</tr>
<tr>
<td>Rat islets and human islets</td>
<td>Mas stimulated a high-affinity GTPase activity in the secretory granule fraction</td>
<td>46</td>
</tr>
<tr>
<td>Rat islets, human islets, HIT-T15 cells, and rat insulinoma cells</td>
<td>Mas stimulated nucleotide diphosphate kinase (NDPK) activity. Interestingly, Mas-17 an inactive analog of Mas, also stimulated NDPK activity</td>
<td>43, 50</td>
</tr>
<tr>
<td>βTC3 cells</td>
<td>Mas analogs, but not Mas-17, stimulated insulin secretion in a Ptx-sensitive manner. Mas also stimulated a GTPase activity associated with insulin secretory granules</td>
<td>34</td>
</tr>
<tr>
<td>RINm5F cells</td>
<td>In contrast to glyceraldehyde-, A-23187-, or carbachol-induced insulin secretion, Mas-stimulated insulin release was unaffected by pancreastatin</td>
<td>20</td>
</tr>
<tr>
<td>Normal rat islets, human islets and clonal β-cells</td>
<td>Mas, but not Mas-17, stimulated P-His phosphorylation in the membrane and secretory granule fractions</td>
<td>52</td>
</tr>
<tr>
<td>Normal rat islets and islets from the Goto-Kakizaki rat</td>
<td>Galparan, a peptide consisting of galanin (1–13 residues) and Mas, stimulated insulin secretion from control and diabetic rat islets. Stimulatory effects of galparan were insensitive to Ptx pretreatment</td>
<td>76</td>
</tr>
<tr>
<td>Rat and human pancreatic islets</td>
<td>Mas-stimulated insulin secretion in the absence of extracellular calcium. Under these conditions, it also augmented glucose-stimulated secretion. Both effects of Mas were Ptx insensitive</td>
<td>90</td>
</tr>
<tr>
<td>Normal rat islets and islets from the Goto-Kakizaki rat</td>
<td>Unlike abnormalities in glucose-, calcium-, or mitochondrial fuel-induced insulin secretion, Mas-stimulated secretion was completely normal in the diabetic GK rat islets</td>
<td>67</td>
</tr>
<tr>
<td>Rat pancreatic islets</td>
<td>Mas-stimulated insulin secretion was not affected by cerulenin, an inhibitor of protein acylation</td>
<td>109</td>
</tr>
<tr>
<td>MIN6 cells</td>
<td>Mas-stimulated release of insulin and GABA. Overexpression of syntaxin 1A and SNAP-25 markedly reduced Mas-stimulated insulin release from these cells</td>
<td>74</td>
</tr>
<tr>
<td>Insulin-secreting βHC-9 cells</td>
<td>Overexpression of Cdc42 markedly increased Mas-stimulated insulin secretion in these cells in a Ptx-independent manner</td>
<td>11</td>
</tr>
<tr>
<td>Normal rat islets, human islets, and clonal β-cells</td>
<td>Mas, but not Mas-17, its inactive analog, stimulated a novel histone-4 phosphorylating histidine kinase activity</td>
<td>37</td>
</tr>
<tr>
<td>INS-1 cells</td>
<td>Expression of dominant negative mutant of Rac1 (N17 Rac1) markedly attenuated mas-induced insulin secretion</td>
<td>3</td>
</tr>
</tbody>
</table>
via modulation of its isoprenylation. Transfection of dominant negative Rac (N17 Rac) markedly attenuated Mas-induced (3) or glucose- and forskolin-induced (57) insulin secretion from clonal β-cell preparations, suggesting that Rac plays an important role in insulin secretion elicited by different secretagogues. Recent investigations by Daniel et al. (11) have also identified Cdc42 as one of the proteins involved in Mas-stimulated insulin secretion. Mas and Mas-17 (its inactive analog) are also proven to be as valuable probes in recent studies (Fig. 3), which addressed the insulin-secretory abnormalities in islets derived from the Goto-Kakizaki (GK) rat, a model for non-insulin-dependent diabetes mellitus (NIDDM) (67). We reported that, whereas glucose- and potassium-induced insulin secretion was reduced significantly in islets from the GK rat, the Mas-induced insulin secretion remained unaltered in these islets. In GK islets, we also observed significant defects in the functional activation of nucleoside diphosphate kinase (NDPK), and on the basis of these data we proposed that the abnormalities in insulin secretion in the GK rat may lie at the level of an NDPK-analog (NDPK) and on the basis of these data we indicated. Representative data from studies described in our earlier publication (43) were plotted. Data represent means ± SE from 3–5 determinations in each case. *P < 0.001 vs. control.

![Fig. 3. Structure-specific stimulation by mastoparan (Mas) of insulin secretion from isolated rat islets. Insulin release was measured from fresh, isolated normal rat islets in static incubation conditions at 3.3 mM glucose. Thirty micromoles each of Mas or Mas-17 (an inactive analog of Mas) were present during the 45-min incubation period, as indicated. Representative data from studies described in our earlier publication (43) were plotted. Data represent means ± SE from 3–5 determinations in each case. *P < 0.001 vs. control.](image-url)
Table 4. Known histidine kinases, their potential phosphoprotein substrates, and their subcellular localization in insulin-secreting cells

<table>
<thead>
<tr>
<th>Protein</th>
<th>Localization</th>
<th>Phosphorylating Kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDPK (nm 23-H1 and nm-23-H2)</td>
<td>Cytosol and membrane</td>
<td>Autophosphorylation</td>
</tr>
<tr>
<td>NDPK (nm 23-H4) β-subunit of trimeric G proteins</td>
<td>Mitochondria</td>
<td>Autophosphorylation</td>
</tr>
<tr>
<td>Succinyl-CoA synthetase (ATP and GTP specific)</td>
<td>Mitochondria</td>
<td>NDPK</td>
</tr>
</tbody>
</table>

that we have recently identified in the islet β-cell (see Table 4).

NOVEL REGULATORY MECHANISMS FOR THE ACTIVATION OF G PROTEINS IN THE ISLET β-CELL: EVIDENCE FOR THE INVOLVEMENT OF PROTEIN HISTIDINE PHOSPHORYLATION

In most cells, the transduction of extracellular signals involves ligand binding to a receptor, often followed by the activation of one (or more) G proteins and their effector systems (6, 17). The pancreatic β-cell is unusual in that glucose, the major physiological agonist, lacks an extracellular receptor. Instead, events consequent to glucose metabolism promote insulin secretion via the generation and/or altered distribution of diffusible second messengers, such as ions, cyclic nucleotides, and biologically active lipids (44, 48, 59, 60, 73, 81). Changes in calcium concentration not only initiate insulin secretion but also regulate various enzymes, such as protein kinases, phosphodiesterases, adenyl cyclases, and phospholipases, thereby facilitating insulin secretion. In addition to calcium-dependent protein kinase(s), several other kinases, including calmodulin-, cyclic nucleotide-, phospholipid-dependent protein kinases, tyrosine kinases, and mitogen-activated protein kinases have been described in β-cells (see Ref. 27 for a review). The majority of these kinases mediate phosphorylation of endogenous β-cell proteins using ATP as the phosphoryl donor. In addition, we (52) reported evidence for the localization of a novel protein kinase in β-cells that selectively uses GTP as a phosphoryl donor and uniquely phosphorylates specific proteins (e.g., β-subunit of trimeric G proteins) at histidine residues. We (52) further demonstrated that this phosphate, in turn, is transferred to free GDP (or GDP liganded to G proteins) to yield free GTP (or GTP bound to G proteins).

Protein Histidine Kinases

To date, the most phosphorylated amino acids identified include serine (P-Ser), threonine (P-Thr), and tyrosine (P-Tyr). Phosphoamino acids exhibit differential sensitivities to acidic and alkaline pH conditions (62). P-Ser and P-Thr, which form O-p (alcoholic O-monoester) linkages, are stable at acidic pH and are fairly unstable under alkaline conditions. P-Tyr, which forms O-p (phenolic O-monoester), is stable under acidic and alkaline conditions. Therefore, because of their stability under acidic conditions, P-Ser, P-Thr, and P-Tyr are readily identified after acid hydrolysis of phosphorylated proteins. However, acid-labile phosphoramidate linkage has been reported (62, 63) in histidine (P-His), arginine (P-Arg), and lysine (P-Lys). It is not surprising that very little information is available on the number of proteins with P-His, since its phosphate is rapidly lost during identification of phosphoamino acids under standard acid hydrolysis conditions or under conditions used for SDS-PAGE (52, 62, 63, 104). It is estimated that P-His may account for 6% of total protein phosphorylation in eukaryotes. In this context, it has also been shown that P-His undergoes rapid dephosphorylation in crude cellular extracts (28, 30), including pancreatic islet cell lysates, as we reported in Ref. 52.

Several recent studies have investigated protein histidine phosphorylation in multiple cell types. For example, Huang et al. (25) purified a monomeric histidine kinase from *Saccharomyces cerevisiae* with an apparent molecular mass of 32 kDa. This kinase exhibited specificity toward ATP (also GTP, but with minimal affinity) to phosphorylate histone-4. This enzyme required divalent cations for maximal activity; spermine or spermidine was ineffective. Motojima and Goto (70) reported histidine phosphorylation of a 36-kDa protein by a histidine kinase in liver extracts. They also reported localization of an okadaic acid-resistant phosphatase activity (with an apparent molecular mass of 45 kDa). Using an HPLC method, they demonstrated copurification of the kinase and p36 substrate at a 70- to 75-kDa size. These data indicate that the liver histidine kinase may be different from the yeast enzyme originally described by Huang et al. Along similar lines, Urushidani and Nagao (96) also reported autophosphorylation, at a histidine residue, of a 40-kDa protein localized in the membrane fraction derived from rabbit gastric mucosa. Sequence analyses data indicated that this protein might represent the α-subunit of an extramitochondrial isoform of succinyl-CoA synthetase (SCS) or its homolog. Autophosphorylation of this protein was stimulated by GDP, Ras (a small G protein), and myelin basic protein and was rapidly dephosphorylated in the presence of ATP, succinate, and CoA. Hegde and Das (19) showed that Ras stimulated the phosphorylation of a 36-kDa protein at a histidine residue in liver membranes. More recently, Besant and Attwood (5) purified and characterized a histone 4-phosphorylating histidine kinase activity from porcine thymus. This enzyme appears to have certain similarities with the yeast enzyme, including the molecular mass, which was estimated to be ~34–41 kDa. Together, these studies identified localization of a histidine-phosphorylating enzyme(s) that appears to be regulated under various experimental conditions (e.g., in the presence of Ras). The reader is referred to several recent reviews (1, 30, 71, 79, 89, 93) that
describe potential regulatory roles of various histidine kinases in cellular regulation and function.

Using SDS-PAGE and the nitran filter paper assay, we (37) recently characterized a protein histidine kinase in the lysates of normal rat islets, human islets, and clonal β-cell (HIT-T15 and INS-1) cell preparations. The β-cell histidine kinase is sensitive to ATP as well as GTP, with an apparent molecular mass of 60–70 kDa. Noticeable similarities appear to exist between the β-cell and the yeast histidine kinases. For example, both use ATP as well as GTP as phosphoryl donors, and both enzymes exhibit similar metal ion requirements and were resistant to polyamines. The principal difference appears to be the size of the enzyme. The β-cell enzyme is ~60–70 kDa in size in contrast to the yeast enzyme, which has been shown to be ~32 kDa. On the basis of our additional observations in the β-cell, we suggest that phosphohistidine phosphorylation may be important in insulin exocytosis from the β-cell. In support of this formulation, we demonstrated (37) that the β-cell histidine kinase is activated in a structure-specific manner by Mas. Mas or Mas-7, but not Mas-17 (an inactive analog), is a potent activator of insulin secretion (Table 2). We observed similar specificities for the activation by Mas analogs of histidine kinase activity, as well as the β-subunit phosphorylation and insulin secretion, in rat islet homogenates (37). Although several previous studies, including our own (see Table 3), have demonstrated insulinotropic effects of Mas, our data suggest for the first time that Mas-mediated signaling events could include activation of protein histidine phosphorylation in the pancreatic β-cell. Furthermore, these data establish a biochemical link between activation of histidine kinase and activation of phosphorylation of the β-subunit through the use of Mas, a global G protein activator. Additional studies are needed to understand precisely the regulation of this enzyme by nutrient insulin secretagogues and G protein-coupled receptor agonists to conclusively establish a link between activation of G proteins (via activation of this or other related histidine kinases) and insulin secretion from isolated β-cells. On the basis of our data on histidine kinase-mediated phosphorylation of the β-subunit of trimeric G proteins, we propose a model for the activation of trimeric G proteins in the β-cell involving protein histidine phosphorylation (Fig. 4).

We propose that physiological insulin secretagogues (e.g., glucose) elicit effects on functional activation of specific G proteins via receptor-independent mechanisms. Our model predicts that glucose and other nutrient secretagogues stimulate histidine phosphorylation of specific "transmitter" proteins (e.g., the β-subunit of trimeric G proteins) and that this phosphate, in turn, is transferred to a "receiver" protein, such as the α-subunit (in its GDP-bound inactive conformation) to yield its GTP-bound active conformation. In support of our hypothesis that cellular metabolism leads to rapid protein histidine phosphorylation, Crovello et al. (8) provided the first direct evidence for the induction of rapid and reversible histidine phosphorylation in mammalian cells upon activation. Using human platelets, they demonstrated transient phosphorylation of P-selectin at a histidine residue by thrombin or collagen. Although the activation mechanism proposed in Fig. 4 pertains to trimeric G proteins, it is also likely

![Fig. 4. Proposed mechanism for receptor-independent activation of trimeric G proteins in the pancreatic β-cell by glucose. Trimeric G proteins remain inactive when their α-subunit is bound to GDP. We propose that a histidine kinase phosphorylates the β-subunit of trimeric G proteins at a histidine residue via a phosphoramidate linkage. This phosphate in turn is relayed to the GDP-bound α-subunit and transphorylates the GDP to GTP. Then, the α-subunit bound to GTP dissociates from the βγ-complex for regulation of its effector proteins. Ample experimental evidence identified multiple effector proteins for the α-subunits as well as the βγ-complex in several cellular systems. After hydrolysis of GTP by GTPase activity intrinsic to the α-subunit, α-GDP reassociates with the βγ-complex to complete one activation cycle. Not shown here is the possibility of nucleoside diphosphate kinase (NDPK) subserving the role of histidine kinase in mediating the phosphorylation of the β-subunits (see text for additional details).]

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that similar activation mechanisms are operable in the context of small G proteins. This may be mediated via the NDPK-catalyzed reaction. In the following sections, we propose a model (Fig. 5) that predicts nutrient-mediated regulation of NDPK, which in turn generates GTP in the "vicinity" of candidate small G proteins necessary for their activation. Alternatively, NDPK could subserve the function of transphosphorylating the GDP bound to G proteins (i.e., their inactive conformation) to their GTP-bound, active conformation.

**NDPK**

The enzyme NDPK catalyzes the transfer of terminal phosphates from nucleoside triphosphates (e.g., ATP) to nucleoside diphosphates (e.g., GDP) to yield their respective nucleoside triphosphates (e.g., GTP). The transfer of terminal phosphates occurs by a two-step, ping-pong reaction involving the formation of a transient high-energy phosphoprotein intermediate form of NDPK, due to phosphorylation at a histidine residue, followed by transfer of that phosphate to a suitable acceptor (29). In addition to the generation of nucleoside triphosphates, NDPK has been implicated in the direct activation of certain G proteins as well as phosphorylation and/or regulation of several key enzymes of intermediary metabolism (e.g., ATP citrate lyase, aldolase, pyruvate kinase, glucose-6-phosphatase, and SCS) (15, 53, 89, 96, 99, 100).

Although multiple roles have been described for NDPK [the reader is referred to recent reviews on NDPK describing potential regulatory roles of this enzyme in regulation of cellular function (29, 89)], one of the unique roles of NDPK (in the context of this current review and β-cell metabolism) is its ability to contribute toward the synthesis of GTP and the subsequent activation of specific G proteins. The latter is thought to occur via channelling of GTP to the "vicinity" of candidate G proteins for their functional activation. It has also been shown that NDPK mediates transphosphorylation of GDP bound to G proteins (inactive conformation) to their GTP-bound (active conformation) of G proteins (43). Original studies from our laboratory (43) have characterized NDPK activity in normal rat and human islets as well as clonal β-cell preparations. More recent studies (53) have identified at least three isoforms of NDPK in the pancreatic β-cell. They include nm23-H1, a predominantly membrane-associated form of NDPK, and nm23-H2, with a membranous as well as soluble localization. In addition, a mitochondrial isofrom of NDPK (nm23-H4) has been identified in the islet β-cell (53). Potential roles of these isoforms and significance of their subcellular distribution have also been described in Ref. 53. On the basis of our current understanding of the biochemical properties and physiological regulation of this enzyme in the islet β-cell, we propose a model for potential contributory roles of NDPK in glucose-stimulated insulin secretion, specifically at the level of activation of G proteins (Fig. 5). We propose that glucose-induced increases in the GTP/GDP ratio (as demonstrated earlier in Refs. 12 and 66) may in part be due to the activation of NDPK, which generates GTP via transphosphorylation of GDP from ATP. This increase in GTP concentrations favors either increase in GTP/GDP exchange on a relevant G protein[s] or channelling of GTP to candidate G protein[s], culminating in their activation. In addition, it is likely that glucose also activates the histidine kinase (as described in the previous section), resulting in stimulation of the phosphorylation of key regulatory proteins, including the β-subunits of trimeric G proteins at a histidine residue. Such a phosphate, in turn, is transferred to the GDP bound to the α-subunits of trimeric G proteins via the phospho-relay mechanism (52, 71, 79, 105) that is given in Fig. 4. We also propose that glucose-mediated activation of NDPK might result in histidine phosphorylation of other proteins, such as SCS, aldolase, and ATP-citrate lyase, which is required for their functional activation, and subsequent insulin secretion. For example, SCS catalyzes the substrate level phosphorylation of ADP or GDP. In the context of SCS regulation in the islet β-cell, we have recently shown that the α-subunit of SCS undergoes phosphorylation at a histidine residue, which may be catalyzed by NDPK-mediated phosphotransfer mechanisms (36, 53). In support of this, we have demonstrated colocalization as a complex of mitochondrial NDPK and SCS in the β-cell mitochondria. Using the mitochondrial extracts from clonal β-cells (INS-1 and HIT-T15), we have been able to quantitate the formation of succinyl-CoA from succinate, CoA, and ATP or GTP. Furthermore, using im-

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**Fig. 5.** Proposed mechanisms for glucose-stimulated activation of G proteins involving members of the histidine kinase family. We propose that, in addition to increasing GTP biosynthesis, glucose activates NDPK to facilitate transphosphorylation of GDP to GTP. Such an increase in GTP levels, specifically in the "vicinity" of candidate G proteins, results in activation of those G proteins, leading to stimulation of insulin secretion (left). On the basis of recent data (reviewed in the text), it is also likely that NDPK activation leads to direct activation of specific G proteins, which remain complexed with "activated" NDPK (middle). We propose that glucose also activates islet endogenous histidine kinase, which we have shown to phosphorylate the β-subunit of trimeric G proteins (Fig. 2), thereby facilitating the activation of cognate trimeric G protein. Glucose could also mediate histidine phosphorylation of other proteins (e.g., ATP citrate lyase, aldolase, succinyl thio kinase) that are critical to glucose metabolism, thereby generating signals necessary for insulin secretion.
munological methods, we localized α- and β-subunits of ATP- as well as GTP-sensitive isoforms of SCS in the β-cell. In addition, using [γ-32P]ATP as a phosphoryl donor, we observed that the α-subunit of SCS undergoes autophosphorylation at a histidine residue; coprovision of exogenous succinate and CoA resulted in pronounced dephosphorylation of the phosphorylated α-subunit of SCS. Taking these observations together, we provide evidence for the localization of two distinct subunits of SCS. Taking these observations together, we provide evidence for the localization of two distinct subunits of SCS. Taking these observations together, we provide evidence for the localization of two distinct subunits of SCS. Taking these observations together, we provide evidence for the localization of two distinct subunits of SCS.

As I review these cited studies, I think that the relay of high-energy phosphates as a consequence of protein histidine phosphorylation constitutes an important non-receptor-mediated activation of specific G proteins (and other proteins relevant to nutrient metabolism) by physiological stimuli such as glucose. Additional studies are required to substantiate such a hypothesis. In this context, two recent studies have provided additional support to our original formulation (52) for the non-receptor-dependent activation of G proteins involving protein histidine phosphorylation and high-energy phosphate transfer. First, Cuello et al. (9) reported activation of trimeric G proteins by a high-energy phosphate transfer from the histidine-phosphorylated NDPK to the β-subunit of trimeric G proteins. Using bovine retinal and brain preparations, these investigators observed that the B isoform of NDPK forms complexes with the βγ-subunits of trimeric G proteins and contributes to the activation of the respective G protein by increasing the high-energy phosphate transfer from a transiently phosphorylated Hist266 in the β-subunit to the GDP bound to the α-subunit, to yield an active conformation. In the second study, Hippe et al. (24) demonstrated the existence of a complex between NDPK (B isoform) and the βγ-complex of trimeric G proteins, and they implicated a role for NDPK in the phosphorylation of the β-subunit, which is then transferred to the GDP bound to the α-subunit, resulting in its active, GTP-bound conformation. Interestingly, these findings are compatible with our recent observations on the existence of NDPK and succinyl thio kinase complexes in β-cells (53), on the basis of which we proposed a role for NDPK in the functional regulation of succinyl thio kinase. It is likely that the mitochondrial NDPK might interact with other mitochondrial proteins as well. This is plausible, especially in light of recent observations of Søre and coworkers (87, 98) that clearly indicated the existence of complexes (appropriately termed “metabolons”) of sequential metabolic enzymes involved in the tricarboxylic acid cycle. Together, it appears likely that the histidine kinase and various isoforms of NDPK that we characterized recently (Table 3) could subserve the function of histidine phosphorylation of key proteins (e.g., monomeric G proteins or subunits of trimeric G proteins), leading to the generation of appropriate signals necessary for physiological insulin secretion (37, 52, 53).

Several recent studies have identified additional roles for NDPK, such as its ability to interact with guanine nucleotide exchange factors for specific G proteins and subserve the function of activating specific GTPases (77, 78, 111). Although these regulatory mechanisms have not been fully studied in the islet β-cell, we (95) and others (4) have obtained evidence to indicate localization of such factors (e.g., the guanine nucleotide exchange factor 1, or GRF1) in insulin-secreting cells. We (45) also described localization of similar exchange factors in normal islet and clonal β-cells, which appear to be regulated by phospholipase-derived mediators of insulin secretion (e.g., arachidonic acid, lysophosphatidylycholine, and phosphatidic acid). In this context, we observed (unpublished observations) potential regulation of the islet NDPK activity by lipid messengers of insulin secretion (e.g., arachidonic acid). Although it seems likely, it remains to be seen whether nutrient-stimulated insulin secretion involves interplay between lipid messengers of insulin secretion, NDPK, guanine nucleotide exchange factors, and their effector G proteins within confines of a stimulated β-cell. Furthermore, studies by Wagner and Vu (101) have identified roles for NDPK in the phosphorylation of farnesyl and geranylgeranyl triphosphates, which form precursors for G protein isoprenylation. In conclusion, a growing body of evidence is emerging to suggest critical regulatory roles for this enzyme, which was originally believed to play the role of a “housekeeping gene.” On the basis of the above-mentioned reasons, it is logical to expect an increased interest in the area of putative regulatory roles of protein histidine phosphorylation in metabolic function and stimulus-secretion coupling, not only of the β-cell but of other endocrine cells as well.

**ISLET G PROTEINS IN MODELS OF IMPAIRED INSULIN SECRETION**

Recent evidence from multiple laboratories appears to suggest abnormalities in the expression and/or function of G proteins in animal and in vitro models of impaired insulin secretion. The majority of these studies were aimed at understanding the functional status of trimeric as well as monomeric G proteins. A relatively large body of evidence is emerging on alterations in the expression and function of G protein metabolism in islets derived from the GK rat, a widely accepted genetically determined rodent model for human type 2 diabetes. For example, we previously reported (67) that insulin secretion elicited in the presence of stimulatory concentrations of glucose, succinic acid methyl ester, or a depolarizing concentration of KCl was significantly impaired in GK rats. Interestingly, insulin secretion elicited by Mas was markedly increased above and beyond the stimulatory effects of this compound in control Wistar rat islets. We also demonstrated a significant reduction in the ATP- as well as GTP-sensitive phosphorylation and catalytic function of NDPK in
islets derived from the GK rat. On the basis of these findings, we suggested that a defect in the late signaling steps in these islets, possibly occurring at the site of activation by NDPK of a Mas-sensitive G protein-dependent step, might contribute toward impaired insulin secretion in this animal model. More recent evidence from our laboratory (38) has also indicated significant defects in ATP-and GTP-sensitive histidine kinase activity in these islets, which we have implicated in the activation of specific G proteins (Figs. 4 and 5). Together, these data tend to support the viewpoint that abnormalities in protein histidine phosphorylation might lead to impaired insulin secretion.

Alterations in the expression and function of trimeric G proteins have also been reported in islets from the GK rat. Using immunohistochemical techniques, Frayon et al. (16) reported altered expression of adenylate cyclase isoforms and Go,olf in two models of NIDDM, namely, the GK rat and the neonatally treated streptozotocin (nSTZ)-induced diabetic rats. Interestingly, relative abundance of the adenylate cyclase II and adenylate cyclase III isoforms was clearly increased in both types of rats. The expression of Go,olf was increased in GK rat islets, whereas it was markedly attenuated in islets derived from the nSTZ rat. On the basis of these data, the authors concluded that alterations in the expression of G protein isotypes could contribute to the diabetic phenotype. Along similar lines, studies by Portela-Gomes and Abdel-Halim (80) indicated significantly higher expression of GoS and Go,olf and adenylate cyclase I and III isoforms in GK rat islets compared with control rat islets, suggesting possible alterations in the signaling mechanisms involving Gs proteins to adenylate cyclase isoforms in islets from the diabetic animals.

Yaekura et al. (107) provided additional insights into putative regulatory roles of small G proteins, specifically, Rab3A, in insulin secretion. They observed that Rab3A (a small G protein)-null mice developed fasting hyperglycemia and glucose intolerance. Insulin secretion in response to arginine was similar in control and Rab3A knockout mice, indicating a phenotype akin to insulin-secretory abnormalities demonstrable in type 2 diabetes. Despite no major differences in β-cell mass and insulin production between the control and null groups, secretagogue-induced insulin secretion was impaired significantly in islets derived from Rab3A-null mice. Furthermore, glucose oxidation and glucose-induced increments in intracellular calcium concentrations were comparable in the two groups. On the basis of these data, the authors concluded that Rab3A plays a major role in glucose-induced insulin secretion by replenishing the readily releasable pool of insulin-laden secretory granules.

Experiments by Srivastava et al. (88) in the Anx7 (a gene that encodes for a calcium-activated GTPase) knockout mouse have provided evidence into calcium signaling through inositol triphosphate stores in glucose-induced insulin secretion. These investigators demonstrated significant reduction in glucose-stimulated insulin secretion from the islets derived from the knockout mouse despite considerably high (8- to 10-fold) insulin content in mutants compared with their control counterparts. Even though the glucose-induced increases in intracellular calcium concentrations were comparable between control and knockout mice, the ability of inositol triphosphate-generating agonists to mobilize intracellular calcium was significantly attenuated in Anx 7(+/−) knockout mouse islets. These studies thus established a link between putative contributory roles for inositol triphosphate-mobilizable calcium stores in glucose-stimulated insulin secretion and its regulation by a calcium-activated GTPase in the islet β-cell.

In summary, even though the list of studies that addressed the issue of possible contributory roles of G proteins in models of impaired secretion is somewhat short, it is my hope that this area will gain further recognition and momentum in the coming years, specifically with the availability of more advanced methodology, including the gene array technology. Together, on the basis of data derived from experiments involving specific inhibitors of G protein functions (e.g., inhibitors of posttranslational modification as well as clostridial toxins), gene depletion approaches, and transgenic animal models, it is reasonable to draw an overall conclusion that small G proteins play key regulatory roles in the signal transduction mechanisms leading to insulin secretion, and that abnormalities in the expression and/or functional activation of these signaling proteins lead to impairment in insulin secretion. In the following section, I will briefly review our current understanding of the roles of these signaling proteins in cytokine-mediated dysfunction and demise of the pancreatic β-cell, which is a well-accepted model for insulin-dependent diabetes mellitus. Other aspects of putative roles of GTP and its binding proteins in β-cell mitogenesis and survival have been reviewed in Ref. 65.

**G PROTEINS IN CYTOKINE-INDUCED β-CELL DYSFUNCTION AND DEMISE**

It is well established that insulin-dependent diabetes mellitus develops as a consequence of the selective destruction of insulin-secreting β-cells, and it has also been proven beyond doubt that demise of the β-cell is mediated by cytokines (e.g., IL-1β) secreted by the infiltrating immune cells (10, 61, 64). Several lines of experimental evidence suggest that the demise of the pancreatic β-cells due to immune attack could be due to the apoptotic and necrotic pathways. In the context of apoptosis, numerous studies have demonstrated the involvement of low-molecular-mass G proteins in multiple cell types (see Ref. 49 for a recent review). However, very little is known with respect to the regulatory roles of small G proteins in IL-1-induced β-cell dysfunction and demise. In this context, we have recently begun to address the issue of small G proteins in cytokine-mediated dysfunction and demise of the islet β-cell, and data along these lines of investigation provided convincing evidence to indicate that Ras plays a
significant role in IL-1-mediated nitric oxide release from isolated rat islets and clonal β-cell preparations (47, 94, 95). Again, as above, specific inhibitors of posttranslational modifications of G proteins, as well as bacterial toxins, were utilized to decipher the role of Ras in this phenomenon. Compatible with these observations are other reports that suggested key regulatory roles for GTP in the survival of the islet β-cell (see Ref. 65 for a review). Together, these data clearly provide the initial evidence, in the context of the β-cell, that GTP and G proteins play very important functional roles in the normal functioning of the islet, and that proapoptotic G proteins (e.g., Ras) play roles in the propagation of cellular events responsible for the cytokine-induced loss of β-cell mass, leading to the onset of insulin-dependent diabetes mellitus (47, 94, 95).

Clearly, this area is in its infancy, and additional studies are needed to identify these candidate pro-and antiapoptotic G proteins. This is an important area of investigation, since such data could provide valuable insights into the development of therapeutic intervention modalities for the prevention of loss of β-cell mass.

CONCLUSIONS AND FUTURE DIRECTIONS

From the discussion above, it is apparent that small-molecular-mass G proteins play key regulatory roles in the stimulus-secretion coupling of the islet β-cell. These conclusions were reached on the basis of studies using mostly biochemical, physiological, and limited gene depletion approaches. We propose that glucose-mediated, receptor-independent activation of these G proteins requires the intermediary of protein histidine phosphorylation and subsequent relay of the high-energy phosphate to GDP bound to G proteins to yield their respective GTP-bound active conformation. It also appears that alterations in the expression and/or functional activation of these proteins lead to impaired insulin secretion. Furthermore, specific G proteins (e.g., Ras) seem to play proapoptotic roles in the islet β-cell after exposure to cytokines. It will be necessary to develop systems for the overexpression of G proteins or application of antisense approaches for specific G proteins (and their modifying enzymes), not only to deduce the physiological functions of these proteins in modulating insulin secretion but also to develop potential therapeutic approaches to states of perturbed metabolic status and insulin release. For these reasons, there appears to be an immediate need for the development of novel inhibitors of G protein functions, especially for those proteins that control and propagate signal transduction steps leading to the generation of nitric oxide, and consequently leading to the metabolic dysfunction and demise of the pancreatic β-cell. In addition to these pharmacological probes, identification of candidate G proteins might help us in the development of novel bioengineered cell lines, which are resistant to immune attack, for the treatment of diabetes in humans (14, 72).

I thank all of my former colleagues at the University of Wisconsin-Madison and my current associates at Wayne State University-Detroit who contributed to the work that I have described in this review.

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