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Review

Aspects of Novel Sites of Regulation of the Insulin Stimulus-Secretion Coupling in Normal and Diabetic Pancreatic Islets

Åke Sjöholm

Department of Molecular Medicine, Karolinska Institute, Karolinska Hospital, Stockholm, Sweden

Noninsulin-dependent diabetes mellitus (NIDDM), a major health care problem in the Western world, is a disease typified by a relative deficiency of insulin, leading to vast derangements in glucose and lipid homeostasis with disastrous vascular complications. Despite immense research efforts aimed at a clear understanding of the etiology of this complex disease, the molecular mechanisms causing the disorder still remain elusive. This article reviews extant data from recent publications implicating novel signal transduction pathways as important regulators of the insulin stimulus-secretion coupling in the pancreatic β-cell. The significance of nitric oxide and serine/threonine protein phosphatases, and their inactivation by insulin secretagogues, glucose metabolites, ATP, GTP, glutamate, and inositol hexaphosphate in this arena is scrutinized. Additionally, also presented is the growing concept that an important signal for insulin secretion may reside in the inextricable interplay between glucose and lipid metabolism, specifically the generation of malonyl-CoA, which inhibits carnitine palmitoyltransferase 1 with the attendant accumulation of long-chain acyl CoA esters. Moreover, attention is directed towards novel intracellular actions of hypoglycemic sulfonylureas in the β-cell. Finally, the importance of "lipotoxicity" and aberrations in glucose uptake and metabolism in β-cell dysfunction is given consideration. Future research efforts should aim at further characterization of effects of second messengers on protein phosphorylation elements in β-cells. Additionally, long-term regulation by glucose and the diabetic state (e.g., fatty acids and ketones) on β-cell protein phosphatases, pyruvate dehydrogenase, and carnitine palmitoyltransferase 1 needs to be explored in greater depth. Clearly, the detrimental impact of diabetic hyperlipidemia on β-cell function has been a relatively neglected area, but future pharmacological approaches directed at preventing lipotoxicity may prove beneficial in the treatment of diabetes.

Key Words: Insulin secretion; pancreatic islet; diabetes mellitus; protein phosphatase; carnitine palmitoyltransferase 1.

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Author to whom all correspondence and reprint requests should be addressed: Dr. Åke Sjöholm, Department of Molecular Medicine, The Endocrine and Diabetes Unit, The Rolf Luft Center for Diabetes Research, Karolinska Institute, Karolinska Hospital (L6:01B), S-171 76 Stockholm, Sweden.

Introduction

Noninsulin-dependent diabetes mellitus (NIDDM), a major health care problem in the Western world, is a disease characterized by a relative deficiency of insulin, leading to vast derangements in glucose and lipid homeostasis with ravaging vascular complications. Despite immense research efforts aimed at a clear understanding of the etiology of this complex disease, the molecular mechanisms causing the disorder still remain elusive. In order to achieve a more complete understanding of the pathogenetic factors, it is necessary first to gain a thorough insight into the mechanisms that normally maintain glucose homeostasis. The pancreatic islet β-cell serves as a unique fuel-sensing organ and is the sole producer of insulin, the key hormone in maintenance of normoglycemia, whose release is tightly controlled by the ambient plasma glucose concentrations *(1–11)*. Since insulin is produced and secreted into the bloodstream exclusively by the pancreatic islet β-cell, it is logical to consider lesions of this cell as a possible event in the development of diabetes *(12–16)*.

This article reviews extant data implicating a deficiency in β-cell insulin stimulus-secretion coupling in the pathogenesis of NIDDM. Furthermore, novel aspects of the regulation of normal and diabetic β-cell insulin secretion will be discussed, and some therapeutical options related to improvements in diabetes therapy will be given consider-

ation. Notwithstanding the pathogenetic significance of impairments in β-cell regenerative capacity *(17,18)*, insulin gene expression and intracellular trafficking *(19–21)*, disturbances in insulin oscillatory secretion *(22,23)*, or insulin sensitivity in the peripheral target cells *(24)*, these phenomena fall outside of the scope of this article and will not be discussed. However, several excellent reviews have been published on this topic. Additionally, because of space limitations imposed, the reader is frequently referred to more extensive reviews.

Glucose Metabolism Promotes Insulin Release

The mechanisms controlling exocytotic release of insulin are finely tuned by a complex set of incoming signals, e.g., nutrients and hormones carried via the blood, neuronal input from surrounding nerve terminals, and paracrine influences from neighboring islet cells. The exocytotic release of insulin is closely regulated by different intracellular signaling systems, encompassing phosphoinositide hydrolysis *(25–30)*, increases in cytosolic Ca^{2+} *(5,22,23,28, 31–34)* and cyclic AMP (cAMP) generation *(5,7,9)* (summarized below). Glucose is a cardinal stimulator of insulin release and is capable of potentiating the action of other secretagogues *(4,6–8,35,36)*. In healthy β-cells, glucose uptake through the facilitative glucose transporter protein GLUT-2 is not considered rate-limiting, although it may become so in diabetics *(see below)*. The β-cell senses subtle changes in ambient glucose concentration by intracellular levels of glucose catabolites *(10,35–38)*. Recently, the rapid induction of genes encoding several glycolytic enzymes (e.g., phosphofructokinase-1C, glyceraldehyde-3-phosphate dehydrogenase, and L-pyruvate kinase) by glucose was demonstrated in clonal β-cells *(39)*. Metabolism of the sugar seems to be necessary for its insulin-releasing effect, as inferred from the combined observations of an attenuated secretory response to glucose by mannoheptulose, which inhibits glycolysis, and the lack of insulin release in the presence of nonmetabolizable sugars *(7)*. The major route of glucose metabolism involves its initial phosphorylation by hexokinase and the high K_m glucokinase (by some considered the β-cell "glucose sensor" *[35–37]*), further conversion into pyruvate and acetyl-CoA by the glycolytic pathway and funneling into mitochondria for subsequent oxidative metabolism in the Krebs cycle *(11,35–40)*. By contrast, metabolic flux through the pentose shunt represents no more than ≈2% of glucose metabolism *(8,35)*. Mitochondrial oxidative metabolism of glucose seems to be required for the insulin-releasing effect of the sugar, because it can be blocked by inhibitors of electron transport or by uncouplers of mitochondrial ATP production *(35)*. Recently, it was suggested that NADH, produced in the glyceraldehyde dehydrogenase step, represents the critical signaling event, possibly by providing reducing equivalents to sites 1 and 2 of the electron transport chain by means of the malate-aspartate and glycerol phosphate-dihydroxyacetone phosphate shuttles, respectively *(41)*. Glucose induces a rapid enhancement of pyruvate dehydrogenase activity *(42)*, a major determinant of glucose oxidation rates, as well as a long-lasting increase in pyruvate dehydrogenase mRNA expression in islets *(43,44)*. It has, moreover, been postulated that glucose metabolites stimulate the glycerol phosphate shuttle, catalyzed by FAD-linked glycerophosphate dehydrogenase, which functions by shifting reducing equivalents into the mitochondria and may thus promote pyruvate oxidation by stimulation of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase *(7,8,35,40)*. In fact, by this mechanism, the glycerol phosphate shuttle is crucial in favoring mitochondrial oxidative events relative to total glycolysis with attendant ATP generation *(12,45)*. The secretory signal(s) emanating from glycolysis may promote mitochondrial oxidation or anaplerosis, but their precise nature has remained elusive *(10)*. However, it was recently shown that intermediates of anaerobic glycolysis between fructose 1,6-diphosphate and phosphoenolpyruvate are essential for β-cell glucose sensing *(38)*. Interestingly, certain glucose metabolites (i.e., fructose-1,6-bisphosphate and 2,3-diphosphoglycerate) are able to potentiate inositol trisphosphate-induced Ca^{2+} mobilization in rat islets *(46)*, possibly through inhibition of inositol trisphosphate-5-phosphomonoesterase activity *(47)*. Additionally, it was recently suggested that unknown signals generated in the proximal part of the glycolytic pathway are of essential importance for a sustained insulin release in response to glucose *(48)*. Finally, unknown signals generated by glucose metabolism modulate dihydropyridine-sensitive Ca^{2+} channels *(49)*. Complementary to this, glucose signaling involves generation of derivatives of acyl CoA, e.g., malonyl-CoA and diacylglycerol *(see below)*.

Glucose-Stimulated Insulin Release Involves Closure of β-Cell ATP-Regulated K^+ Channels

Previous findings have revealed the existence in β-cell plasma membrane of ATP-regulated K^+ channels that control transmembrane K^+ fluxes *(50–54)*. ATP may thus provide a link between glucose metabolism and changes in β-cell electrical activity elicited by glucose. More specifically, ATP generated by glucose metabolism may close K^+ channels resulting in depolarization and subsequent influx of Ca^{2+} through voltage-activated Ca^{2+} channels *(50–54)*. This influx of Ca^{2+} increases the cytosolic Ca^{2+} concentration, an event that sets in motion the exocytotic discharge of insulin into the bloodstream *(31)*. Another mechanism has been uncovered by the Henquin laboratory *(55,56)*, who showed that glucose retains an excellent ability to release insulin when K^+ channels are kept open using diazoxide and β-cells maximally depolarized by high concentrations of K^+, suggesting the existence of other regulatory pathways controlling the stimulus-secretion

coupling. It was shown that the islet energy state (ATP/ADP ratios) correlated well with glucose-stimulated insulin release in this model, whereas it occurred independently of cyclic AMP, inositol phosphates, or protein kinase C (PKC) activity *(55,56)*.

Glucose Promotes β-Cell Phosphoinositide Breakdown and PKC Activation

Another way of increasing cytosolic Ca^{2+} is via mobilization of Ca^{2+} from intracellular stores. This phenomenon has attracted great interest since the discovery of inositol trisphosphate ($InsP_3$) as a second messenger, which conveys receptor-mediated signals and is capable of releasing Ca^{2+} from the endoplasmic reticulum *(57)*. $InsP_3$ is generated during phospholipase C-catalyzed hydrolysis of phosphoinositides present in the plasma membrane *(5)*, an event mediated by heterotrimeric GTP-binding proteins *(58,59)*, which are expressed in islets *(60)*. $InsP_3$ then diffuses into the cytosol and binds to a specific receptor on the endoplasmic reticulum, resulting in mobilization of Ca^{2+}, an effect also demonstrated in the β-cell *(26)*. Recently, it was reported that the NAD^+ metabolite cyclic ADP-ribose purportedly can release Ca^{2+} from intracellular stores in β-cells *(61)*. However, others *(62)* have refuted this assertion, which cannot easily be reconciled with the lack of effects of $InsP_3$ reported by the same authors *(61)*. The second product of the phospholipase C-catalyzed reaction is diacylglycerol, which functions as an endogenous activator of the Ca^{2+}- and phospholipid-dependent PKC *(63)*. The importance of PKC in regulation of insulin secretion has been subject to intense scrutiny (*see* refs. *5,8,28,29,64,65)* for reviews), its role being explored by tumor-promoting phorbol esters, which are potent stimulators of PKC when added acutely. It has been found that phorbol esters are able to increase insulin secretion as well as β-cell PKC activity *(66–75)*. Moreover, endogenous β-cell PKC activity was increased by exposure to a high glucose concentration *(76–78)*, and immunocytochemically PKC-α was translocated to the plasma membrane by glucose stimulation *(77,78)*. The source of diacylglycerol generated by glucose seems to be different than that produced by carbachol activation of phospholipase C and possibly related to glucose metabolism itself *(79)*. PKC may, in turn, modulate other signaling pathways, e.g. phospholipase D *(80)*, voltage-dependent Ca^{2+} channels, and phospholipase C *(74,75)*. Additionally, previous reports indicate that early fetal islets, that have not yet become glucose-sensitive do not express the PKC-α isozyme, and that the development of a glucose-sensitive insulin secretion correlates temporally with the appearance of this isozyme in the β-cells *(81)*. However, when PKC is downregulated by prolonged phorbol ester treatment, there is still an intact response to glucose with respect to insulin release and increase in cytosolic Ca^{2+} *(64,65,69)*. These results therefore imply that PKC activation apparently is of no major regulatory importance in glucose-regulated insulin release, although it may be required for amplification of insulin release in response to other secretagogues by sensitizing the stimulus-secretion coupling to Ca^{2+}. The issue remains, however, to clarify whether any particular PKC isozyme may specifically be required for β-cell glucose sensing. Additionally, ceramide, generated during sphingomyelin hydrolysis induced by interleukin-1β *(82)*, was recently shown to exert actions opposite to those of diacylglycerol in the β-cell, i.e., inhibition of β-cell mitogenesis and insulin production *(83)*.

Glucose Stimulates β-Cell cAMP Formation

Glucose also causes an activation of the β-cell adenylyl cyclase system, possibly through a Ca^{2+}/calmodulin mechanism *(84)*, resulting in the formation of cAMP *(7,9)*, a well-known second messenger. cAMP in turn activates type I and II protein kinases A, which phosphorylate a variety of target proteins in the β-cell. Moreover, cell-permeant cAMP analogs stimulate insulin release *(5,9,28,72,85–89)*, whereas antagonists of cAMP-dependent protein kinases seemingly are not able to counteract the insulin release elicited by glucose while preventing the secretory response to the adenylyl cyclase activator forskolin *(90)*. In permeabilized β-cells, natural cAMP also promoted insulin release *(90)*. In their entirety, these data indicate that cAMP itself is sufficient to trigger insulin release by activating a phosphorylation cascade, but the nucleotide does not seem to be necessary in transducing the secretory signal of glucose. On the other hand, extant data indicate that glucagon and the incretin glucagon-like peptide 1, a novel antidiabetogenic drug (*91,92*; *see below*), acts specifically and solely through cAMP formation *(93)*.

Insulin Secretagogues Transiently Inhibit β-Cell Protein Phosphatase Activity

Compared to protein kinases, relatively little attention has been paid to the role of serine/threonine protein phosphatases (PPases), which catalyze protein dephosphorylation *(94)*, in the β-cell. However, both type 1 and 2A serine/threonine PPases, as well as the cyclosporin target calcineurin (PPase-2B), were recently identified in β-cells by Western blotting and by enzymatic assay *(75,95,96)*. Additionally, the specific PPase inhibitor, okadaic acid *(97)*, was shown to promote Ca^{2+} entry and insulin release, possibly through hyperphosphorylation (and thereby activation) of voltage-activated L-type Ca^{2+} channels *(98)*. Likewise, inhibition of insulin release by neurotransmitters was recently reported to occur through activation of PPase-2B *(99)*. Furthermore, in intact β-cells, several insulin secretagogues evoked a rapid and transient inhibition of PPase activity, presumably contributing to a hyperphosphorylated state of β-cell regulatory proteins *(100)*. Our general belief is that PPase inhibition results in an increase in a cellular phosphoprotein regulating insulin secretion.

We also speculate that PPase inhibition probably enhances the actions of protein kinases, allowing secretion to be "tuned" by many signaling pathways. For example, a stimulus leading to kinase activation that is not "strong" enough to initiate secretion by itself may be enhanced to a point sufficient to induce secretion if a second signal (i.e., one leading to the inhibition of the PPase[s] that counter the actions of that kinase) is also present. Alternatively, it is equally possible that PPase inhibition affects entirely different pathways from those known to be affected by protein kinases. Nonetheless, since the activation of a process by dephosphorylation will likely require phosphorylation to turn it off, it is again likely that there will be cooperative effects of protein kinases and phosphatases. Consistent with this model is the recent finding that the 36-kDa catalytic subunit of PPase-$2A_c$ undergoes carboxyl-methylation, an effect accompanied by increased PPase-2A activity and suppressed insulin secretion *(101)*.

Adenine and Guanine Nucleotides Inhibit β-Cell Protein Phosphatase Activity

Previous investigations showed that GTP and other guanine nucleotides stimulate insulin secretion from permeabilized β-cells *(102,103)*, nutrient insulin secretagogues rapidly increase β-cell ATP and GTP levels through oxidative metabolism prior to initiation of insulin release *(104,106)*, and islet GTP is required (and can be rate-limiting) for insulin release *(107–109)*. Furthermore, small elevations of glucose concentrations redirect and amplify GTP synthesis *(110)*, which may potentiate Ca^{2+}-induced insulin secretion in intact islets *(111)*. However, the molecular mechanisms by which these nucleotides stimulate insulin release are not fully understood. Previous investigators have shown that guanine nucleotide-induced insulin secretion cannot fully be explained by $PtdInsP_2$ breakdown, Ca^{2+}, cyclic AMP, or pertussis toxin-sensitive GTP-binding proteins N_s, N_i, or N_o *(102)*. Concordant with this, we have shown that physiological concentrations of natural adenine and guanine nucleotides (ATP > ADP, GTP > GDP) were found to inhibit type-2A, and to a lesser extent type-1, PPase activity in β-cell homogenates or purified PPases in a dose-dependent fashion *(100,112)*. Therefore, adenine and guanine nucleotides cause concentration-dependent inhibitory effects on β-cell PPase activities, which may contribute to the increase in phosphorylation state that occurs during stimulation of insulin release. Thus, PPase inhibition may be one important mechanism, complementary to effects on protein kinases and ion channels, by which these nucleotides promote insulin release.

Glutamine and Glutamate Inhibit β-Cell Protein Phosphatase Activity and Stimulate Insulin Release

There is a linear correlation between the ability of an amino acid to activate glutamate dehydrogenase and its ability to release insulin from β-cells *(113,114)*. Thus, although the insulin-releasing properties of L-leucine in part involves its metabolism with subsequent ATP generation, the amino acid also allosterically activates glutamate dehydrogenase. Furthermore, the nonmetabolizable analog of L-leucine, β-2-aminobicyclo[2.2.1]heptane-2-carboxylate also activates glutamate dehydrogenase and is almost as potent as L-leucine in promoting insulin release *(115)*. The metabolism of L-glutamine involves its conversion by glutaminase into glutamate, which in turn is oxidatively deaminated by glutamate dehydrogenase in mitochondria ensuring the formation of the Krebs cycle intermediate α-ketoglutarate. We recently showed that stimulating intact β-cells with L-glutamine caused a rapid and transient decrease in the activity of PPases *(100)*. Our findings also indicate that glutamate is a potent inhibitor of β-cell PPase activities, exhibiting an IC_{50} of ≈0.4 m*M* *(112)*, well within physiological concentrations in islets *(113)*. Moreover, a cell-permeable glutamate dimethyl ester was found to be a potent insulin secretagogue *(116)*. Recent findings indicate that islet glucagon-producing α-cells, as well as intrapancreatic ganglia, stain intensely for glutaminase *(117)*, suggesting that glutamate may influence islet β-cells in a paracrine manner, being released from adjacent α-cells or neurons. In addition to acting possibly via ionotropic glutamate receptors recently discovered on β-cells *(117)*, our data suggest that glutamate, exogenously derived or generated from L-glutamine, may provoke insulin release by direct inhibition of β-cell PPase activity in addition to effects on metabolism and ATP formation *(100,112)* (*see* Fig. 1 for overview).

Glucose Metabolites and Inositol Hexaphosphate Inhibit β-Cell Protein Phosphatase Activity

We have found that addition to β-cell homogenates of certain glucose metabolites, generated in glycolysis and the Krebs cycle, inhibits the activities of type 1 and 2A cation-independent PPases in a dose-dependent fashion and at concentrations that promote insulin secretion *(112)*. When insulin secretion is stimulated by glucose, these metabolites rapidly accumulate inside the β-cell *(37)*. IC_{50} values were for phosphoenolpyruvate 0.25 m*M* and for oxalate ≈5 m*M* *(112)*. Additionally, fructose-1,6-bisphosphate, 3-phosphoglycerate, and citrate were potent inhibitors of β-cell PPase activities *(112)*. D-Glucose, fructose-6-phosphate, β-glycerophosphate, 2-phosphoglycerate, isocitrate, α-ketoglutarate, succinate, fumarate, malate, NAD(H), and NADP(H) had little or no effects on PPase activity when added to β-cell homogenates. Interestingly, fructose-2,6-bisphosphate and glucose-1,6-bisphosphate, which are known to activate phosphofructokinase allosterically, the rate-limiting enzyme in the glycolytic pathway, have recently been reported to have inhibitory effects on porcine heart PPase-2A *(118)*. Thus, these glucose metabolites, in addition to Ca^{2+} and ATP, might sustain the insulin secretory response to glucose in β-cells by contributing to

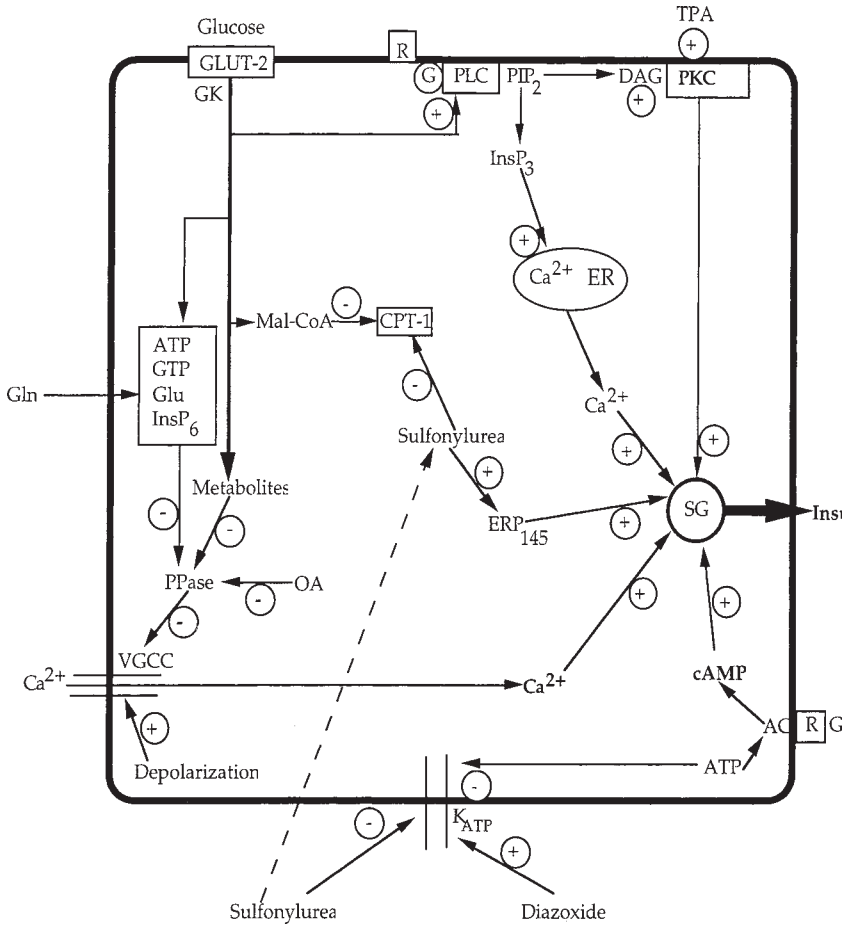

Fig. 1. Current working model depicting mechanisms regulating insulin secretion in β-cells. +, Stimulation; –, inhibition. AC, adenylyl cyclase; ATP, adenosine trisphosphate; cAMP, adenosine 3', 5'-cyclic monophosphate; CPT-1, carnitine palmitoyltransferase 1; DAG, 1,2-diacylglycerol; ER, endoplasmic reticulum; ERP_{145}, exocytosis-regulating protein; G, GTP-binding protein; GK, glucokinase; Gln, L-glutamine; GLP-1, glucagon-like peptide 1; Glu, glutamate; GLUT-2, glucose transporter 2; GTP, guanosine trisphosphate; $InsP_3$, inositol 1,4,5-trisphosphate; $InsP_6$, inositol hexaphosphate; K_{ATP}, ATP-regulated K^+ channel; OA, okadaic acid; mal-CoA, malonyl coenzyme A; PIP_2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PLC, phospholipase C; PPase, serine/threonine protein phosphatase; R, receptor; SG, secretion granule; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; VGCC, voltage-gated Ca^{2+} channel. *See text* for details.

a hyperphosphorylated state of β-cell proteins. Indeed, previous studies have shown that 3-phosphoglycerate not only increases protein phosphorylation *(119)*, but also stimulates insulin release from permeabilized β-cells *(120)*.

We have recently shown that inositol hexaphosphate ($InsP_6$) is a potent inhibitor of β-cell PPase activities, PPase-2A being most susceptible to inhibition *(123)*. IC_{50} values were ≈6 and 2 μ*M* for PPase-1 and 2A, respectively, effects reproduced on PPases purified to apparent homogeneity. By contrast, all other inositol polyphosphate species produced little or no consistent effects. The $InsP_6$ effects were occurring at physiological concentrations (≈45 μ*M*) previously reported in RINm5F insulinoma cells *(27)*. Therefore, PPase inhibition may be a novel biological role of $InsP_6$ of possible critical regulatory significance in the insulin secretory process. Additionally, $InsP_6$ may activate PKC subspecies, as was recently shown in islets and other tissues *(121,122)*, possibly leading to a direct stimulation of exocytosis by recruitment of insulin secretory granules to the site of exocytosis. Interestingly, $InsP_6$ levels were increased by glucose, suggesting that it may be of physiological significance *(123)*. Additionally, $InsP_6$ was found to increase the current through voltage-gated L-type Ca^{2+} channels *(123)*, thus mimicking the action of the specific PPase inhibitor okadaic acid *(98)*. Therefore, it is conceivable that an increase in islet $InsP_6$ evoked by glucose stimulation contributes to the secretory response to the sugar by inactivating PPases, thereby promoting entry of Ca^{2+} through voltage-gated L-type Ca^{2+} channels.

Glucose Inhibits β-Cell Fatty Acid Oxidation and Increases Long-Chain Acyl-CoA Esters Through Malonyl-CoA

It has been known for some 20 years that, in mouse islets, glucose inhibits β-oxidation of endogenous fatty acids, instead favoring their esterification *(124,125)*. Among metabolic coupling factors in addition to ATP and reduced

pyridine nucleotides (NAD[P]H), a great deal of interest has recently been focused on malonyl-CoA *(126–129)*, which is formed as citrate is generated from glucose in the Krebs cycle *(127,130)*. Malonyl-CoA is a potent inhibitor of the β-cell enzyme carnitine palmitoyltransferase 1, which diverts β-cell fatty acid metabolism from β-oxidation into generation of long-chain acyl-CoA esters *(129)*. These, in turn, may complex with α-glycerophosphate to form PKC-activating diacylglycerol, or exert effects of their own on the insulin stimulus-secretion coupling, e.g., modulating intracellular Ca^{2+} handling by lowering the set point for Ca^{2+} release *(131)* or directly stimulating PKC or ion channels *(127)*. Glucose and several other nutrient secretagogues, provoke a rapid increase in β-cell short-chain acyl-CoA profile that preceded the onset of insulin secretion *(126,127,129)*. Likewise, specifically inhibiting carnitine palmitoyltransferase 1 resulted in an increased insulin release *(127)*. Conversely, by pharmacologically inhibiting the penultimate step in malonyl-CoA synthesis, catalyzed by citrate lyase, glucose-induced insulin secretion was totally obtunded, indicating the profound functional importance of malonyl-CoA in glucose-stimulated insulin secretion *(128)*. Additionally, long-term glucose exposure stimulates acetyl-CoA carboxylase gene expression in a pancreatic β-cell line, thereby providing the carbon flux ensuring malonyl-CoA formation *(132)*. Likewise, in islets and in insulinoma cells, glucose rapidly increases acetyl-CoA carboxylase activity prior to initiation of insulin release *(133)*. In contrast to the abundancy of acetyl-CoA carboxylase and the anaplerotic enzyme pyruvate carboxylase, which controls replenishment of Krebs cycle intermediates, fatty acid synthase is poorly expressed in β-cells *(133)*. Therefore, it appears that β-cell metabolic signal transduction by malonyl-CoA functions primarily to regulate mitochondrial β-oxidation of fatty acids at the expense of providing substrates for phospholipid biosynthesis *(133, 134)*. Furthermore, diazepam binding inhibitor, a polypeptide produced in islet δ-cells with paracrine inhibitory effects on insulin secretion *(135)*, even in permeabilized β-cells *(136)*, has been identified as an acyl-CoA binding protein *(136)*. Interestingly, in other cells, in diabetes and starvation (which impedes glucose-stimulated insulin release *[137]*) carnitine palmitoyltransferase 1 becomes resistant to inhibition by malonyl-CoA *(138)*. Whether this phenomenon, presumably inhibiting PKC because of the metabolic switch favoring fatty acid β-oxidation at the expense of phospholipid synthesis, would impact negatively on glucose-stimulated insulin release in vivo in diabetics remains to be seen.

Role of GTP Binding Proteins

Heterotrimeric GTP-binding proteins (G proteins) serve as coupling factors between cell-surface receptors and intracellular effector enzymes, e.g., adenylyl cyclase, guanylyl cyclase, and phospholipase C (reviewed in ref. *58*). Several inhibitory, as well as stimulatory, G proteins have been identified and characterized also in β-cells *(59,60,139)*. On ADP-ribosylation, a conformational change is induced, whereupon the GTP binding affinity, and functional activity of the protein are altered. A useful means of pharmacologically manipulating their activity originated in the discovery of "islet-activating protein," the toxin of *Bordetella pertussis*, which stimulates islet cAMP synthesis and insulin secretion by alleviating adenylyl cyclase from an inhibitory constraint by ADP-ribosylating an inhibitory G protein *(140)*. Conversely, cholera toxin may be employed to activate adenylyl cyclase directly. In β-cells, it has been shown that the suppressive effects of α_1 and α_2 receptor agonists phenylephrine and clonidine *(88,141–143)*, somatostatin *(144)*, galanin *(139)*, and E-type prostaglandins *(59)*, but not those of the cytokine interleukin-1β *(85,87,145)*, at least in part seem to be conveyed through interference with pertussis toxin-sensitive heterotrimeric G proteins regulating islet cAMP and Ca^{2+} metabolism. Additionally, the mitogenic action of lithium in the β-cell *(146)* appears to be conveyed through pertussis toxin-sensitive G proteins *(147)*. Recently, a novel regulatory mechanism for the 37-kDa β-subunit of trimeric G proteins in β-cell membranes and insulin secretory granules was proposed by the Metz laboratory *(148)*. According to this model, which contrasts to the classical receptor-agonist mechanisms described above, a GTP-specific protein kinase may transiently phosphorylate a histidine residue in the β-subunit through a classical ping-pong mechanism, thereby altering its functional activity. Moreover, galanin and prostaglandin E_2 have been shown to activate a high-affinity GTPase in β-cell membranes *(149)*.

Additionally, monomeric small (23–27 kDa) GTP binding proteins, which are believed to shuttle between a soluble and membrane-bound state, have recently attracted a great deal of attention in the β-cell as modulators of intracellular trafficking and insulin exocytotis by regulating vectorial movement of secretory granules *(150,151)*. In clonal β-cells, approx 10 proteins structurally related to the *rho* and *ras* protooncogenes have been identified, the majority being associated with secretory granule-enriched fractions *(151)*. Among these, one protein crossreacted with a monoclonal antibody to rab3a. Since a synthetic rab3a peptide was found to stimulate insulin release, rab3a may thus constitute a potential novel candidate for regulation of exocytosis is the β-cell *(151)*. It seems that in insulinoma cells, these small GTP binding proteins complex with GDP dissociation inhibitor proteins in the cytosol *(151)*, particularly of the *rho* types.

Nitric Oxide Differentially Inhibits the Insulin Stimulus-Secretion Coupling

Some 10 yr ago, it was shown that supernatants from activated macrophages are cytotoxic towards β-cells in vitro *(152)*. These islet-infiltrating leukocytes have been

shown to produce a number of cytokines *(152,153)*, most notably interleukin-1β (IL-1), and extant data indicate that IL-1 is able to exert both inhibitory and cytotoxic actions on islet β-cells in vitro *(85,87,152)*. IL-1 inhibits insulin secretion at least in part by selectively interfering with β-cell mitochondrial substrate oxidation, leading to a reduced energy production *(154)*. A considerable amount of compelling evidence suggests that a primary event in IL-1 action involves generation of the noxious gas nitric oxide (NO) *(155–158)*. Being a highly reactive, low-mol-wt radical, NO is able to interact rapidly with a variety of target molecules *(159–161)*.

The influence of the NO donor 3-morpholino-sydnonimine (SIN-1) on the regulation of discrete parts of the stimulus-secretion coupling in isolated pancreatic islets has been investigated *(86)*. The insulin secretory response to the cardinal insulin secretagogue glucose was preferentially impeded following SIN-1 exposure, resembling the situation noted with IL-1. One striking feature of NO is that it is able to bind to iron-sulfur enzymes, and thereby modulate their biological activity *(159)*. One such example is the Krebs cycle enzyme *cis*-aconitase, which was previously shown to be a major inhibitory target for IL-1 and NO in the β-cell, and possibly accounting for the reduced glucose oxidation by IL-1 *(154)*. Obviously, inhibition of this enzyme would result in an impaired ATP production with secondary effects on ATP-dependent K^+ channels and Ca^{2+} entry. An alternative effect was proposed in a recent report *(162)* in which it was suggested that NO inhibits glucose-induced insulin release by opening ATP-sensitive K^+ channels through suppression of the activity of the glycolytic enzyme phosphofructokinase. Additionally, by ADP ribosylation, NO can enhance incorporation of NAD into glyceraldehyde-3-phosphate dehydrogenase by modifying a cysteine at the active site of the enzyme, thereby potentially depressing glycolysis *(163)*. Another biological feature of NO includes its ability to stimulate *S*-nitrosylation of various target proteins containing "critical" SH groups *(160,161)*, and it is conceivable (although not yet proven) that NO directly affects the β-cell ATP-dependent K^+ channel, which is known to contain functionally important reactive SH groups *(164)*. The precise nature of which proteins in the stimulus-secretion coupling that are targeted by NO in the thiol-dependent redox mechanisms reported *(86)* remains to be elucidated in forthcoming studies. However, in this context, it is of interest to note that reversible redox reactions are of great regulatory significance in β-cell function (reviewed in *165*).

Previous findings indicate that the stimulation of insulin secretion with carbachol was partially countered by SIN-1 *(86)*, suggesting that NO may have modified phospholipase C by means of *S*-nitrosylation, as was reported previously in other tissues *(166)*. Indirect evidence in favor of this view comes from the fact that coincubation with the disulfide reducing agent dithiothreitol (DTT) partially offset the inhibitory action of SIN-1 *(86)*. Stimulating insulin release by directly activating heterotrimeric GTP binding proteins, connected to phospholipase C and adenylyl cyclase, by AlF_3 was not affected by SIN-1 *(86)*. This finding indicates that NO apparently does not affect the GTP binding proteins regulating insulin release in this system. By contrast, in other cells, NO has been reported to influence the function of GTP binding proteins *(166)*.

SIN-1 impeded the insulin secretory response to PKC activation by phorbol ester *(86)*, suggesting PKC inactivation by NO, possibly through *S*-nitrosylation. This is an attractive hypothesis, since PKC has critical thiol residues that influence its kinase activity and that can be oxidized by NO *(167)*. However, somewhat surprising, coaddition of DTT failed to reverse the inhibitory effect of SIN-1 on PKC-regulated insulin release *(86)*. This indicates either that SIN-1 acts unrelated to *S*-nitrosylation in this case, or alternatively, that NO permanently inactivated PKC, as has been reported previously *(167)*.

cAMP-stimulated insulin release was not influenced by SIN-1 *(86,168)*. This finding differs from previous data in other tissues that showed NO inhibition of adenylyl cyclase activity by *S*-nitrosylation of vicinal thiols in the calmodulin binding domain of the cyclase, an effect reversed by DTT or glutathione *(169)*. However, it appears that in the β-cell, the cAMP system is less sensitive to thiol oxidation than other parts of the stimulus-secretion coupling *(165,168)*.

There are several reports indicating that NO can regulate Ca^{2+} channels in cardiac myocytes *(170,171)*, although it is not clear whether this is achieved through *S*-nitrosylation. However, contrasting to these reports, the β-cell Ca^{2+} channel seems more resistant to NO, since SIN-1 exerted no discernible effects on K^+-stimulated insulin release *(86)*.

In conclusion, these data suggest that NO inhibits insulin secretion from β-cells partly by *S*-nitrosylation of vicinal thiol residues, likely forming intramolecular disulfides, in key proteins in the stimulus-secretion coupling *(86)*. The results seem to exclude heterotrimeric GTP binding proteins, adenylyl cyclase, or voltage-activated Ca^{2+} channels, being inhibited by NO in the β-cell *(86)*. By contrast, enzymes involved in glucose catabolism, phospholipase C, PKC, or proteins regulating intracellular Ca^{2+} handling may be targeted by NO. These adverse effects of NO on the β-cell stimulus-secretion coupling may be of importance for the development of the impaired insulin secretion characterizing diabetes mellitus.

Novel Mechanisms of Action of Hypoglycemic Sulfonylureas

Hypoglycemic sulfonylureas, such as glyburide, are widely used in the management of NIDDM patients, and it is currently believed that they chiefly stimulate insulin secretion by closing β-cell ATP-dependent K^+ channels, thereby triggering Ca^{2+} influx *(50–52,172–177)*. However, recent studies have shown that 90% of β-cell sulfonylurea

Table 1
Metabolic Abnormalities in Pancreatic β-Cells in NIDDM[a]

Enzyme, corresponding	Comment	Refs.
GLUT-2	Glucose transporter	*186,194,207–209*
Glucokinase	In MODY patients	*196*
Glucose cycling		*197–199*
Pyruvate dehydrogenase	In hyperlipidemia	*212–218*
ATP-dependent K^+ channel		*219*
FAD-linked glycerophosphate dehydrogenase	In GK rats	*185*
Intracellular Ca^{2+} handling		*200*

[a]This table summarizes metabolic anomalies reported in pancreatic β-cells in NIDDM animal models.

binding takes place intracellularly *(178–180)*, and a 145-kDa sulfonylurea binding, exocytosis-regulating, protein has been identified in β-cells *(178)*. Sulfonylureas are also able to stimulate insulin release from permeabilized β-cells, or in voltage-clamped conditions where the membrane potential is held constant *(178)*. Recent findings show that glyburide, in therapeutic concentrations, potently inhibits β-cell carnitine palmitoyltransferase 1, thus giving rise to diacylglycerol and subsequent PKC activation *(112,138)*. In concordance with this, previous studies have shown that PKC inhibitors can abolish the secretagogueic action of glyburide *(178)*. Furthermore, specific pharmacological inhibitors of carnitine palmitoyltransferase 1 and fatty acid oxidation (e.g., etomoxir) not only stimulate insulin secretion in vitro *(128)*, but are also hypoglycemic in vivo in diabetic states *(181)*. It should be noted, however, that the suggestion of intracellular, Ca^{2+}-independent, secretagogueic actions of sulfonylureas has been challenged recently *(182)*.

The Pancreatic Islet β-Cell in NIDDM

In humans suffering from NIDDM, as well as in genetic animal models for this disease (e.g., the GK rat *[183–185]*), a striking feature is the selective impairment in the insulin secretory response to glucose, whereas the hormone output by other types of secretagogues seem essentially intact *(12,14–16,37)*. It has been shown that islets from diabetic GK rats exhibit several abnormalities in glucose metabolism and glucose uptake, including downregulation of the high-K_m facilitative glucose transporter protein GLUT-2 *(184,186)*. In Table 1, some metabolic abnormalities in diabetic β-cells are summarized. Previous studies have shown that glucose-insensitive fetal islets also exhibit reduced expression of GLUT-2 and an impaired glucose metabolism *(187–190)*, while retaining a normal function of the ATP-regulated K^+ channels *(191)*. All diabetic animal models examined to date have shown reduced β-cell GLUT-2 expression and glucose uptake, often paralleled by similar reductions in GLUT-2 mRNA, whereas mutations in the GLUT-2 gene in diabetic patients rarely occur *(186,192)*. Is this GLUT-2 deficiency the cause or consequence of diabetes? This important issue was reviewed by Unger *(186)*. It appears that in animal models for obesity-associated NIDDM, insulin infusion markedly depresses GLUT-2 mRNA and activity, although it was believed that the hyperinsulinemia did not cause this in its own right *(186)*. Conversely, chronic glucose infusion in vivo or culturing islets in high glucose concentrations in vitro resulted in increments in GLUT-2 levels, excluding hyperglycemia *per se* causing GLUT-2 downregulation in diabetes *(186)*. Additionally, preventing hyperglycemia in rats with obesity-related NIDDM by the α-glucosidase inhibitor acarbose did not appreciably upregulate GLUT-2 *(186)*. Concordant with this, stable transfection of normally glucose-insensitive insulinoma cell lines *(70,193)* with GLUT-2 cDNA confers glucose-stimulated insulin release *(186)*. Interestingly, experiments by Thorens et al. showed that transplantation of normal islets into diabetic recipients resulted in downregulation of their GLUT-2 expression and glucose-stimulated insulin release; conversely, grafting GLUT-2-deficient islets from diabetic animals into healthy recipients caused GLUT-2 upregulation and restoration of glucose-stimulated insulin release *(194)*. These findings clearly indicate that some unknown factor other than glucose and insulin in the diabetic environment (fatty acids?) suppresses β-cell GLUT-2 expression. However, also nonobese animal models for NIDDM, such as the GK rat, neonatally streptozotocin-treated rats, normal rats with steroid-induced diabetes, and rats with extreme insulin resistance, exhibit GLUT-2 deficiency (reviewed in *186*). It should be noted, however, that in human islets GLUT-1 seems to be much more abundantly expressed than GLUT-2 *(195)*.

In addition to this, genetic studies have disclosed that a certain familiar form of maturity-onset diabetes in youth (MODY pedigrees) is characterized by glucokinase gene mutations *(37,196)*. MODY patients with glucokinase mutations exhibit alterations in the pattern of glucose-stimulated insulin release, inasmuch as their β-cells show a right-shifted threshold for glucose-stimulated insulin release in comparison to normal subjects *(37,196)*. In transgenic mice expressing glucokinase antisense ribozyme, there was a similar right-shifted glucose-stimulated insulin release; however, these animals remained normoglycemic *(8)*.

Furthermore, in genetically diabetic obese mice, evidence suggests the existence of glucose cycling, in which ATP is consumed *(197–199)*. Therefore, this futile cycling of glucose, consisting of phosphorylation of glucose to glucose-6-phosphate and its subsequent dephosphorylation back to glucose may result in the consumption of ATP leading to a reduction in the cytosolic ATP/ADP ratio and thereby causing an impairment in glucose-sensitive insulin release. A similar increase in glucose cycle activity was also demonstrated in the GK rat and after glucocorticoid treatment *(197–199)*. Also, in the GK rat mitochondrial oxidative metabolism of glucose is impaired *(184)*, possibly because of a deficient activity of FAD-linked glycerophosphate dehydrogenase, which regulates β-cell glycerol phosphate shuttle *(185)*. Since it was concluded that the GLUT-2 underexpression observed in β-cells of GK rats could not be sufficient to explain entirely the lowered insulin release and the apparent normal activity of glucokinase/hexokinase retained in GK rat β-cells, the impaired glycerophosphate dehydrogenase activity may constitute an important defect in these islets with potentially significant pathophysiological implications. A similar reduction in glycerophosphate dehydrogenase activity was noted in islets of *db*/*db* mice and streptozotocin-diabetic rats *(185)*. By contrast, the activity of other enzymes, e.g., glutamate dehydrogenase, glutamic acid decarboxylase, and α-ketoglutarate dehydrogenase remained unaltered in diabetic islets *(185)*. The secretory response to glucose seems to have been preferentially impeded in these islets, because Krebs cycle substrates remained excellent insulin secretagogues *(185)*. Finally, in a series of thought-provoking studies, it has been suggested that intracellular Ca^{2+} handling may be deficient in diabetic β-cells *(41,200–202)*. Thus, in this model, endoplasmic reticulum Ca^{2+} stores were proposed to regulate membrane potential since the Ca^{2+} ATPase inhibitor, thapsigargin, was found not only to promote secretion but also to increasing Ca^{2+} influx *(202)*. Additionally, it was proposed that glucose-induced intracellular Ca^{2+} release is dependent on depolarization alone *(201)*, and that islets of *db*/*db* mice lacked the normal pattern of Ca^{2+} oscillations, possibly owing to a deficiency in endoplasmic reticulum Ca^{2+} ATPase functional activity *(200)*.

Impact of Lipotoxicity on the β-Cell in the Diabetic State

Among the metabolic derangements resulting from insulin deficiency in NIDDM, hyperglycemia with accompanying "glucose toxicity" has traditionally received the most attention *(186,203,204)*. However, disturbances in lipid homeostasis have been a relatively neglected area. Despite hyperinsulinemia, primary adipocyte resistance to insulin-mediated antilipolysis seems to coexist with other metabolic abnormalities in obesity-associated NIDDM *(205,206)*. It is becoming increasingly clear that hyperlipacidemia may be just as deleterious as hyperglycemia and also impacts negatively on the β-cell *(207–209)*. Thus, by chronically infusing normal rats with a fat emulsion (Intralipid™), it was shown that the resulting increase in plasma free fatty acid exerted detrimental actions on β-cell function *(210)*, an effect broadly reproduced in later studies with palmitate *(211)*. The intracellular events conveying these inhibitory effects in the normal and diabetic β-cell were recently elucidated by in vitro studies *(212–215)*. These studies indicate that long-term exposure to long-chain fatty acids increased basal insulin release, whereas glucose-stimulated insulin release was obtunded *(212–215)*. Additionally, β-cell insulin biosynthesis and glucose oxidation rates were impeded under these conditions *(212)*. Importantly, the inhibitory effects were offset by the carnitine palmitoyltransferase 1 inhibitor, etomoxir *(212)*. It was moreover found that such a chronic in vitro fatty acid exposure impaired rat and human β-cell pyruvate dehydrogenase activity, partly because of increments in pyruvate dehydrogenase kinase activity *(213,215)*. Additionally, in human islets, ketone bodies also suppressed glucose-stimulated insulin secretion *(214)*. In genetically diabetic, nonobese, GK rats *(184)*, the impaired glucose-sensitive insulin secretion coincided with attenuated pyruvate dehydrogenase activity and increased pyruvate dehydrogenase kinase activity *(215)*, and a similar deficiency was noted in the obese *db*/*db* mouse *(216)*. However, whether this downstream enzymatic deficiency is merely secondary to the GLUT-2 defect in these islets *(186,194)* remains to be seen. At any rate, these findings indicate the existence of a glucose–fatty acid cycle in β-cells chronically exposed to fatty acids, resembling the metabolic situation in obesity-associated NIDDM, with the concomitant impairment in pyruvate dehydrogenase activity. Additionally, it was recently shown that fatty acids induce carnitine palmitoyltransferase 1 gene expression in the clonal β-cell line INS-1, an effect coinciding with an impaired glucose oxidation rate *(217)*. Therefore, these aberrations in β-cell function may be of relevance both in obese and lean NIDDM patients. Moreover, the impairment in β-cell glucose sensitivity induced by fasting was recently attributed to a fatty acid-induced inhibition of pyruvate dehydrogenase activity *(218)*. Recently, another attractive hypothesis concerning the molecular mechanisms responsible for glucose intolerance in obesity-associated NIDDM was launched. It was shown that high levels of long-chain acyl-CoA esters, which accumulate in the β-cell in diabetes because of hyperglycemia and hyperlipidemia, activate the ATP-sensitive K^+ channel of the β-cell *(219)*. This activation would be expected to result in an impaired insulin release and may thus be an important mechanism accounting for the selective glucose-insensitivity characterizing the β-cell in diabetes. Additional targets for long-chain acyl-CoA esters in the β-cell, with potential relevance for the insulinopenia in diabetes, include glucokinase, the endoplasmic reticulum

Ca^{2+}-ATPase, carnitine palmitoyltransferase-1, acetyl-CoA carboxylase, the adenine nucleotide translocase, and the sodium pump *(219)*.

Glucoincretins and Other Compounds as Potential Novel Antidiabetic Drugs

Glucose-induced insulin secretion is known to be considerably greater when the sugar is ingested compared to an intravenous glucose load *(220)*. This phenomenon has launched the search for gut hormones, "incretins," capable of potentiating the effect of glucose. One major incretin is glucagon-like peptide 1 *(7–36)* (GLP-1) amide, which has attracted a great deal of interest since the discovery of its potent antidiabetogenic effect (*221* reviewed in *91,92,222*). Having the advantage over insulin and the sulfonylureas of being active only under conditions of hyperglycemia, undesired hypoglycemic side effects are avoided *(223)*. GLP-1 fulfills all the criteria of a glucoincretin; it delays gastric emptying and, like insulin, also suppresses glucagon secretion, thereby depressing hepatic glucose production *(223)*. When administered subcutaneously to patients with NIDDM before a meal, GLP-1 reduced both postprandial hyperglycemia and insulin requirements, and may prove to be especially suitable for treatment of overweight patients with secondary failure to sulfonylureas *(223)*. On the cellular level, GLP-1 confers glucose competence to glucose-unresponsive β-cells *(92,93)*. Possibly this effect is mediated by the increased cAMP content of the β-cells, because it was blocked by a cAMP antagonist and mimicked by an agonist of this pathway *(93)*. Since the incretins were extensively reviewed recently *(92)*, they will not be discussed in greater detail presently.

Additional novel insulin secretagogues that are efficient in diabetic islets include cell-permeant methyl esters of glycolytic and Krebs cycle intermediates, such as succinate, glutamate, and 3-P-glyceraldehyde, which seem to act by bypassing the metabolic deficiency in diabetic islets *(12,224–226)*. Given the present findings on PPases *(see above)*, these metabolites may act in part by directly (or by ATP) inhibiting β-cell PPase activities, in addition to generating ATP during their metabolic breakdown *(100,112)*. Whether any of these compounds will become of clinical utility remains to be seen.

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