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Review Article

Diabetes and the Role of Inositol-Containing Lipids in Insulin Signaling

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Abstract

Among metabolic diseases, diabetes is considered one of the most prevalent throughout the world. Currently, statistics show that over 10% of the world's aged population (60 years and older) suffers from diabetes. As a consequence, it consumes a considerable proportion of world health expenditure. This review considers both past and current research into the molecular basis of insulin resistance found in type II diabetes and focuses on the role of inositol-containing phospholipid metabolism. It has been firmly established that the activation of phosphatidylinositol 3–kinase (PI3-K) is important for the propagation of the metabolic actions of insulin. In addition to the 3-phosphorylated phosphatidylinositols formed via the action of PI3-K, the glycosyl-phosphatidylinositol/inositol phosphoglycan (GPI/IPG) signaling component is also strongly implicated in mediating numerous metabolic actions of insulin. Although all the elements within the type II diabetes phenotype have not been fully defined, it has been proposed that defects in insulin transmembrane signaling through malfunction of inositol-containing phospholipid metabolism and absenteeism of the generation of phospholipid-derived second messengers may be associated with the appearance of the type II diabetic phenotype. Pharmaceutical approaches using synthetically produced IPG analogues, which themselves mimic insulin's actions, alone or in combination with other drugs, may lead the way toward introducing alternative therapies for type II diabetes in the coming years.

Introduction

Diabetes mellitus is the term used to define the absolute or partial impairment of insulin action on the cells of target tissues that leads to abnormally elevated blood glucose concentrations. A positive diagnosis of diabetes is made when a fasting plasma glucose level is >7.8 mM or a plasma glucose level is >11.1 mM during an oral glucose tolerance test (measured at two time points, before 2 hr and at 2 hr) (1–3). The consequence of such impairment of insulin function is the tendency toward the onset of chronic com-

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plications such as premature cardiovascular disease, retinopathy, neuropathy, nephropathy, and macrovascular disease (4,5). When no treatment against diabetes is administered, severe organ damage gradually leads to the death of afflicted subjects.

There are two types of diabetes. Type I diabetes (also termed insulin-dependent diabetes mellitus) is the result of autoimmune destruction of the pancreatic beta cells, which gives rise to a deficiency in insulin secretion (6–8). To circumvent this lack of insulin secretion, insulin is usually self-administered subcutaneously to maintain an approximately normal blood glucose concentration. Type II diabetes (also known as non-insulin–dependent diabetes mellitus) is caused by both insulin deficiency and insulin resistance. This insulin resistance, particularly within skeletal muscle, liver, and adipose tissue, leads to pronounced hyperglycemia as glucose transport into cells is severely reduced (3,9). Despite the fact that the etiology of type II diabetes is less well understood than that of type I, available evidence points to both genetic and nutritional/behavioral factors (type of diet, obesity, physical exercise) as possible regulators of the onset of type II diabetes (10,11).

Molecular Mechanisms of Insulin Action: Via Proteins and Phospholipids

Insulin delivers its signal through binding to its high-affinity cell surface receptor. This protein is a tetrameric complex consisting of two α and two β subunits. The intracellular domains of the β subunits contain intrinsic protein tyrosine kinase activity and are involved in the initiation of insulin-dependent transmembrane signaling events. The early signal transduction events involve the autophosphorylation of its receptor on tyrosine residues and of the insulin receptor substrates 1, 2, and 3 (IRS-1, -2, and -3), leading to the generation of docking sites on both the insulin receptor and the IRS proteins for SH2 and SH3 domain–containing proteins. These proteins, which include both enzymes and adapters, initiate a multitude of downstream signals that regulate the phosphorylation state of a wide variety of proteins and some phospholipids (12–14).

Despite our vast knowledge of the role of enhanced protein phosphorylation/dephosphorylation in response to insulin, relatively little is known about insulin's effects on phospholipid metabolism and its possible role in explaining insulin resistance in type II diabetes. The connection between insulin-mediated signal transduction and phospholipids received its breakthrough in 1986 when Cuatrecasas' laboratory provided evidence that the previously described "insulin-stimulated water-soluble modulators of enzyme activity" were in fact derived from glycolipids (15). This led to the rapid appearance of numerous reports within the literature confirming that insulin, and other growth factors and classical hormones, stimulated the hydrolysis of similar glycolipids (glycosyl-phosphatidylinositol, now termed GPI) generating water-soluble second messengers termed inositol phosphoglycan (IPG) (16,17). This process, drawn schematically in Figure 1, indicates GPI phospholipid hydrolysis leading to the generation of IPG. As interest in the GPI/IPG signaling system during insulin signal transduction grew, investigators in diabetic research attempted to understand the metabolically altered phenotype by considering phospholipid-derived second messengers and their role in the control of intermediary metabolism (18).

IPG: A Requirement for Functional Metabolic Actions of Insulin?

Positive insights into a relationship between the insulin GPI/IPG signaling system and type II diabetes soon became apparent after examining differences between insulin-stimulated GPI hydrolysis in cells isolated from diabetic and normal rats. Three independent investigations revealed that, in addition to less GPI being present, insulin-stimulated GPI hydrolysis in cells exhibiting insulin resistance was absent or severely impaired (19–21). IPG, the product of GPI hydrolysis, is considered a potential insulin second messenger on the basis of three lines of evidence: (*1*) by itself it can mimic a large number of the metabolic actions of insulin (both in vivo and in vitro, see later and Table 1), (*2*) anti-IPG antibodies can attenuate several in vivo actions of insulin, and (*3*) synthetically produced chemical IPG analogues exhibit in vitro and in vivo insulin-like activities (see later and ref. 17). The first of these three lines of evidence has been widely used to investigate differences in gluconeogenesis and lipogenesis between normal and diabetic experimental models. In doing so, the results have underlined the importance of this novel insulin-stimulated signaling pathway. At least

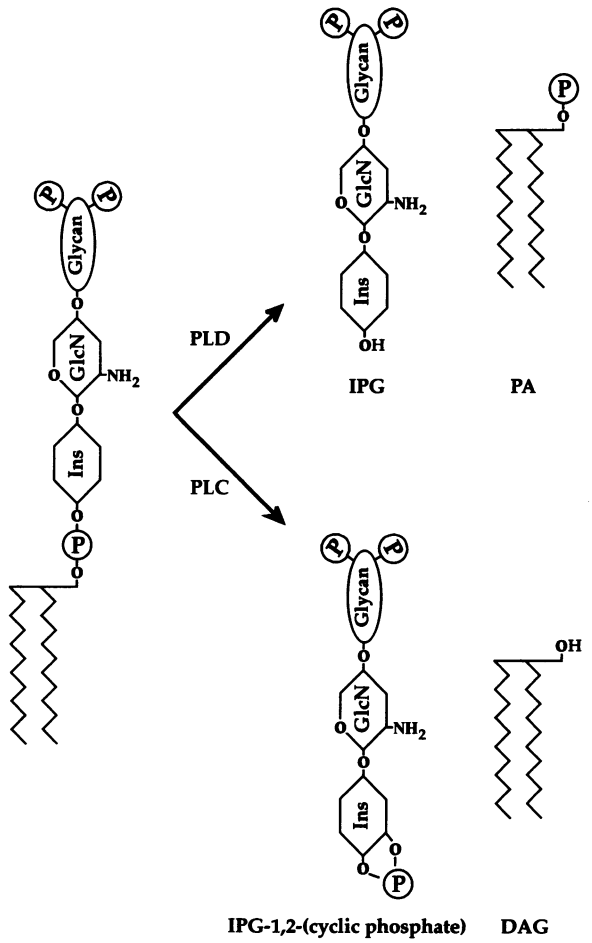

Fig. 1. Structure and hydrolysis of a GPI phospholipid by phospholipases D (PLD) and C (PLC) generating IPG type A and PA, and IPG-1,2-(cyclic phosphate) type A and DAG. Originally it was proposed that the responsible enzyme was a PLC, however, recent studies suggest that PLD is also capable of generating biologically active IPG type A from GPI (24,25). Ins, *myo*-inositol; GlcN, glucosamine; PA, phosphatidic acid; DAG, diacyl glycol.

two types of IPG exist. The structures of the IPGs have been refined but not totally elucidated. IPG type A contains *myo*-inositol and glucosamine (22), whereas IPG type P contains α-pinitol (3-O-methyl D-*chiro*-inositol) and galactosamine as core components (23). Both types of IPG have been shown to additionally contain neutral sugars and phosphate residues. The origin of IPG type A is thought to be *myo*-inositol-containing GPI, as both phospholipase C (PLC)- and phospholipase D (PLD)-mediated hydrolysis of GPI yield biologically active IPG molecules (17,22,24,25). The same cannot be said, however, for IPG type P, as important differences are evident. Stereochemically, the favored configuration of *chiro*-inositol within phosphoinositides for phospholipase C–mediated hydrolysis is that of the L-diastereomer (26). However, Larner's group has claimed that IPG type P contains D-*chiro*-inositol (23,27), thereby putting in doubt its phospholipid origin as D-*chiro*-inositol-containing phosphoinositide-specific PLCs have thus far remained elusive. Within a few years of the publication of a preliminary structure of GPI, a synthetic chemical analogue of IPG type A (1-D-6-O-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1,2-(cyclic phosphate)) was reported. This compound exhibited both insulin-mimetic properties (stimulation of lipogenesis in rat adipocytes, ref. 28) and insulin-like growth factor I (IGF-I)–mimetic properties (stimulated mitogenesis and *Jun* and *Fos* oncogene expression in the developing chicken inner ear, refs. 29–31; I. Varela-Nieto, D. R. Jones, Y. León, H. N. Caro, T. W. Rademacher, unpublished observations). It appears that the *myo*-inositol 1,2-(cyclic phosphate) portion of the above IPG type-A analogue is important, as (1-D-6-O-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-phosphate is an inactive IPG type-A analogue (32). The opening of a *myo*-inositol 1,2-(cyclic phosphate) moiety to form a *myo*-inositol 1-phosphate within IPG type A could be a method for the cessation of its biological activity in vivo. This decyclization could be achieved by the action of the enzyme cyclic inositol phosphohydrolase (33). In addition to 1-D-6-O-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1,2-(cyclic phosphate), the synthesis of 6-0-(2-amino-2-deoxy-α-D-glucopyranosyl)-D-chiro-inositol-1-phosphate, an analogue of IPG type P, has been reported. This latter compound also shows both insulin-mimetic (34) and distinct IGF-I-mimetic (e.g., differentiation of the cochleovestibular ganglion in the developing chicken inner ear, ref. 35) attributes. A variation on the structure of the above-mentioned IPG type-P analogue has recently been reported. The pseudodisaccharide consisting of α-pinitol linked to galactosamine, termed INS-2, is able to stimulate testosterone production in human thecal cells to an extent similar to that of insulin (36,37). Furthermore, synthetic inositol-glycan moieties mimic insulin action, as discussed in detail below. An inspection of the biological properties of IPG type-A and type-P analogues reveals that they show contrasting biological effects that correspond to those of purified IPG subtypes (17,38,39). These observations reinforce the claim that IPG is a true second messenger and is essential in many metabolic, mitogenic, and differentiation processes.

Table 1. Insulin mimetic effects of IPG type A (A), IPG type P (P), and their analogues from different sources

Whole Cells		Cell Extracts	
Biological Activity	**Effect**	**Enzymatic Activity or Phosphorylation**	**Effect**
Lipolysis (A, A*, A•)	Inhibition	cAMP phosphodiesterase (A)	Stimulation
Lipogenesis (A, A*, A•)	Stimulation	Pyruvate dehydrogenase (A)	Stimulation
GPAT (A*, A•)	Stimulation	PDH phosphatase (P)	Stimulation
Phospholipid methyltransferase (A)	Inhibition	Glucose-6-phosphatase (A)	Inhibition
Steroidogenesis (A, P*)	Stimulation	Fructose-1,6-biphosphatase (A)	Inhibition
Glucose transport (A, A*, A•)	Stimulation	Adenylate cyclase (A)	Inhibition
GLUT4 translocation (A*, A•)	Stimulation	cAMP-kinase (A)	Inhibition
Acetyl-CoA carboxylase (A)	Stimulation	Casein kinase II (A)	Biphasic
Glycogen phosphorylase a (A)	Inhibition	Glycerol-3P acyltransferase (P)	Stimulation
Pyruvate kinase (A)	Stimulation	ATP citrate lyase (A)	Stimulation
Glucose oxidation (A)	Stimulation	Galactolipid sulfotransferase (A)	Inhibition
Glucose production (A)	Inhibition	Protein phosphatase 2C (P)	Stimulation
Lactate accumulation (A)	Stimulation		
Glycogen synthesis (A, P, A*, A•)	Stimulation		
Glycogen synthase kinase-3 (A*, A•)	Inhibition		
Tyrosine aminotransferase (A)	No effect		
Protein phosphorylation (A, A*, A•)	Stimulation Inhibition		
cAMP levels (A)	Inhibition		
PI 3-kinase (A*, A•)	Stimulation		
Myelin basic protein kinase (A•)	Stimulation		
Mitogen activated kinase (A•)	Stimulation		
Protein kinase B phosphorylation (A•)	Stimulation		
Fructose-2,6-P2 levels (A)	Stimulation		
Ion channels (A)	Modulation		
Ca^{2+}-Mg^{2+} ATPase (A)	Stimulation		
Ca^{2+} entry (A)	Inhibition		
Amino acid transport (A)	Stimulation		
Protein synthesis (A, A•)	Stimulation		
Specific mRNA levels (A)	Stimulation Inhibition		
DNA and RNA synthesis (A)	Stimulation		
Cell proliferation (A, P)	Stimulation		
Insulin secretion (A)	Inhibition		
Cell differentiation (neurogenesis) (P)	Stimulation		

Adapted and updated from Jones and Varela-Nieto (17). A*, A phosphoinositolglycan-peptide (PIG-P) (57,58,61); A•, various chemically synthesized PIG-P analogues (38,39); GPAT, glycerol-3-phosphate acyltransferase P*, an analog of IPG type P (INS-2) consisting of α-pinitol and galactosamine (36) PDH, pyruvate dehydrogenase.

Despite the uncertainty of the precursor and/or origin of IPG type P, the role of *chiro*-inositol and IPG type P in type II diabetes has been the subject of multiple and enlightening investigations. Kennington and colleagues reported that in patients suffering from type II

diabetes and in diabetic Rhesus monkeys there was a decreased urinary excretion of *chiro*-inositol compared to that seen in healthy controls. Furthermore, *chiro*-inositol in IPG type-P preparations from type II diabetic human biopsy muscle tissue was undetectable (40). A follow-up report by Asplin and co-workers indicated that in type II diabetics both the amount of IPG type P and its bioactivity were reduced in hemodiasylate, urine, and muscle compared to normal subjects (41). These and other reports (which have included results of the analysis of inositol isomer excretion and of IPG preparations from diabetics of differing nationalities) tentatively suggest that a decrease in *chiro*-inositol and IPG type-P biosynthesis may be underlying features of type II diabetes and that the urinary *chiro*-inositol-to-*myo*-inositol ratio could be considered a clinical marker for predicting the onset of diabetes at an early stage (42). Another direct report indicating the abnormality of IPG type-P production in diabetics is that from Shaskin and colleagues (43), whose results clearly indicate that in a group of insulin-resistant subjects, glucose ingestion failed to increase the serum concentration of IPG type P compared to that seen in healthy controls. Pak and colleagues have addressed the metabolism of both *myo*- and *chiro*-inositol in the diabetic Goto-Kakizaki rat compared to its normal healthy counterpart (44). Their findings indicate that there exist significant differences in the in vivo interconversion of the inositol isomers in their free unconjugated form and at the phosphoinositide level between the two rat phenotypes. This clearly underlines the importance of correct inositol isomer homeostasis, particularly in insulin-sensitive tissues.

The two biochemical assays principally used to define the biological activity of the IPG subtypes are the IPG type A–mediated inhibition of protein kinase A and the stimulation of pyruvate dehydrogenase phosphatase by IPG type P. As mentioned previously, IPG molecules possess insulin-mimetic properties. These properties, which have been extracted from more than a decade of research literature, are listed in Table 1. Clearly, in most of the insulin-mimetic observations, IPG type A was used because it could be prepared through the in vitro hydrolysis of purified GPI from various sources (22,45). Also, IPG type P was perhaps viewed at first with caution, as less information was available concerning its structure and its source was undefined, thereby limiting its use in experimental studies. As techniques have improved for the purification of both types of IPG, during the last 5 years, exciting evidence has shed new light on the role of IPG subtypes in the mechanism of insulin action. The first in vivo effects of IPG in animal models showed that both IPG subtypes were able to stimulate glycogen production in the diaphragm of normal rats and transiently reduce the elevated blood glucose in streptozotocin-diabetic rats (46). This later observation was reinvestigated using prolonged intravenous delivery of IPG type P, which was able to normalize the plasma glucose level in diabetic rats to an extent to that observed with insulin (23). Two further papers from Larner's laboratory have indicated that insulin-dependent regulation of glucose metabolism through IPG production is indeed more complicated than first thought. This claim is substantiated by the finding that in the plasma of type II diabetics there exists an elevated (compared to control subjects) concentration of an antagonist of insulin-stimulated glucose oxidation (47). Structural information concerning this antagonist revealed that it contained *myo*-inositol 1,2-(cyclic phosphate), galactosamine, and mannose residues, indicating attributes very similar to those of the two IPG subtypes identified thus far. The biological activity of this inhibitor was found to be due to the inhibition of IPG type P–stimulated pyruvate dehydrogenase phosphatase activity by shifting the magnesium ion requirement of the enzyme to the right (48).

Evidence suggests that the in vivo regulation of IPG production in diabetic subjects must be somehow perturbed, compared to that in healthy controls. Directly or indirectly, upstream of insulin-stimulatable GPI-hydrolyzing enzymes lay the insulin receptor and the IRS proteins. Point-mutated and kinase-deficient insulin receptors fail to couple to the generation of IPG type A through GPI hydrolysis (49,50), which implies that in some way, insulin-stimulated tyrosine phosphorylation events control GPI hydrolysis. It is not known however, if this is due to a lack of tyrosine phosphorylation of a phospholipase or to malfunction of an adapter protein linking the receptor to such an enzyme involving a tyrosine phosphorylation event. In the case of insulin, this tyrosine-dependent GPI hydrolysis may be related to IRS protein expression and function. A decreased level of IRS protein expression and serine/threonine residue hyperphosphorylation of IRS proteins give rise to signals that attenuate insulin signal transduction and, furthermore, are characteristic of insulin-resistant cells (51,52). In

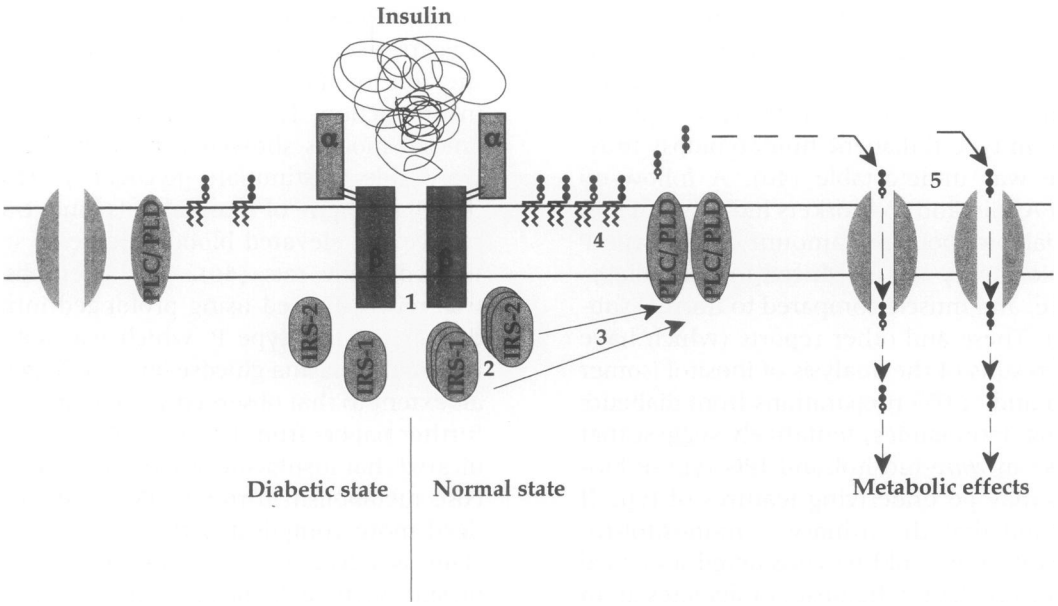

Fig. 2. Schematic indicating a possible route through which insulin could stimulate IPG type-A generation in normal insulin-responsive cells. Points within the proposed route at which a blockade would result in the formation of insulin-resistant cells are indicated. (1) Insulin receptor protein tyrosine kinase (point-mutated, kinase domain–deleted, serine/threonine hyperphosphorylation). (2) IRS proteins (expression levels, phosphorylation state). (3) Phospholipases C (PLC) or D (PLD) (expression levels, coupling). (4) GPI in the plasma membrane (quantity, localization). (5) IPG type-A transporter/receptor IPG type-A signal "transducing protein" (expression levels, functionality). The open symbols represents IPG derived from GPI-PLD-mediated GPI hydrolysis. The closed symbols represent IPG derived from GPI-PLC-mediated GPI hydrolysis.

addition, and equally intriguing, in IRS-2-knockout mice the type II diabetic phenotype was observed, suggesting that insulin signaling through this molecule is important for avoiding extracellular hyperglycemia (53).

One of the signal transduction pathways that is rapidly initiated by insulin is the activation of PI3-K through its interaction with the IRS proteins (54,55). The intricacies of this signaling pathway are further complicated by the very recent finding that insulin-dependent activation of PI3-K may be compartmentalized within cells. This subcellular activation of PI3-K in distinct cell compartments appears to be dependent on the localized expression of IRS proteins, whose pattern is determined by the metabolic status of the cell; this, in turn, is inferred by the diet of the animal (56). Interestingly, an IPG type A–like glycopeptide termed PIG-P, isolated from *Saccharomyces cerevisiae,* was able to elevate both IRS-1-associated PI3-K activity and the tyrosine phosphorylation state of IRS-1 without increasing insulin receptor phosphorylation, indicating a clear bypass of the insulin receptor (57). This was a significant advance, in that it described a defined signal transduction pathway for PIG-P, which led to a physiological response: the translocation of GLUT4 glucose transporters to the plasma membrane for the uptake of glucose from the extracellular space (58). These GLUT4 glucose transporters may be localized in membrane caveolae (59), from which GPI and IPG have been isolated (60). The mechanism by which PIG-P exerts its insulin mimetic properties has been investigated. A membrane-spanning protein labile to high-concentration salt, tryptic action, and *N*-ethylmaleimide may act as a "transducer protein" with the cooperation of accessory proteins, including caveolin, to cause the activation of a cytoplasmic tyrosine kinase that in turn could phosphorylate the IRS proteins, thereby leading to the early activation of PI3-K. This PI3-K activation may represent the point at which insulin-stimulated signal transduction via its receptor and PIG-P action through the "transducer protein" converge (61). A series of back-to-back reports immediately followed which examined the structural requirements for PIG-P biological activity through the use of an extensive range of chemically synthesized analogues.

Interestingly, 1-D-6-O-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1,2-(cyclic phosphate)-containing analogues exhibited the greatest insulin-mimetic properties in rat adipocytes and diaphragms (stimulation of PI3-K, stimulation of PKB/Akt, stimulation of glucose transport, inhibition of glycogen synthase kinase-3 activity, stimulation of glycogen synthase, stimulation of the MAP kinase pathway, and stimulation of protein synthesis), which underline the importance of this structure (38,39). Earlier studies with an IPG-like molecule (similar to that isolated from *Saccharomyces cerevisiae*) derived from the GPI anchor of the variant surface glycoprotein of *Trypanosoma brucei* demonstrated insulin-mimetic characteristics (inhibition of lipolysis in rat adipocytes and gluconeogenesis in hepatocytes) (62). This compound, again resembling that of IPG type A, was subsequently shown to cause the activation of the protein serine/threonine phosphatase type 1 in rat adipocytes (63). New light has recently been shed on the mechanism of insulin-mediated activation of protein serine/threonine phosphatases. Results concerning insulin-mediated activation of glycogen synthase and inhibition of glycogen phosphorylase in young, lean, adult Rhesus monkey liver have implicated the involvement of activated protein serine/threonine phosphatases types 1 and 2C. These activations may be mediated by IPG type P (64,65). At the molecular level, activation of protein phosphatase type 1 may be more complex because of the dual control exerted by both IPG type A and type P. When threonine phosphorylated, DARRP-32 and INH-1 were potent inhibitors of protein phosphatase type 1. Very recently, IPG type A was found to inhibit the PKA-mediated phosphorylation of DARRP-32 and INH-1, whereas IPG type P was observed to directly stimulate protein phosphatase-2C, thereby causing the dephosphorylation of phosphorylated DARRP-32 and INH-1 (66). With all the information available in the literature, we were able to tentatively align a pathway of molecular events, starting at the insulin receptor and culminating at the point of IPG generation in normal insulin-responsive cells. This chain of events is illustrated in Figure 2. Within the proposed scheme, possible points, including the insulin receptor protein tyrosine kinase, IRS proteins, phospholipase type C (or D), membrane GPI, and the IPG transporter or IPG "transducer protein" at which defects could exist, are indicated. A malfunction of any of these components could be associated with the onset of type II diabetes.

Closing Remarks

Without doubt, insulin signaling is a complex event that involves the divergence of multiple pathways downstream from its activated receptor. Some of these signaling cascades converge to control enzymes directly involved in intermediary metabolism, whereas others lead to the control of protein translation (67). Although the roles of IPG types A and P are not completely understood, we believe that they play important roles in insulin signaling. Perhaps the most direct evidence comes from the findings that, if the enzyme responsible for the synthesis of *myo*-inositol-containing GPI is knocked out, then insulin fails to stimulate glycogen, suggesting that the presence of the GPI/IPG signaling system is a prerequisite for the process in cells that use IPG type A for glycogen production (68). However, because IPG type P has been shown to stimulate glycogen production in vivo in other cell types (23,46), it seems plausible to suggest that there are important cell-to-cell type differences in both the generation and utilization of IPG subtypes to mediate, in some cases, the same metabolic effects (36,69; I. Varela-Nieto, D. R. Jones, Y. León, H. N. Caro, T. W. Rademacher, unpublished observations). In terms of metabolic control, insulin elicits many of its effects through the activation of PI3-K (54,55). In light of recent work in which insulin-mimetic PIG-P and synthetic inositol-glycan analogues have been shown to activate various enzymes through the obligatory participation of PI3-K, it could be possible that the type II diabetic phenotype may manifest itself when the levels of PI3-K or its functionality are below those in normal models (70–72).

It is hoped that in the near future, further insights into the GPI/IPG signaling pathway will emerge to allow us to extend our knowledge of how diabetes causes its unwelcomed aberations of intermediary metabolism and, in particular, how chemically defined agents, such as IPG analogues, may provide a possible starting point for the design of drugs to combat type II diabetes.

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