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MINI-REVIEW

The Role of Glycosyl-Phosphoinositides in Hormone Action

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Abstract

Despite significant advances in the past few years on the chemistry and biology of insulin and its receptor, the molecular events that couple the insulin–receptor interaction to the regulation of cellular metabolism remain uncertain. Progress in this area has been complicated by the pleiotropic nature of insulin's actions. These most likely involve a complex network of pathways resulting in the coordination of mechanistically distinct cellular effects. Since the well-recognized mechanisms of signal transduction (i.e., cyclic nucleotides, ion channels) appear not to be central to insulin action, investigators have searched for a novel second messenger system. A low-molecular-weight substance has been identified that mimics certain actions of insulin on metabolic enzymes. This substance has an inositol glycan structure, and is produced by the insulin-sensitive hydrolysis of a glycosyl-phosphatidylinositol in the plasma membrane. This hydrolysis reaction, which is catalyzed by a specific phospholipase C, also results in the production of a structurally distinct diacylglycerol that may selectively regulate one or more of the protein kinases C. The glycosyl-phosphatidylinositol precursor for the inositol glycan enzyme modulator is structurally analogous to the recently described glycosyl-phosphatidylinositol membrane protein anchor. Preliminary studies suggest that a subset of proteins anchored in this fashion might be released from cells by a similar insulin-sensitive, phospholipase-catalyzed reaction. Future efforts will focus on the precise role of the metabolism of glycosyl-phosphatidylinositols in insulin action.

Key Words: Glycolipids; Protein phosphorylation; phospholipase; inositol.

Introduction

Despite intensive scrutiny, our understanding of the molecular events that link the insulin receptor to the regulation of cellular metabolism lags far

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behind that of other peptide hormones. One likely explanation for this slow progress may lie in the complicated nature of insulin action. The wide number and broad diversity of responses makes it improbable that a single mechanism could account for all of the actions of insulin. Rather, it is more likely that insulin action involves a network of interrelated and independent pathways with differing levels of divergence regarding mechanisms of regulation.

As it became clear that the well-recognized mechanisms of signal transduction (i.e., cyclic nucleotides, ion channels) were not primarily responsible for explaining the actions of insulin, many investigators focused on the role of protein phosphorylation. Insulin simultaneously produces both the dephosphorylation of some proteins (i.e., glycogen synthetase, pyruvate dehydrogenase, hormone-sensitive lipase) and the phosphorylation of others (i.e., ribosomal S6, ATP citrate lyase). The dephosphorylation reactions induced by insulin result in the regulation of carbohydrate and lipid metabolism. Although it is not clear whether insulin-induced serine phosphorylation leads to changes in the catalytic activities of any enzymes, processes such as glucose transport and protein synthesis may require insulin-induced kinase reactions (Denton, 1986). These and other observations have led to the emerging concept that two basic pathways, activation of protein phosphatases and kinases, may mediate many of insulin's actions.

Two major hypotheses have been proposed for coupling the insulin receptor to intracellular changes in protein phosphorylation: (i) a phosphorylation cascade, initiated by the tyrosine kinase activity of the receptor (Kasuga *et al.*, 1982), and (ii) the generation of a low-molecular-weight, diffusable second messenger. Evaluation of the phosphorylation cascade hypothesis has centered on site-directed mutagenesis and antireceptor antibody experiments which suggest that the receptor tyrosine kinase is necessary for the full expression of insulin's activities (Chou *et al.*, 1987; Ellis *et al.*, 1986; Morgan and Roth, 1987). The search for an insulin-dependent "second messenger" has been under way since the early 1970's. An insulin-sensitive substance was first detected in skeletal muscle that could acutely modulate glycogen synthetase activity *in vitro*. Similar kinds of extracts or substances of elusive chemical identity were subsequently identified in a variety of cell types, reported capable of regulating the activites of several insulin-sensitive enzymes (Jarett and Kiechle, 1984). It is important to note that these two pathways need not be mutually exclusive, and in fact they may operate synergistically to coordinate a pattern of protein phosphorylation in response to insulin.

Biological Characterization of a Putative Insulin Second Messenger

A low-molecular-weight substance was identified that was released from hepatic plasma membranes in response to insulin (Saltiel and Cuatrecasas,

1986). The purification of this enzyme-modulating activity relied mainly on ion exchange, molecular sizing, and phase partitioning procedures. The substance was negatively charged, insoluble in organic solvents, and not adsorbed to reversed phase columns, indicating a relatively high degree of polarity. The chemical properties inferred from chromatographic behavior and susceptibility to specific chemical modification suggested an oligosaccharide-phosphate structure.

Initial studies on the biological activities of this substance focused on the modification of the activity of the low-K_m *c*AMP phosphodiesterase in fat cell membranes. The activity of this *c*AMP phosphodiesterase was activated acutely by the modulator, as reflected by an increase in the V_{max} of the enzyme, with no appreciable effect on its K_m. The purified substance could also modify *in vitro* other insulin-sensitive enzymes assayed in subcellular fractions, including adenylate cyclase, pyruvate dehydrogenase, phospholipid methyltransferase, *c*AMP-dependent protein kinase, and acetyl CoA carboxylase (Saltiel and Cuatrecasas, 1988). Although the precise biochemical mechanism(s) by which this substance elicits its effects on these enzymes is unclear, the regulation of the activity of each of these enzymes might be explained by alterations in the state of phosphorylation of the enzyme or closely related regulatory factors. The specific regulation of protein phosphatase activity was observed in lysates from fat, brain, and liver, although it is unknown whether the modulator produces the direct allosteric regulation of specific protein phosphatases.

Preliminary compositional analyses of the enzyme-modulating substance suggested the existence of inositol as a component. Several well known inositol phosphate-containing compounds were evaluated, but none exhibited significant enzyme-modulating activity, and they did not share the chemical properties, chromatographic or electrophoretic behavior, or insulin sensitivity of the enzyme modulator. These results suggested that the enzyme modulator might be an unusual derivative containing inositol phosphate. A potential clue was identified when a novel glycosylated derivative of inositol was found in certain cell surface proteins. This novel molecular species was shown to result from a covalent bond between certain proteins and phosphatidylinositol (PI) (Low and Saltiel, 1988). This unusual linkage at the carboxy terminus of these proteins serves as an anchor for attachment to the plasma membrane. The protein is coupled via an amide bond to ethanolamine which is then attached through a phosphodiester linkage to an oligosaccharide that exhibits a terminal non-N-acetylated hexosamine glycosidically linked to the inositol ring of PI. The membrane-bound form of the protein can be converted to a water-soluble form that contains a C-terminal glycosyl-inositol phosphate by digestion with a bacterial PI-specific phospholipase C (PLC), with the simultaneous liberation of diacylglycerol.

To evaluate the possibility that the insulin-dependent enzyme modulator might arise from the phosphodiesteratic hydrolysis of a structurally similar glycolipid, the PI-specific bacterial phospholipase C was added to liver plasma membranes, and the release of the modulator into the medium was assayed. In this series of experiments, PI-PLC was found to reproduce the effect of insulin in facilitating the generation of the enzyme modulator. The PI-PLC digestions generated a substance which was chromatographically, electrophoretically, and chemically identical to that produced by insulin treatment, suggesting a basic inositol phosphate-glycan structure. Moreover, a potential precursor of the PI-PLC-generated substance could be extracted from liver membranes and chromatographically resolved from other known phosphoinositides. This precursor glycolipid was identified in a number of cell types and appeared to contain PI linked to a glycan through a non-N-substituted hexosamine. These experiments suggested that the enzyme modulator was produced as a result of a hormone-stimulated hydrolysis of this novel membrane-associated glycosyl-PI.

Considerable interest has been focused on the precise structure and biosynthesis of the glycosyl-PI precursor, and on its possible relationship to the glycosyl-PI protein anchor. Comparison of compositional analyses from several laboratories has indicated certain conserved and variant features. The precursor glycolipid appears to contain a basic core structure of PI-hexosamine. The T lymphocyte or BC_3H1 cell-derived glycolipid appears to contain diacylglycerol as the major glycerolipid moiety (Saltiel *et al.*, 1986, 1987; Gaulton *et al.*, 1988; Farese *et al.*, 1988), although in hepatoma cells a 1,2-alkylacylglycerol structure has been suggested (Mato *et al.*, 1987b). More recent studies have indicated another possible structural variation in the liver-derived glycosyl-PI, the presence of significant but variable amounts of *chiro*inositol, which perhaps accounts for the apparent lack of [^{3}H]*myo*-inositol labeling in hepatoma cells (Mato *et al.*, 1987a; Larner *et al.*, 1988). Another minor structural variation regards the presence of galactosamine in lieu of glucosamine (Larner *et al.*, 1988). Distal to the hexosamine there appear to be considerable differences in glycan composition reported by different laboratories. Whether these apparently different structures represent molecules with distinct cellular functions or subcellular localizations remains to be determined.

The insulin-sensitive glycosyl-PI appears to exhibit considerable similarity to the glycosyl-PI protein anchor. The two types of glycolipid contain similar glycerolipid domains, sensitivity to PI-PLC and nitrous acid, and the presence of inositol, nonacetylated glucosamine, and a variable glycan region. However, the insulin-sensitive glycosyl-PI apparently lacks two of the features commonly observed in the protein anchor, ethanolamine and amino acids. Additionally, the molecular size of the insulin-sensitive glycosyl-PI is smaller than similar

molecules bound to protein. Despite the similarities between the insulin-sensitive glycosyl-PI and the cell surface protein anchor, the topological distribution of the insulin-sensitive glycolipid in the plasma membrane is uncertain. Some studies have suggested a cytoplasmic orientation, since treatment of cells with PI-PLC (presumably exhaustive) did not block the insulin-induced intracellular accumulation of the inositol glycan. Gaulton and Pawlowski (1988) have reported that fluorescently labeled monoclonal antibodies to the insulin-sensitive glycosyl-PI show cytoplasmic staining, indicating an intracellular localization. In contrast, Alvarez *et al.* (1988) suggested an extracellular location for the lipid, since 85% of the total cellular glycosyl-PI was apparently surface amidinated with isethionyl acetimidate. Although the free release from cells of the inositol glycan in response to insulin has not been reported, the exogenous addition of PI-PLC to intact cells produces an insulin-like activity, exogenous addition of purified inositol glycan to intact cells mimics many of the effects of insulin, and insulin may lead to the release from the cell surface of certain glycosyl-PI anchored proteins (see below). Thus, the issue of topology of the relevant glycolipid must remain open until definitive evidence is available.

The possibility that the protein-bound and free forms of glycosyl-PI are located on opposite sides of the plasma membrane leads to further uncertainty regarding their respective biosynthetic processing. One possibility of explaining this apparent dilemma is that the early stages of glycosylation of PI occur on the cytoplasmic aspect of the endoplasmic reticulum. Upon attaining a certain level of glycosylation, a fraction of the glycolipid molecules ultimately destined for protein anchoring might be translocated from the cytoplasmic face of the endoplasmic reticulum membrane to the luminal side, in analogy to the translocation of the $(Man)_5(GlcNAc)_2$-lipid utilized for N-linked glycosylation of proteins. This translocation step may serve to segregate further biosynthethic modifications of the lipid molecules ultimately destined for protein attachment from those that will remain on the cytoplasmic face. Alternately, a final processing event, such as addition of a terminal sugar-phosphate, may serve to segregate those molecules not destined for translocation. In either case, the subsequent membrane trafficking to the cell surface might then result in a cytoplasmically oriented free glycolipid and a cell surface-oriented protein-anchored glycolipid.

Regulation of Glycosyl-PI Hydrolysis

While insulin is known to cause increased labeling of several phospholipids, it has not been found to immediately stimulate the hydrolysis of PI or the polyphosphoinositides, and it does not induce calcium mobilization

through the generation of inositol trisphosphate. In contrast, insulin does stimulate hydrolysis of glycosyl-PI, with the simultaneous production of the [^{3}H]inositol glycan and diacylglycerol that contains predominantly saturated fatty acids, but little if any arachidonic acid. The rapid production of this specific species of labeled diacylglycerol is not observed with agonists known to stimulate the hydrolysis of polyphosphoinositides (Saltiel *et al.*, 1987). Thus, the unique species of diacylglycerol and the inositol glycan probably arise from the specific, insulin-sensitive hydrolysis of the free glycosyl-PI.

The production by insulin of a structurally distinct species of diacylglycerol in the absence of calcium mobilization may help explain the perplexing role of protein kinase C in insulin action. A number of conflicting observations regarding kinase C may be accommodated by invoking the involvement of distinct chemical forms or metabolic pools of diacylglycerol. Most agonists that cause kinase C activation do so by stimulating the hydrolysis of the polyphosphoinositides, leading to the generation of inositol phosphates and diacylglycerol that contains arachidonate in the C2 position. The absence of phosphoinositide turnover in response to insulin, as well as the scarcity of arachidonate in the insulin-generated diacylglycerol, may cause a selective activation of kinase C depending upon the cell type, extent of activation, enzyme compartmentalization, substrate specificity, or susceptibility to proteolysis. Perhaps the most interesting possibility includes the selective activation of isoforms of kinase C by structurally distinct diacylglycerols. Multiple forms of the enzyme were predicted by the cloning of multiple *c*DNA's, and several isozymes have now been chromatographically resolved. Some evidence suggests that these isoforms may exhibit distinct regulatory properties, especially with regard to calcium and diacylglycerol sensitivity as well as substrate specificity. Moreover, these isoforms may exhibit different tissue and/or subcellular distributions, or may be differentially susceptible to down regulation or proteolysis. Thus, the selective activation of protein kinase C or a fraction of isozymes may explain the apparent discrepancies between the biological actions of phorbol esters and insulin.

The observation that insulin specifically stimulated the hydrolysis of glycosyl-PI led to the search for a specific phospholipase C. Such an enzyme was isolated from a plasma membrane fraction of liver, using as an assay the liberation of diacylglycerol from the glycosyl-PI anchored variant surface glycoprotein from *T. brucei* (Fox *et al.*, 1987). The catalytic activity appears to reside in a single polypeptide with an apparent molecular weight of about 52,000 daltons. The enzyme is calcium-independent, and is specific for glycosyl-PI; no hydrolysis of PI, PIP_2, or other phospholipids is observed under a variety of conditions. Although there is still no information available on the primary sequence or subcellular localization of the mammalian glycosyl-PI-PLC, the *c*DNA for an enzyme with similar specificity was cloned and sequenced from *T. brucei* (Hereld *et al.*, 1988). Interestingly, both

immunohistochemical data and the predicted amino acid sequence indicate a cytoplasmic orientation for this parasitic enzyme.

How is the regulation of glycosyl-PI hydrolysis coupled to the activity of the insulin receptor? Although a phospholipase C capable of catalyzing this reaction has been purified, it has thus far been difficult to directly demonstrate the activation of this enzyme by insulin. Recent studies with antireceptor antibodies or site-directed mutagenesis indicate that the tyrosine kinase activity of the receptor may be necessary for the expression of all of the biological actions of insulin (Rosen, 1987). In cells transfected with mutant insulin receptors that lack tyrosine kinase activity, glycosyl-PI hydrolysis is not observed in response to insulin, whereas cells transfected with wild type receptors respond normally (O. Rosen, personal communication). This suggests that the activation of the glycosyl-PI-specific phospholipase C by the receptor might occur as a consequence of a tyrosine kinase-induced cascade, possibly leading to changes in the state of phosphorylation of the enzyme or an associated regulatory factor. Alternatively, the autophosphorylation of the receptor on tyrosine residues could catalyze an intramolecular conformational change that initiates a membrane coupling event, involving noncovalent interactions with a regulatory factor. The latter possibility is supported by recent studies demonstrating that certain monoclonal antibodies to the insulin receptor which do not stimulate the receptor tyrosine kinase activity retain other insulin-mimetic properties regarding metabolic regulation (Hawley *et al.*, 1989). Perhaps these antibodies induce a conformational change in the receptor similar to that caused by autophosphorylation, resulting in some intramembrane coupling event. The coupling factor might be a specific GTP-binding protein, which in turn could activate the PLC. The involvement of a G protein in insulin action has been suggested in studies demonstrating that pertussis toxin (Elks, 1987; Goren *et al.*, 1985; Luttrell *et al.*, 1988) or antibodies to the GTP-binding *ras* p21 protein (Korn *et al.*, 1987) can block certain actions of insulin. Moreover, insulin inhibited the pertussis toxin-catalyzed ADP-ribosylation or a 41,000 M_r substrate in liver membranes (Rothenberg and Kahn, 1988). Certain of the G proteins are relatively good substrates for the insulin receptor kinase *in vitro* (Zick *et al.*, 1986). Although the direct phosphorylation of a G protein on tyrosine residues in response to insulin has not been observed *in vivo*, these *in vitro* data suggest at least the possibility of a high-affinity interaction between certain G proteins and the receptor.

Nerve Growth Factor Stimulates Glycosyl-PI Hydrolysis

Although insulin and insulin-like growth factors are somewhat unique in their potent anabolic activities, there are a limited number of other peptides

that share insulin-like biological activities. Among those is nerve growth factor (NGF). NGF, like insulin, promotes neurite outgrowth and enhances survival of certain neurons (Recio-Pinto *et al.*, 1984). Additionally, insulin and NGF share many long and short-term effects, including stimulation of amino acid and glucose transport, proteoglycan production, and induction of certain proteins and proto-oncogenes. Like insulin, reports have suggested an involvement of protein kinase C in NGF action, although there appears to be no effect of the hormone on polyphosphoinositide hydrolysis. Because of these similarities, the effect of NGF on glycosyl-PI metabolism was explored in the pheochromocytoma clonal line, PC12 (Chan *et al.*, 1989). NGF was found to stimulate the production of a [^{3}H]myristate-labeled species of diacylglycerol, with no effect on arachidonyl-labeled diacylglycerol. This was accompanied by the simultaneous NGF-stimulated hydrolysis of glycosyl-PI, along with the intracellular production of the inositol glycan. The specificity of this action was confirmed by the failure of NGF to stimulate diacylglycerol or inositol glycan production in a receptor-negative genetic variant of the PC12 line (Chan *et al.*, 1989).

In addition to explaining, in part, some of the anabolic effects of NGF, hydrolysis of glycosyl-PI might also provide a mechanism for activation of protein kinase C without PIP_2 hydrolysis or calcium mobilization. This possibility was examined by evaluating the effects of a kinase C inhibitor on the NGF-dependent induction of the *c-fos* gene. The kinase C inhibitor staurosporin attenuated this effect of NGF in a dose-dependent manner, suggesting that active protein kinase C is necessary for the induction of certain proto-oncogenes by NGF (Chan *et al.*, 1989).

Despite the similarities in these biological actions of insulin and NGF, there are significant differences in the basic structures of their receptors. Molecular cloning of the high-affinity NGF receptor revealed a sequence that predicted a monomeric structure containing a single transmembrane domain (Chao *et al.*, 1986). Moreover, there was no evidence for tyrosine kinase activity in the cytoplasmic domain of the receptor. This predicted structure is unlike the basic form thought to be required for receptors that couple to G proteins (seven membrane-spanning domains), and also unlike many other growth factor receptors and proto-oncogene products that contain a tyrosine kinase activity in the cytoplasmic domain wich catalyzes the autophosphorylation of the receptor. In addition to NGF, human growth hormone exhibits certain insulin-like effects (Isaksson *et al.*, 1985), although the receptor for this hormone is similar to the NGF receptor, in that its sequence predicts a single transmembrane-spanning domain with no apparent tyrosine kinase in the cytoplasmic domain (Leung *et al.*, 1987). Thus, it will be important to determine the precise biochemical events distal to receptor binding at which the actions of insulin (tyrosine kinase) and NGF (nontyrosine kinase) receptors

converge to elicit similar biological responses. This information may help to elucidate the molecular events in transmembrane signaling for these hormones.

Hormone-Stimulated Release of Glycosyl-PI Anchored Proteins

Another result of the insulin-induced hydrolysis of glycosyl-PI might be the release of glycosyl-PI-anchored proteins. Insulin has been reported to cause reduced levels of cellular alkaline phosphatase activity in BC_3H1 cells (Romero *et al.*, 1988). Like PI-PLC, insulin caused the release of the glycosyl-PI anchored heparan sulfate proteoglycan from rat hepatocytes (Ishihara *et al.*, 1987). A form of lipoprotein lipase may be directly or indirectly anchored to the cell surface by a glycosyl-PI anchor, since enzyme activity or LPL protein labeled metabolically with [^{3}H]glucosamine or [^{32}P]orthophosphate can be specifically immunoprecipitated from the media of 3T3-L1 cells after treatment with PI-PLC. Additionally, membrane-associated lipoprotein lipase can be labeled at the cell surface with biotin and subsequently solubilized with PI-PLC. The kinetics of release of lipoprotein lipase activity from 3T3-L1 cells by insulin and PI-PLC are identical, indicating that the acute phase release by insulin may be due to activation of a glycosyl-PI-specific phospholipase (Chan *et al.*, 1988). The observation that lipoprotein lipase is itself anchored or is tightly coupled to a glycosyl-PI anchored protein is of special significance, since this represents the first example of a protein anchored in this fashion that is known to be released from cells in response to hormones. Tissue or circulating levels of certain of the glycosyl-PI anchored proteins are altered in diabetic states, including alkaline phosphatase, 5′-nucleotidase, lipoprotein lipase, and heparan sulfate proteoglycan. However, it is unclear whether insulin will lead to the release of all accessible glycosyl-PI anchored proteins, or only a specific subset. Recent studies demonstrated that acute exposure of BC_3H1 cells to insulin or serum caused the selective loss of glycosyl-PI anchored proteins (of M_r 35 and 130 kDa) from the cell surface (Lisanti *et al.*, 1989). The further exploration of this issue may help to resolve whether there are distinct hormonally sensitive and insensitive "structural" pools of glycosyl-PI, similar to what has been proposed for metabolic pooling of the inositol lipids. Although it is not yet known whether the insulin-induced release of these glycosyl-PI anchored proteins is due to a phospholipase C, the hydrolysis of glycolipid molecules on opposite sides of the membrane in response to insulin also raises questions concerning the polarity or orientation of the relevant hydrolytic enzymes. If the free glycosyl-PI is located on the cytoplasmic face of the plasma membrane, it may be necessary to involve at least two separate enzymes, located on different sides of the membrane. One possibility is that the hormone-sensitive,

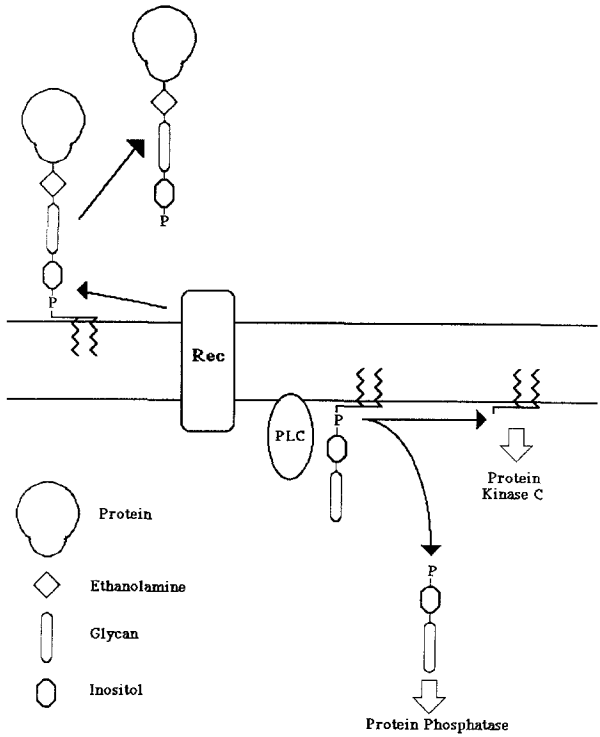

Fig. 1. The metabolism of glycosyl-PI in insulin action. A hypothetical model is presented illustrating the hydrolysis of glycosyl-phosphoinositides in plasma membranes. The interaction of insulin with its receptor causes the activation of the receptor tyrosine kinase, probably the initial signal for receptor function. The activated receptor is then coupled by an unknown mechanism that may involve an intermediate G protein to the stimulation of one or more phospholipases C specific for glycosyl-PI. This enzyme or group of enzymes then catalyzes the hydrolysis of a free glycosyl-PI that might be on the cytoplasmic side of the plasma membrane, resulting in the intracellular generation of the enzyme-modulating inositol phosphate glycan and a species of diacylglycerol that may cause a selective activation of the protein kinases C. Similarly, the activation of the insulin receptor might also cause the release of anchored proteins like heparan sulfate proteoglycan, alkaline phosphatase, or lipoprotein lipase, through an unknown mechanism that may involve an anchor-degrading enzyme.

cell surface-oriented enzyme is a specific phospholipase D (Low and Prasad, 1988).

Inositol Glycan as a Second Messenger of Insulin Action

Despite the progress made in identifying the structure and biogenesis of the inositol glycan, it is still premature to regard this compound as a second

messenger for any of the actions of insulin. The apparent insulin dependency and the rapidity and extent of the generation of the inositol-glycan are consistent with the properties expected for second messengers. However, many questions remain concerning precise chemical structure, as well as the nature of the insulin-mimetic properties. Thus far, the actions of the inositol glycan have been explored mainly in subcellular assays, so that the extent to which this molecule reproduces the actions of insulin in intact cells remains unclear. In recently studies, these issues have been addressed by evaluating the effects of the inositol glycan in fat and liver cells. Purified preparations of these compounds mimic the lipogenic (Saltiel and Sorbara-Cazan, 1987) and antilipolytic (Kelly *et al.*, 1987) actions of insulin, as well as the regulation of phosphorylate *a* (Alvarez *et al.*, 1987), pyruvate kinase (Alvarez *et al.*, 1987), *c*AMP levels (Alvarez *et al.*, 1987), and pyruvate dehydrogenase (Gottschalk and Jarett, 1988). Moreover, they produce specific protein phosphorylation patterns similar to those produced by insulin in intact fat or liver cells (Alemany *et al.*, 1987). Interestingly, preparations of the inositol glycan also caused the direct inhibition of insulin release in isolated rat islet cells (Malaisse *et al.*, 1989). However, the compound does not appear to modulate glucose transport activity or ribosomal S6 kinase. An oligosaccharide with chemical and chromatographic properties similar to the inositol glycan has recently been isolated from conditioned media of Reuber hepatoma cells (Witters and Watts, 1988). This substance stimulates both [^{3}H]thymidine uptake and activation of acetyl CoA carboxylase in a manner kinetically indistinguishable from and not additive with insulin. In contrast, this glycan does not stimulate amino acid uptake or tyrosine aminotransferase induction in hepatoma cells nor glucose transport in 3T3-L1 cells. It is especially interesting to note that the insulin-mimetic actions of the inositol glycan appear to be limited to those anabolic activities that are (1) more or less insulin-specific (i.e., are generally not reproduced by other growth factors like EGF or PDGF) and (2) are probably mediated by enhanced dephosphorylation (i.e., stimulation of protein phosphatase). Thus, the selective ability of the inositol glycan to mimic only a subset of the actions of insulin provides further evidence for diverse pathways of signal transduction in the actions of the hormone.

Although the inositol glycan appears to be a promising candidate for a second messenger of insulin action, a number of issues remain to be resolved. The ultimate proof of a role for this compound as a second messenger will critically depend, among other things, on the determination of its precise structure. This may be complicated by the chromatographic resolution of multiple species of these molecules, perhaps reflecting the existence of distinct forms with different enzyme-modulating functions or subcellular distributions. It will also be important to produce these compounds in large quantity and

homogeneous form, perhaps by organic synthesis, in order to reevaluate each of their biological activities in detail. Moreover, the biosynthetic route, mode of production and degradation, relationship to the insulin receptor, and the precise biochemical actions of these molecules need further exploration. It will be especially important to determine the topological distribution of the glycosyl-PI precursor in the plasma membrane. More detailed molecular characterization of the glycosyl-PI-specific phospholipase C will be necessary. Development of inhibitors or neutralizing antisera to this enzyme, and eventually site-directed mutagenesis studies, should help to define the functional role of this reaction in the pleiotropic actions of insulin and related hormones.

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