MINIREVIEW

Insulin-Sensitive Phospholipid Signaling Systems and Glucose Transport. Update II[1]

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Insulin provokes rapid changes in phospholipid metabolism and thereby generates biologically active lipids that serve as intracellular signaling factors that regulate glucose transport and glycogen synthesis. These changes include: (i) activation of phosphatidylinositol 3-kinase (PI3K) and production of PIP_3; (ii) PIP_3-dependent activation of atypical protein kinase Cs (PKCs); (iii) PIP_3-dependent activation of PKB; (iv) PI3K-dependent activation of phospholipase D and hydrolysis of phosphatidylcholine with subsequent increases in phosphatidic acid (PA) and diacylglycerol (DAG); (v) PI3K-independent activation of glycerol-3-phosphate acylytansferase and increases in *de novo* synthesis of PA and DAG; and (vi) activation of DAG-sensitive PKCs. Recent findings suggest that atypical PKCs and PKB serve as important positive regulators of insulin-stimulated glucose metabolism, whereas mechanisms that result in the activation of DAG-sensitive PKCs serve mainly as negative regulators of insulin signaling through PI3K. Atypical PKCs and PKB are rapidly activated by insulin in adipocytes, liver, skeletal muscles, and other cell types by a mechanism requiring PI3K and its downstream effector, 3-phosphoinositide-dependent protein kinase-1 (PDK-1), which, in conjunction with PIP_3, phosphorylates critical threonine residues in the activation loops of atypical PKCs and PKB. PIP_3 also promotes increases in autophosphorylation and allosteric activation of atypical PKCs. Atypical PKCs and perhaps PKB appear to be required for insulin-induced translocation of the GLUT 4 glucose transporter to the plasma membrane and subsequent glucose transport. PKB also appears to be the major regulator of glycogen synthase. Together, atypical PKCs and PKB serve as a potent, integrated PI3K/PDK-1-directed signaling system that is used by insulin to regulate glucose metabolism.

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The regulation of glucose transport by insulin is of critical importance for controlling glucose uptake and its subsequent metabolism or storage in glycogen, or its conversion to fat in muscle and adipose tissues. Derangements in glucose transport are of major importance in the pathogenesis of acquired forms of insulin resistance in overt forms of diabetes mellitus and obesity, and also in the pathogenesis of the initial genetic defect in insulin action that precedes the appearance of clinically apparent glucose intolerance and type II diabetes mellitus, and that presumably underlies the development of Syndrome X, a relatively common state of insulin resistance characterized by obesity, hypertension, hyperlipidemia, atherosclerosis, and, in women, polycystic ovary disease.

Needless to say, the cause of the initial genetic defect in glucose transport is still uncertain. On the other hand, during the past few years we have gained considerable insight into intracellular signaling factors that regulate glucose transport. In particular, it now seems clear that phosphatidylinositol (PI) 3-kinase (3K) is required for insulin effects on both glucose transport and subsequent storage of glucose in glycogen. Recent findings further suggest that atypical protein kinase C (PKC) isoforms, ζ and λ, and, perhaps, protein kinase B (PKB or Akt), serve as downstream effectors for PI3K in controlling the rate-limiting step in glucose transport, via the translocation of GLUT4 glucose transporters from the endoplasmic reticulum to the plasma membrane. The latter process is exceedingly complex and most likely requires input from multiple signaling factors. However, some of the more important factors appear to have

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been recently identified, and it is probably only a matter of time before we understand how they work in concert with each other and possibly other factors to regulate glucose transport. With such understanding, we will ultimately be in a position to identify the causes for acquired and genetic forms of insulin resistance.

In the present review I will focus on certain of our more recent findings on insulin-sensitive phospholipid-dependent signaling systems, and attempt to integrate these findings with other previous and more recent findings, particularly as they relate to the activation of glucose transport. These signaling systems include: (i) activation of atypical PKCs, ζ and λ, through PI3K-dependent increases in D3-PO_4 polyphosphoinositides; (ii) activation of PKB through PI3K-dependent increases in D3-PO_4 polyphosphoinositides; (iii) activation of phospholipase D (PLD) and subsequent hydrolysis of phosphatidylcholine (PC) via a PI3K-dependent mechanism requiring small G-proteins, Rho and ARF; (iv) activation of *de novo* synthesis of phosphatidic acid (PA) and diacylglycerol (DAG) independently of PI3K; and (v) activation of diacylglycerol (DAG)-sensitive PKCs via PC-PLD and *de novo* PA/DAG synthesis. This summary will largely focus on the roles of atypical PKCs and PKB in the activation of glucose transport by insulin.

General Aspects of Insulin Action

In addition to controlling glucose transport and storage of glucose in glycogen in skeletal muscle and adipose tissue, insulin controls many other biological/biochemical processes in a variety of cell types. Most notably, insulin stimulates lipid synthesis and inhibits lipid hydrolysis in adipose tissue, promotes glycogen synthesis and inhibits gluconeogenesis and glucose release in liver, and promotes protein synthesis and general RNA and specific mRNA synthesis in virtually all insulin-sensitive tissues. In health, insulin uses multiple, but obviously well-coordinated signaling mechanisms to control these diverse metabolic processes. Absence of such effective coordination leads to states of clinical insulin resistance.

General Aspects of Lipid-Dependent Insulin Signaling Systems

Initially, insulin interacts with the α-subunit of the insulin receptor and thereby activates tyrosine kinase in the β-subunit. This leads to tyrosine phosphorylation of cytoplasmic domains of the insulin receptor itself, and a number of extra-receptor proteins, including insulin receptor substrates (IRSs) 1, 2, 3, and 4, GAB-1, and Src homology 2/2-collagen related (SHC) (Fig 1). Tyrosine-phosphorylated (pY) motifs, pYXXM, in these proteins then interact with SH2 domains in a second set of adapter proteins, including the p85 subunit of PI3K, GRB2, SYP, and NCK (1).

The interaction of IRS-1/2/3/4 with the p85 subunit of PI3K leads to activation of the p110 subunit of PI3K (1), thereby causing increases in the conversion of PI-4,5-$(PO_4)_2$ to PI-3,4,5-$(PO_4)_3$ (PIP_3) within plasma and other cellular membranes. PIP_3 in turn either activates or facilitates the action of 3-phosphoinositide-dependent protein kinase-1 (PDK-1), which controls the phosphorylation of critical phosphate residues in the activation loops of PKC-ζ/λ ki-

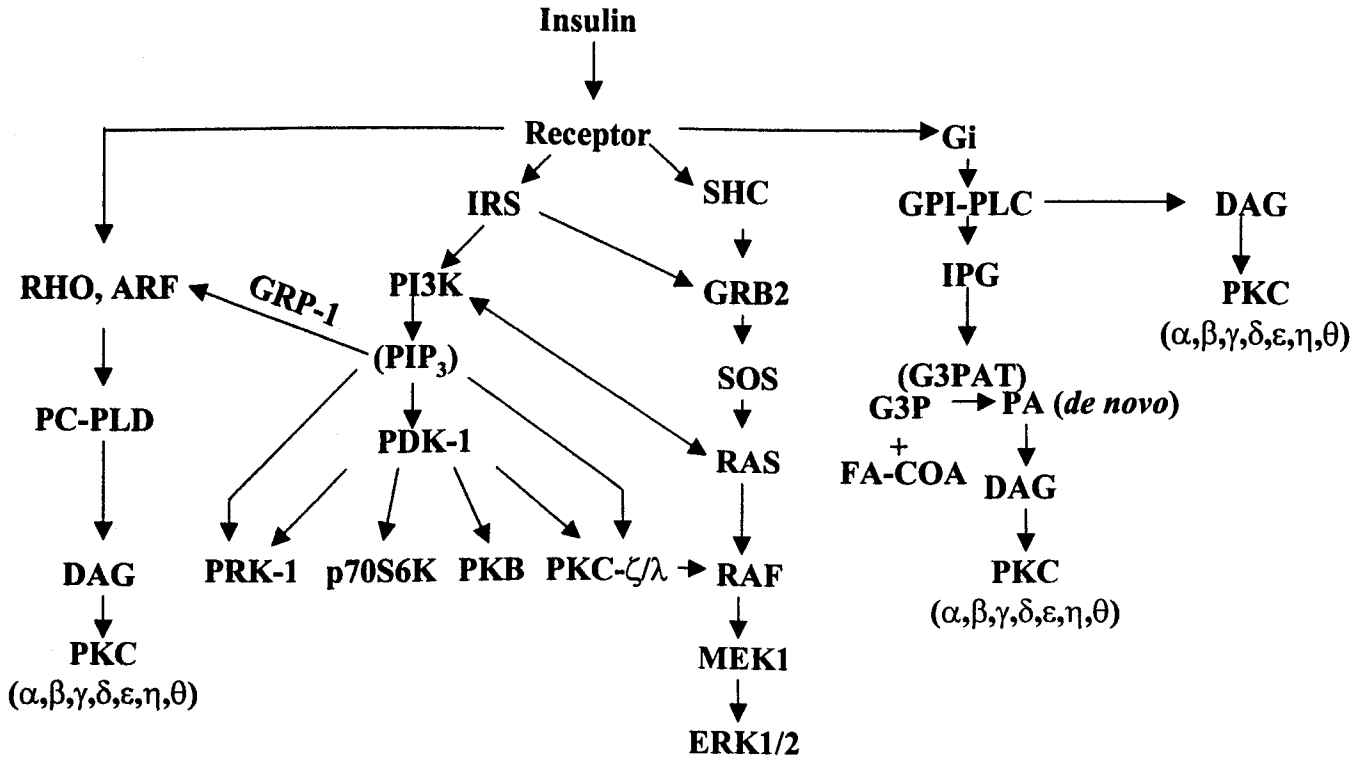

Figure 1. Insulin-sensitive lipid signaling systems.

nase (2, 3) and PKB, as well as other PKCs, PKC-related kinase-1 (PRK-1, also called PKN), and p70S6 kinase.

The interaction of IRS family members or SHC with SH2 groups on the adapter protein, GRB2, leads to an interaction of its SH3 groups with proline-rich sequences in son of sevenless (SOS), which in turn stimulates GTP/GDP exchange in RAS (4). Activated GTP-RAS then interacts with RAF through a still poorly understood mechanism requiring so-called 14•3•3 proteins. RAF thereupon activates MEK1, a dual function kinase that phosphorylates extracellular signal-regulated kinases 1 and 2 (ERK1/2) on tyrosine and threonine residues. Activated ERK1/2, phosphorylates MAP kinase-activated protein kinases 1 and 2 (MAPKAP-1/2) on serine residues, and activated MAPKAP-1/2 regulates a number of genes that control many cellular processes, including cellular differentiation, proliferation, and survival/apoptosis (4). The activation of the GRB2/SOS/RAS/RAF/MEK/ERK pathway by insulin in some cell types is independent of PI3K. However, in other cells, PI3K, PDK-1, and PKC-ζ/λ are required, along with GRB2, SOS, RAS, the onco-protein RAF, and MEK1, for activation of ERK1/2 (Fig. 1). ERK1 and 2 are not required for insulin-stimulated glucose transport, but may activate glucose transport during the action of certain other agonists.

With respect to the activation of DAG-dependent PKCs, insulin activates a plasma membrane PLD that hydrolyzes PC (5) through a mechanism requiring small G-proteins Rho and ARF (6, 7) and PI3K (5), perhaps for translocation of Rho and ARF (6) or activation of the ARF exchange factor, GLP-1 (8). PLD action on PC generates PA, which is rapidly converted to DAG or to PI (Fig. 2). DAG so formed activates conventional PKCs (cPKCs α, β, and γ) and novel PKCs (nPKCs δ, ε, η, and θ). DAG produced through the *de novo* pathway (Figs. 1 and 2) can also activate cPKCs and nPKCs.

The *de novo* pathway appears to be to activated independently of PI3K through release of an inositol-phosphoglycan (IPG) mediator from the head group of glycosyl-PI (GPI) through the action of a specific plasma membrane phospholipase C (PLC) (9, 10). This mediator activates microsomal glycerol-3-PO_4 acyltransferase (G3PAT), which increases *de novo* PA/DAG/PI/PIP_3 synthesis (11, 12). The activation of GPI hydrolysis (Fig. 2) and G3PAT are both sensitive to pertussis toxin and therefore appear to be dependent upon a heterotrimeric Gi-protein(s).

Phosphatidylcholine Hydrolysis

Insulin-induced activation of PC-PLD in the plasma membrane has been observed in rat adipocytes (5, 13) and other cell types (13, 14). In rat adipocytes, the activation of PC-PLD by insulin is dependent on PI3K (5) and apparently requires small G-proteins, Rho, and/or ARF (6, 7). The translocation of Rho and ARF to the plasma membrane of rat adipocytes is inhibited by wortmannin (6), and can be stimulated by addition of polyphosphoinositides *in vitro* (6). Presumably, acidic head groups of membrane-localized D3-PO_4 polyphosphoinositides interact with basic residues in Rho and ARF, and the resultant translocation seems to be important for PLD activation in the plasma membrane (6, 7). GTP-loading of Rho, however, is not inhibited by wortmannin (6) and therefore appears to be effected by a PI3K-independent mechanism. ARF activation and translocation

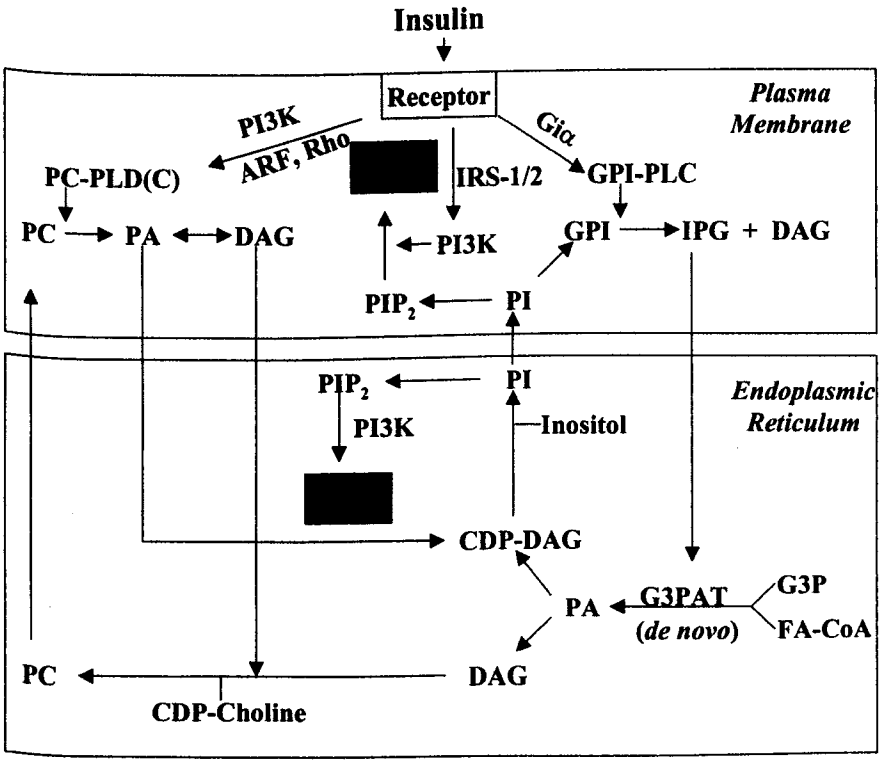

Figure 2. Insulin-sensitive pathways of phospholipid synthesis and degradation.

may occur in conjunction with PI3K-dependent translocation and activation of GRP-1 (8), which, like ARNO, serves as a GTP/GDP exchange factor for ARF. Polyphosphoinositides, including PI3K-dependent D3-PO_4 polyphosphoinositides, are required for PLD activation, but it is not clear if this reflects a requirement for translocating and activating Rho and/or ARF, or a direct effect of these lipids on PC-PLD or its interaction with substrate, PC. Whereas phorbol esters (via PKC) activate PC-PLD in some cells, they do not mimic insulin effects on PC-PLD in rat adipocytes (6). Whereas PLD activation has been observed in rat adipocytes, rat hepatocytes, rat-1 fibroblasts, and L6 myotubes, it does not appear to occur in 3T3/L1 adipocytes (15). The latter finding suggests that PLD activation is not essential for activating glucose transport, but, on the other hand, PLD activation has been found to enhance the sensitivity of the glucose transport response, i.e., the Km, to insulin (15). Thus, in cells in which PLD is activated by insulin, PLD may serve as a positive modulator for insulin-stimulated glucose transport. In addition to PLD activation, Rho is required for activation of PRK-1 (PKN), which appears to be required for insulin-stimulated GLUT 4 translocation (see below).

De Novo Synthesis of Phosphatidic Acid, Diacylglycerol, Phosphatidylinositol, and Phosphatidylcholine

Insulin increases *de novo* PA synthesis in microsomal membranes by: (i) increasing substrate availability, i.e., glycerol-3-PO_4 via enhanced glucose uptake and glycolysis, and fatty acyl-COA via enhanced fatty acid synthesis, uptake, and esterification with coenzyme A: and (ii) activation of G3PAT, which transfers fatty acids to 1 and 2 positions of glycerol-3-PO4 (11, 16). G3PAT largely resides in microsomal membranes and appears to be activated by insulin through the release of IPG mediators from GPI through activation of a GPI-specific PLC in the plasma membrane by a mechanism requiring a pertussis toxin-sensitive Gi-protein (11, 12, 16). Unlike PLD-dependent PC hydrolysis, the activation of G3PAT is not dependent upon PI3K (5). Although some findings have suggested that a heterotrimeric Gi-protein is involved in insulin regulation of glucose metabolism (see Ref. 17), the importance of signaling via Gi to GPI-PLC and G3PAT remains unsettled. (However, see below for potential involvement of the G-αq subunit in insulin-stimulated glucose transport.) In our studies we found that pertussis toxin does not significantly inhibit acute effects of insulin on glucose transport, despite inhibiting G3PAT activation in BC3H-1 myocytes (18). We, therefore, do not believe that insulin effects on glucose transport require the activation of G3PAT and *de novo* synthesis of PA and DAG. On the other hand, simple increases in availability of glucose (19–22) or, presumably, fatty acids, can lead to increases in *de novo* PA/DAG synthesis and activation of DAG-dependent cPKCs and nPKCs. Importantly, this activation of DAG-dependent PKCs may explain some aspects of "glucotoxicity" including glucose-induced insulin resistance in diabetic states (also see below).

PI3K Activation

We initially reported that insulin provokes rapid increases in absolute levels of polyphosphoinositides (23, 24) and incorporation of precursors into these lipids (23–25) in rat adipocytes and other cell types. We (23–25) and others (26) at first thought that based upon results from simple thin layer chromatography, increases in polyphosphoinositides reflected increases in PI-4-PO_4 and PI-4,5-$(PO_4)_2$, which at that time were the only polyphosphoinositides known to be present in significant amounts in mammalian cells. However, we subsequently realized that increases in levels of D3-PO_4 polyphosphoinositides, in particular PIP_3, were largely responsible for increases in total polyphosphoinositides in insulin-treated cells (27, 28). We also realized that PI3K was acutely activated by insulin, and that this accounted for increases in levels of polyphosphoinositides (27, 28) in both plasma membranes and microsomal membranes (28).

The activation of PI3K by insulin is effected by the interaction of pYXXM motifs in IRS family members with SH2 domains of the p85 subunit of PI3K, thereupon leading to a conformational change and an activation of the p110 subunit of PI3K (1). Overexpression of both IRS-1 and IRS-2 can lead to the enhanced activation of PI3K-dependent GLUT 4 translocation by insulin in rat adipocytes (29, 30), and, in fact, targeted disruption of the IRS-1 gene diminishes insulin-stimulated glucose transport in mouse adipocytes (31). The activation of IRS-1- and IRS-2-dependent PI3K functions upstream of the activation of PDK-1 and both PKC-ζ/λ and PKB, and as discussed in greater detail below, this pathway is of critical importance in the metabolic actions of insulin.

The importance of PI3K activation in the metabolic actions of insulin was at first uncertain. However, with the availability of relatively specific inhibitors of PI3K, wortmannin, and LY294002, it was soon realized that PI3K is required for insulin-induced increases in glucose transport and glycogen synthesis, and decreases in lipolysis (32–34). In contrast, insulin-induced activation of G3PAT (5) and pyruvate dehydrogenase (34) (which enzymes regulate glucose entry into the *de novo* lipid synthesis and oxidative pathways, respectively) are not inhibited by wortmannin, are therefore independent of PI3K and are unrelated to the activation of glucose transport. Of further note, PI3K is required for insulin-induced activation of ERK1/2 in rat adipocytes (86) and several other cell types (unpublished), but insulin does not appear to activate glucose transport or glycogen synthesis via ERK1/2 (however, as discussed below, the activation of the ERK pathway by certain agonists can activate GLUT 4 translocation and glucose transport).

Activation of DAG-Dependent PKCs by Insulin

Increases in DAG levels in isolated plasma membranes and microsomes and/or total cellular extracts of rat adipo-

cytes have been observed in some (3, 35), but not all (36), studies. Increases in [^{3}H]-glycerol incorporation into DAG have also been observed in rat adipocytes (22, 36), BC3H-myocytes (37, 38), and rat skeletal muscles (39, 40), but such increases in tracer incorporation studies are deceptively small and difficult to correlate with changes in absolute DAG levels, as it is necessary to extensively pre-label precursor pools and thus increase basal incorporation into DAG and other lipids prior to insulin treatment. In addition, it is necessary to measure the specific activity of intracellular glycerol-3-PO_4 to determine actual increases in absolute levels of newly synthesized DAG by isotopic analysis; this has only been done in studies of BC3H-1 myocytes (38) and calculated increases in DAG were in fact relatively large and comparable with actual increases in DAG mass, as measured by an enzymatic assay. In any event, the DAG derived in microsomes from the *de novo* PA/DAG synthesis pathway appears to be much less than that derived from PC hydrolysis in the plasma membrane during initial the moments of insulin action (13). In concert with the latter findings, in GK-diabetic rat adipocytes wherein G3PAT activation is markedly impaired, insulin-induced increases in DAG levels are observed only in the plasma membrane, presumably via PC hydrolysis, and not in the microsomal fraction (12).

Whether from PC hydrolysis or from *de novo* PA/DAG synthesis, insulin-induced increases in DAG in rat adipocytes apparently activate DAG-dependent PKCs. Translocation of PKCs-α, β, δ, and ε to membrane fractions has been observed in rat adipocytes (41) and in certain other, but not all, cell types, and such translocation is generally thought to reflect the activation of PKC. However, increases in cytosolic Ca^{++} and alterations in specific membrane proteins, including the insulin receptor itself, may also cause PKC translocation. Accordingly, evidence other than PKC translocation is needed to be certain that PKC enzyme activity is truly increased. Increases in membrane PKC enzyme activity have in fact been observed in rat adipocytes (42) and other cell types (39, 40, 43–46) following insulin treatment, but the assays used in these studies measured total PKC activity, including that of atypical PKCs. (To specifically measure DAG-sensitive PKC activity, it would be necessary to compare kinase activities in both the presence and absence of DAG or phorbol esters, while keeping other cofactors, Ca^{++} and/or phosphatidylserine, constant to calculate DAG-dependent and DAG-independent PKC activity). As discussed below, at this point only the atypical PKCs that have clearly and consistently been shown to be activated by insulin in all important cell types, undoubtedly because there are covalent changes in these atypical PKCs, i.e., an activating set of phosphorylations that carry over into specific PKC-ζ and PKC-λ immunoprecipitates whose protein kinase activities can be directly measured. This or other covalent activation may or may not occur in specific DAG-dependent PKCs, and if not, the ability to observe increases in immunoprecipitable activity may be precluded. Nevertheless, it is still possible that DAG-dependent PKCs may be activated *in vivo* by membrane-associated DAG during the action of insulin. Further studies are needed to see if immunoprecipitable preparations of specific DAG-dependent PKCs manifest increases in enzyme activity following insulin treatment. In this regard we have recently observed (unpublished) that insulin does in fact provoke increases in the activity of immunoprecipitable PKC-α and PKC-β 2 in rat adipocytes, but this activation is not inhibited by wortmannin and may therefore be largely due to activation of PI-glycan hydrolysis and the *de novo* PA/DAG synthesis pathway.

Another method that has been used to verify the activation of DAG-dependent PKC is to study ^{32}P-labeling of specific substrates in intact cells. In fact, we have reported that insulin stimulates ^{32}P-labeling of the commonly used MARCKS protein PKC substrate in rat adipocytes and soleus muscles (35). However, we now know that MARCKS is phosphorylated by ERK1/2 (47), as well as by PKC, that insulin potently activates ERK1/2 in rat adipocytes by a process requiring PI3K, PDK-1 and PKC-ζ, and that the observed inhibition of insulin effects on MARCKS labeling by the PKC inhibitor RO 31-8220 (35) was subsequently found to correlate closely with inhibition of PKC-ζ enzyme activity (48) and activation of ERK (49). Thus, effects of insulin on MARCKS-labeling may have been due to activation of ERK1/2 by a PKC-ζ-dependent mechanism, rather than via DAG-dependent PKCs (note that PKC-ζ, unlike cPKCs and nPKCs, does not itself phosphorylate MARCKS).

The Role of DAG-Sensitive PKCs in Insulin-Stimulated Glucose Transport

Initial studies showed that insulin effects on glucose transport are blocked by PKC inhibitors (50). However, the concentrations of inhibitors required to inhibit glucose transport effects of insulin were consistently higher than those required to inhibit DAG-dependent cPKCs and nPKCs. The reason for this discrepancy was not apparent until subsequent studies revealed that atypical PKCs are inhibited at relatively high concentrations of most PKC inhibitors, and moreover, there is close correlation between dose-dependent inhibition of PKC-ζ/λ and insulin-stimulated glucose transport (48, 51). Excellent correlations between inhibition of insulin-stimulated glucose transport and inhibition of PKC-ζ/λ enzyme activity by bisindolemaleimide-type PKC inhibitors, RO 31-8220 and LY379196, and by the cell-permeable myristoylated PKC-ζ/λ pseudosubstrate peptide have been observed in rat adipocytes (48), 3T3/L1 adipocytes (45), and L6 and BC3H-1 myocytes (51). Of further note, GO6976, which specifically inhibits cPKCs α, β, and γ, but not nPKCs or atypical PKCs, does not inhibit insulin-stimulated glucose transport in these cell types (48, 51, 52).

Studies in which prolonged treatment with phorbol esters were used to down-regulate DAG-dependent PKCs suggested that these PKCs may be required for insulin-

stimulated glucose transport in rat adipocytes (53, 54), but not in other cell types (55–57). As an explanation for this difference, we initially thought that PKC-β was retained in cells in which insulin effects on glucose transport were retained following prolonged phorbol ester treatment. However, with better immunoblotting methods and availability of more specific anti-PKC-β antibodies, we subsequently realized that previously used antibodies had cross-reacted with proteins other than PKC-β. More recently, with improved incubation conditions for down-regulation of DAG-dependent PKCs we have been able to show that despite the loss of greater than 90% PKC-α, PKC-β1, PKC-β2, PKC-δ, and PKC-ε following overnight phorbol ester treatment, insulin effects on glucose transport and/or GLUT4 translocation are fully retained in rat adipocytes (48, 52), as well as in BC3H-1 myocytes and L6 myotubes (51). Moreover, in the experimental conditions used in earlier studies we had noted that PKC-δ and PKC-ε were not well down-regulated by overnight treatment of rat adipocytes and other cells with phorbol esters (e.g., see Ref. 58), and in light of findings in more recent studies in which better down-regulation was achieved, it now seems likely that activation of these residual PKCs by phorbol esters may have impaired the activation of IRS-dependent PI3K and glucose transport in the early studies (note that in particular, cPKCs and probably nPKCs as well diminish insulin receptor function and perhaps most importantly, IRS-1 dependent activation of PI3K, PKC-ζ/λ and PKB; see below).

Further evidence against a requirement for DAG-dependent PKCs in insulin-stimulated glucose transport was provided by transient cotransfection studies in rat adipocytes. In contrast to strong inhibitory effects of expression of kinase-inactive forms of atypical PKC-ζ and PKC-λ (52) and their upstream activator, PDK-1 (59), on insulin-induced translocation of epitope-tagged GLUT4 to the plasma membrane, kinase-inactive forms of PKC-α, PKC-β2, PKC-δ, and PKC-ε were without effect on this translocation (52). Also, in contrast to stimulatory effects of expression of wild-type and constitutively-active forms of PKC-ζ on insulin-stimulated epitope-tagged GLUT4 translocation, the expression of wild-type and constitutively-active forms of PKC-α, PKC-β1, PKC-β2, and PKC-δ were without effect on this translocation (52).

A major reason for initially thinking that DAG-dependent PKCs are required for insulin-stimulated glucose transport is that DAG analogues, in particular phorbol esters, provoke insulin-like effects on glucose transport, particularly in cells that have only GLUT1 glucose transporters, e.g., as in BC3H-1 myocytes (60). However, in rat adipocytes and 3T3/L1 adipocytes, which have GLUT4 and GLUT1 glucose transporters, effects of phorbol esters on glucose transport and GLUT4 translocation are relatively small as compared to effects of insulin (e.g., see Ref. 52). Moreover, it has recently been shown in rat adipocytes, L6 myotubes, and 3T3/L1 adipocytes that phorbol esters activate PI3K (51, 61, 62) and interestingly, the effects of phorbol esters on glucose transport in L6 myotubes and 3T3/L1 adipocytes are blocked by the PI3K inhibitor, wortmannin (51, 61). Additionally, in rat adipocytes we have found that the relatively small effects of phorbol esters on glucose transport and GLUT4 translocation (52) may be accounted for by similar small effects of phorbol esters on immunoprecipitable PKC-ζ/λ activity (unpublished). The mechanisms responsible for this crosstalk between phorbol esters (presumably acting via DAG-dependent PKCs) and either PI3K (and thus perhaps indirectly on atypical PKCs) or more directly atypical PKCs (independently of PI3K) are presently uncertain. As discussed in greater detail below, ERK activation may in some instances lead to PKC-ζ/λ activation, and phorbol esters appear to activate glucose transport via Raf/MEK-1/ERK in rat adipocytes (unpublished).

Finally, in adipocytes and soleus muscles prepared from mice in which PKC-α (unpublished) or PKC-β genes (63) have been knocked out by gene targeting methods, insulin-stimulated glucose transport is actually enhanced, not diminished, as would be expected if either of these PKCs were required. This seemingly paradoxical enhancement of insulin effects on glucose transport is apparently due to the fact that DAG-dependent PKCs such as PKC-α (64) and PKC-β (65) inhibit initial steps in insulin signaling through the insulin receptors, IRS-1 and PI3K. Indeed, we have found that insulin stimulation of IRS-1-dependent PI3K, PKB, and PKC-λ are increased in PKC-α knockout mice (unpublished).

To summarize, we presently believe that DAG-dependent PKCs are not required for the acute stimulation of glucose transport in muscle and adipose tissues. Moreover, these DAG-dependent PKC's apparently negatively down-regulate this effect of insulin by phosphorylating specific serine/threonine residues on the insulin receptor or IRS-1/2, thereby diminishing signaling to PI3K, PDK-1 PKC-ζ/λ, and PKB. These inhibitory effects of DAG-dependent PKCs may occur during the effects of insulin itself (via activation of PC-PLD and *de novo* PA/DAG synthesis) as a "physiological" feedback mechanism, particularly in states of persistent hyperinsulinemia, as well as in states of diabetes- or obesity-related elevation of plasma levels of glucose and/or free fatty acids (via increases in *de novo* synthesis of PA/DAG).

The Activation of Atypical PKCs by Insulin

Insulin provokes increases in the activity of immunoprecipitable PKC-ζ/λ/ι in 3T3/L1 adipocytes (45, 66), rat adipocytes (48), 32D cells (67), L6 myotubes (51), rat hepatocytes (68), CHO/IR cells (69), and rat and mouse vastus lateralis muscles and human adipocytes (unpublished). Increases in immunoprecipitable PKC-ζ/λ enzyme activity presumably reflect the covalent modification of PKC-ζ and PKC-λ by PDK-1-dependent phosphorylation of activation loop sites, via threonine-410 in rat PKC-ζ and threonine-411 in mouse PKC-λ (2, 3), followed by autophosphorylation of threonine-560 in rat PKC-ζ and threonine-563 in

mouse PKC-λ (70). Both PKC-ζ and PKC-λ are activated by insulin in intact rat adipocytes and by PI-3,4,5-(PO4)3 *in vitro* (70), and it is likely that activation mechanisms for both atypical PKCs are identical as they are 72% homologous and contain identical N-terminal autoinhibitory pseudosubstrate sequences and comparable activation loop and autophosphorylation sites in their catalytic domains. It may be noted that rats contain PKC-ζ, mice contain PKC-λ, and humans contain PKC-ι (which is 98% identical to PKC-λ and which is activated by insulin in human adipocytes via PI3K; unpublished)) as their primary atypical PKC in insulin-sensitive skeletal muscle and adipose tissues.

The activation of PKC-ζ and PKC-λ is blocked by PI3K inhibitors, wortmannin and LY294002, in all cell types tested (45, 48, 51, 66, 67). Further, expression of the dominant-negative mutant form of the p85 subunit of PI3K, so-called Δp85 (which lacks binding sites for the p110 subunit of PI3K), inhibits the activation of PKC-λ by insulin in 3T3/L1 adipocytes (66). Also, endogenous PKC-ζ/λ is activated by addition of an exogenous pYXXM peptide (that activates PI3K) to rat adipocyte homogenates (48), and in addition, epitope-tagged PKC-ζ and PKC-λ, as prepared from immunoprecipitates of rat adipocyte lysates, is activated by direct addition of PI-3,4,5-$(PO_4)_2$ (i.e., PIP_3) and PI-3,4-$(PO_4)_2$ (48, 70) to the *in vitro* assay system. It is clear, therefore, that PI3K is both necessary and sufficient for insulin-induced activation of PKC-ζ and PKC-λ.

In addition to PI3K, PDK-1, which phosphorylates threonine residues in the activation loops of PKC-ζ (2, 3), and presumably PKC-λ and PKC-ι, as well as comparable sites in activation loops of other PKCs, PKB, PRK-1, and p70 S6 kinase, has been suggested to serve as a major factor in transmitting activating signals from PI3K to PKC-ζ and PKC-λ (2, 3). In keeping with this suggestion we found that over-expression of wild-type PDK-1 provokes insulin-like increases, and expression of kinase-inactive PDK-1 inhibits insulin-induced increases in epitope-tagged PKC-ζ enzyme activity in transiently transfected rat adipocytes (59). Further, the expression of a mutant form of PKC-ζ that contains alanine instead of threonine at the 410 loop site and therefore cannot be phosphorylated and activated by PDK-1 also inhibits insulin-induced activation of co-expressed epitope-tagged wild-type PKC-ζ (59). (This mutant PKC-ζ presumably diverts the activating effects of PDK-1 and PIP_3 away from wild-type PKC-ζ.) Although these findings indicate that PDK-1 and its threonine-410 target are required for insulin-induced activation of PKC-ζ, it is presently not entirely certain if PDK-1 is itself activated by insulin. It is also presently uncertain if PDK-1 itself is activated by PIP_3, or whether PIP_3 acts upon PKC-ζ/λ to cause an unfolding and thereby facilitate access for PDK-1 to threonine-410/411 loop sites in PKC-ζ/λ. Although some studies have failed to show an effect of insulin on PDK-1 activity (e.g. Ref. 69), insulin-induced activation of immunoprecipitable PDK-1 has been observed by Quon and coworkers (71). In any event, we have observed that insulin provokes rapid increases in the level of phosphorylation of threonine-410 in PKC-ζ (as per immunoblotting with phospho-specific antibodies;79, 87), and it is therefore clear that PDK-1 activity or its action is enhanced by insulin-induced increases in PI3K and PIP_3.

Although PIP_3 activates PKC-ζ when added to PKC-ζ immunoprecipitates (48, 70) and purified PKC-ζ preparations (72) *in vitro*, it is known (2, 3) that PDK-1 can (but does not invariably; unpublished) bind to PKC-ζ and may therefore be carried over into PKC-ζ immunoprecipitates. Thus, PIP_3 may act *in vitro* as well as *in vivo* by either directly activating PDK-1 or by interacting with the N-terminal lipid-binding domain of PKC-ζ to facilitate the interaction of threonine-410 with the catalytic site of PDK-1. In addition, PIP_3 stimulates autophosphorylation of PKC-ζ, and relieves the autoinhibition exerted by the N-terminal pseudosubstrate sequence on the C-terminal catalytic domain of PKC-ζ (note that the last two actions of PIP_3, as discussed below, may require, but are themselves independent of, PDK-1). In this regard we have found that insulin activates truncated forms of PKC-ζ and PKC-λ that lack the entire N-terminal regulatory domains by a mechanism requiring PI3K (70 and unpublished). Thus, since the PIP_3-binding domain is presumably not present in these truncated forms of PKC-ζ and PKC-λ, these observations raise the possibility that PI3K-dependent increases in $PIP3_3$ activate PDK-1 in intact cells, which in turn activates the expressed truncated form of PKC-ζ. However, another possibility is that insulin activates truncated forms of PKC-ζ and PKC-λ through an initial activation of endogenous full-length PKC-ζ/λ, which in turn leads to transphosphorylation of truncated PKC-ζ/λ.

Revelant to the above considerations, we found that insulin in intact cells and PIP_3 *in vitro* phosphorylate and enzymatically activate a form of PKC-ζ in which threonine-410 has been mutated to anionic, phosphate-mimicking glutamate (70). This mutant is constitutively active at the 410 activation loop site and cannot be further activated by PDK-1 at this site (the only apparent PDK-1 target in PKC-ζ), but nevertheless contains autophosphorylation and autoinhibitory pseudosubstrate sites that apparently can be activated. It may therefore be surmised that insulin in intact cells and PIP_3 *in vitro* activate this threonine-410-to-glutamate-410 mutant PKC-ζ by mechanisms that are clearly independent of, and probably distal to, the action of PDK-1, via autophosphorylation and relief of pseudosubstrate-mediated autoinhibition. Of further note, we have recently found (87) that insulin in intact cells and PIP_3 *in vitro* activate a double PKC-ζ mutant in which both the threonine-410 loop site and the threonine-560 autophosphorylation site have been replaced by phosphate-mimicking glutamate residues. Since this double mutant is not phosphorylated at all by insulin or PIP_3 treatment (as threonine-560 appears to be the only autophosphorylation site; Ref. 87), but is nevertheless enzymatically activated by insulin and PIP_3 (87), it appears that PIP_3 activates this mutant by a phosphorylation-independent mechanism. Further, since

PIP_3 does not directly activate N-terminally truncated forms of PKC-ζ and PKC-λ that lack pseudosubstrate sequences, it may also be surmised that PIP_3 activates this double mutant form of PKC-ζ by binding to its N-terminal domain, thereby relieving the autoinhibition caused by the pseudosubstrate sequence.

Taken together, our findings suggest that insulin, via PIP_3, which most likely binds to the N-terminal regulatory domain and causes molecular unfolding , activates atypical PKCs by a three-step mechanism involving PDK-1-dependent loop phosphorylation, autophosphorylation, and allosterically induced relief of pseudosubstrate-mediated autoinhibition. In keeping with this idea it is interesting to note that glucose transport effects of insulin are only partly mimicked by PKC-ζ mutants that are only partly activated by either replacement of threonine-410 or threonine-560 residues with glutamate, or by mutation of critical residues in the pseudosubstrate sequence, or by deletion of the pseudosubstrate autoinhibitory site (see Refs. 45, 48, 52, 66, 87).

The Role of Atypical PKCs in Insulin-Stimulated Glucose Transport

As discussed above, DAG-activated PKCs do not appear to be required for insulin-stimulated glucose transport in most cell types. Nevertheless, inhibitor studies have consistently suggested a requirement for a PKC or another protein kinase that operates downstream of PI3K during insulin action, is inhibited by relatively high concentrations of PKC inhibitors, including the PKC-ζ/λ pseudosubstrate (which does not inhibit PKB), and is not down-regulated by phorbol esters. Although these early findings were compatible with the possibility that atypical PKCs may be required for insulin-stimulated glucose transport, direct and more convincing evidence for this possibility was not forthcoming until plasmid or viral gene transfer studies were feasible. In our initial transfection study (45) we stably transfected 3T3/L1 fibroblasts and adipocytes and found that expression of kinase-inactive PKC-ζ, but not kinase-inactive PKC-α, PKC-β1, or PKC-β2, inhibited insulin-stimulated increases in GLUT 4 and GLUT 1 translocation and 2-deoxyglucose uptake. Also, stable overexpression of wild-type PKC-ζ and constitutively active PKC-ζ, but again, not PKC-α, β1, β2, or ε, potentiated or provoked insulin-like effects on GLUT 4/1 translocation and 2-deoxyglucose uptake (45). Similar inhibition of insulin effects on GLUT 4/1 translocation and 2-deoxyglucose uptake were subsequently observed in L6 myotubes stably transfected with kinase-inactive PKC-ζ (51).

Although our stable transfection studies suggested that PKC-ζ or PKC-λ may be required for insulin-stimulated glucose transport in 3T3/L1 cells and L6 myotubes, there are inherent caveats in this experimental approach, e.g., stably transfected cells selected by G418 treatment may use aberrant signaling systems. We therefore employed a transient transfection method in which rat adipocytes are transiently cotransfected with plasmids encoding Hemagglutin-(HA) tagged GLUT 4 and various genes of interest (e.g., see Refs.29 and 32). With this approach we found that insulin-stimulated HA-GLUT 4 translocation is inhibited by kinase-inactive forms of both PKC-ζ and PKC-λ (48, 52, 59). Moreover, the inhibitory effects of kinase-inactive forms of PKC-ζ and PKC-λ could be "rescued" by cotransfecting wild-type forms of either PKC-ζ or PKC-λ (52), suggesting that the inhibitory effects are specifically due to the mutation causing the loss of kinase activity in PKC-ζ/λ, or stated differently, the kinase activity of PKC-ζ or λ is specifically required for insulin-stimulated glucose transport. These findings further suggested that the atypical PKCs, ζ and λ, perhaps not surprisingly in view of their homology, can function interchangeably during insulin stimulation of glucose transport in rat adipocytes (which have primarily PKC-ζ; see Ref. 79).

In addition to PKC-ζ/λ, we found that expression of PDK-1, the upstream regulator of PKC-ζ/λ, is required for insulin-stimulated HA-GLUT 4 translocation in transiently transfected rat adipocytes (59). In keeping with this finding, a mutant form of PKC-ζ in which threonine-410 in the activation loop of PKC-ζ is mutated to alanine (thereby destroying the target of PDK-1 and causing this PKC-ζ mutant to be activation-resistant), like kinase-inactive PKC-ζ or PKC-λ, was found to inhibit insulin-stimulated HA-GLUT 4 translocation (59). Of further note, the inhibitory effect of kinase-inactive PDK-1 on HA-GLUT 4 translocation was largely "rescued" by co-expression of wild-type PKC-ζ and expression of both kinase-inactive PKC-ζ and the threonine-410 mutant form of PKC-ζ inhibited the activation of epitope-tagged wild-type PKC-ζ, but not epitope-tagged PKB (59). Collectively, these findings provided further support for the hypothesis that PKC-ζ is required for insulin-stimulated GLUT 4 translocation.

In contrast to kinase-inactive forms of PKC-ζ and PDK-1, overexpression of wild-type forms of both PKC-ζ and PDK-1 and expression of constitutively-active forms of PKC-ζ were found to provoke insulin-sensitizing or insulin-like effects on HA-GLUT 4 translocation in transiently transfected rat adipocytes (48, 52, 59). These findings, along with findings described above, suggested that both atypical PKCs, ζ and λ and their upstream regulator PDK-1, are necessary and "sufficient" for insulin-stimulated GLUT 4 translocation in rat adipocytes. This suggestion does not imply that other protein kinases are not required in this stimulation, and indeed, PKB has been reported to be required for a relatively small component of GLUT 4 translocation in the rat adipocyte (72) and for a relatively large component of GLUT 4 translocation in L6 myotubes (74), but not in 3T3/L1 adipocytes (66). Accordingly, it is possible that different cell types may differentially utilize either or both of these and other protein kinases to regulate glucose transport. In this regard we have found that the small G-protein, Rho, and its downstream effector, PRK-1, perhaps because of homology to PKCs and activation by PDK-1 (PKN) and PIP_3, appear to be required for insulin-

stimulated HA-GLUT 4 translocation in rat adipocytes (75). This apparent requirement for Rho and PRK-1 (PKN) needs to be studied further.

An important role for atypical PKCs in insulin-stimulated glucose transport in 3T3/L1 adipocytes has also been suggested from studies that employed adenoviral gene transfer methods. In these mouse-derived cells, which contain PKC-λ, but little or no full-length PKC-ζ, adenoviral-mediated expression of kinase-inactive PKC-λ inhibits insulin-stimulated GLUT 4 translocation and 2-deoxyglucose uptake (66). In addition, adenoviral-mediated expression of constitutively active PKC-λ provokes insulin-like effects on Glut 4 translocation and glucose transport (66). These findings in 3T3/L1 adipocytes are virtually the same as those observed in transiently transfected rat adipocytes (48, 52), and taken together provide strong evidence for the hypothesis that atypical PKCs are required for insulin-stimulated glucose transport.

We have also used adenoviral gene transfer methods to examine the requirement for atypical PKCs in L6 myotubes. In these cells, expression of kinase-inactive PKC-λ (kindly supplied to us by Dr. Masato Kasuga) completely inhibited insulin effects on GLUT 4 translocation and 2-deoxyglucose uptake (76). Also, expression of constitutively active PKC-λ provoked insulin-like effects and overexpression of wild-type PKC- potentiated insulin effects on GLUT 4 translocation and 2-deoxyglucose uptake (76). Of further note, the levels of GLUT 4 and the activation of PKB were, if anything, enhanced by expression of kinase-inactive PKC-λ in these adenoviral gene transfer studies; thus, inhibitory effects of kinase-inactive PKC-λ could not be explained by nonspecific inhibition of insulin signaling to PI3K/PDK-1/PKB or by decreases in GLUT 4 availability. In addition, the level of expression of various forms of PKC-λ in these studies was closely monitored to avoid or minimize the possibility of gross overexpression and potentially untoward effects of large amounts of exogenous DNA and functionally altered protein. Importantly, alterations in glucose transport appeared to be commensurate with the observed level of expression of the various forms of PKC-λ. In particular, insulin effects on both total PKC-ζ/λ enzyme activation and glucose transport were fully inhibited with only a 3- to 4-fold increase in total cellular PKC-ζ/λ, which includes both endogenous wild-type PKC-ζ/λ and expressed kinase-inactive PKC-λ. If kinase-inactive PKC-λ competes equally with wild-type PKC-ζ/λ, a 2-fold increase in total PKC-ζ/λ should have been attended by a 50% reduction in insulin effects on processes that are fully dependent on PKC-ζ/λ. Accordingly, the observed complete (100%) inhibition of insulin effects on glucose transport with a 3- to 4-fold increase in total PKC-ζ/λ actually exceeds the expected 75% to 80% decrease in insulin-stimulated glucose transport and this probably reflects the fact that insulin effects on activation of PKC-ζ/λ were completely abolished by a 3- to 4-fold increase in total PKC-ζ/λ (76).

Interestingly, adenoviral transfer of wild-type PKC-ζ to skeletal muscles of immunosuppressed rats *in vivo* is also attended by enhanced effects of insulin on glucose transport (77). Since we have found that insulin rapidly activates PKC-ζ in rat and mouse skeletal muscles (unpublished), it appears more than likely that atypical PKCs play an important role in insulin-stimulated glucose transport in skeletal muscle, the major organ for disposal of glucose *in vivo*.

One criticism of experiments that employ expression of kinase-inactive forms of PKC-ζ is that the presence of large amounts of these mutants may diminish the ability of PDK-1 to activate other endogenous targets, including PKB, p70S6 kinase, and PRK-1. This criticism, however, fails to take into account a number of findings. First, the expression of inhibitory mutant forms of PKC-ζ in both transient transfection (59) and adenoviral gene transfer (66, 76) experiments does not inhibit PKB activation by insulin. Second, if this criticism were correct, any form of PKC-ζ/λ, including wild-type, should bind to PDK-1 and inhibit insulin effects on processes that require PI3K/PDK-1, but not PKC-ζ/λ; this is in fact the opposite of what is observed. Third, the rescue of inhibitory effects of kinase-inactive and other inhibitory mutant forms of PKC-ζ/λ by wild-type forms of PKC-ζ/λ strongly suggests that it is the loss of kinase activity of PKC-ζ/λ *per se*, and not the presence of large amounts of PKC-ζ/λ, that is responsible for dominant-negative effects of the inhibitory mutant forms of PKC-ζ/λ on insulin-stimulated glucose transport. Fourth, expression of kinase-inactive forms of PKB (which, like PKC-ζ/λ, should interact with PDK-1) in at least several cases (see below) has been found to have little or no effect on insulin-stimulated GLUT 4 translocation or glucose transport.

The Role of PKB in Metabolic Actions of Insulin

PKB is activated by insulin via a PI3K- and PDK-1-dependent mechanism similar to that used to activate PKC-ζ/λ. PDK-1 phosphorylates threonine-308 in the activation loop of PKB and this is followed by a secondary phosphorylation of serine-473, leading to full activation of PKB (78). The identity of the kinase responsible for phosphorylation of serine-473 is not entirely certain, but may in fact may be PDK-1. There are at least three isoforms of PKB, α, β, and γ, that differ in abundance in various cell types and may or may not subserve different cellular functions. In most circumstances, PKB and atypical PKCs, which are both activated by PI3K/PIP_3 and PDK-1, would be expected to be activated together; however, in our experience their ratios vary depending upon the agonist, its level, the cell type, the presence of pathological insulin resistance, the use of treatment with insulin sensitizers, and probably other modulating factors. In this regard, in states of acquired insulin resistance in experimental animal models, we have found that there are defects in PKC-ζ/λ activation, with little or no apparent alteration in PKB phosphorylation/activation (e.g., see Ref. 79).

The importance of PKB for insulin regulation of glycogen synthase is generally accepted, but not without res-

ervation. Presumably, PKB phosphorylates and thereby inhibits glycogen synthase kinase-3β, which when active, phosphorylates and inhibits glycogen synthase. The importance of PKB for insulin regulation of glucose transport, however, is less certain. On the one hand, it is clear that constitutively active forms of PKB stimulate glucose transport in a variety of cell types, including 3T3/L1 adipocytes (80) and rat adipocytes (81). In addition, in L6 myotubes, plasmid-mediated expression of a triple mutant form of PKBα, which is kinase-inactive and mutated at both phosphorylation sites and is therefore additionally activation-resistant (74), and in 3T3/L1 adipocytes, microinjection of antibodies that target PKBβ, but not PKBα (82), markedly inhibit insulin-stimulated GLUT 4 translocation. On the other hand, viral-mediated expression of a double mutant form of PKBα that is activation-resistant failed to inhibit insulin-stimulated glucose transport and GLUT 4 translocation in 3T3/L1 adipocytes (83), despite inhibiting the activation of both PKBα and PKBβ (84). Also, in rat adipocytes, plasmid-mediated expression of a single mutant kinase-inactive PKBα (73) and both double and triple mutant kinase-inactive and activation-resistant forms of PKBα (unpublished observations) were found to inhibit insulin-stimulated GLUT 4 translocation by only 20% to 25%, as opposed to the 60% to 70% inhibition observed with kinase-inactive or activation-resistant forms of both PKC-ζ/λ and PDK-1 (48, 52, 59). Obviously, there are several findings that seem to be contradictory regarding the role of PKB in insulin-stimulated glucose transport. Perhaps a failure to fully inhibit specific isoforms of PKB, or specific intracellular sites of PKB activation, may explain some of these discrepant findings. In this regard it may be noted that insulin-induced activation of PKBβ exceeds that of PKB-α in rat adipocytes, whereas activation of PKBα exceeds that of PKBβ in hepatocytes, and in skeletal muscle, PKBα appears to be the major insulin-sensitive PKB isoform (85). This contrasts with the situation in L6 myotubes, a skeletal muscle cell line, where PKBγ appears to be the major insulin-sensitive isoform (85) (note that PKBγ does not appear to be significantly activated by insulin in rat adipocytes, hepatocytes or skeletal muscle). Clearly, more information is needed to evaluate the role of specific PKB isoforms and their cellular activation sites in the activation of glucose transport in specific tissues.

Other Phospholipid/Lipid Kinase-Dependent Signaling Systems as Potential Regulators of Glucose Transport

In addition to the IRS/PI3K/PDK/PKC-ζ/λ pathway, there is mounting evidence that other related signaling pathways are used by insulin and other agonists for stimulating glucose transport. For example, two groups have reported that Gαq/11 subunits are required for insulin-stimulated glucose transport in 3T3/L1 adipocytes (88, 89). However, one group has reported that Gαq/11 operates via PI3K and atypical PKC-λ, rather than PKB (89), whereas the other group has suggested that PI3K is neither activated by Gαq/11, nor required for Gαq/11 action on glucose transport (88). It remains to be seen if this difference in findings is due to differences in 3T3/L1 cell lines or other experimental factors. It also remains to be seen how Gαq/11 or other heteromeric G-protein subunits are activated by insulin, whether such G-protein activation occurs in other cell types, and whether PKC-ζ/λ or PKB functions downstream of these G-proteins and accounts for their effects on glucose transport.

In addition to the IRS/PI3K/PDK-1 and Gαq/11-dependent pathways, we have recently found that some (e.g., phorbol esters; see above), but not all (e.g., insulin; see above), agonists that activate ERK, can via a mechanism requiring the activation of the ERK pathway, activate PKC-ζ/λ, and thereby activate GLUT 4 translocation and glucose transport, independently of PI3K, PDK-1, and PKB (unpublished observations). The mechanism whereby PKC-ζ/λ is activated in conjunction with the ERK pathway and the agonists that operate via this PI3K/PDK/PKB-independent ERK-dependent pathway are presently under study in our laboratory.

Concluding Remarks

Perhaps the issue of whether PKC-ζ/λ/ι, PKBα/β/γ, or both kinases is/are of greater or lesser importance for insulin-stimulated glucose transport will only be clarified by comparing their relationship with alterations of glucose transport in specific tissues in various physiological and pathophysiological conditions. Although it is still too early to be sure of the final resolution of this issue, in initial studies we have found that impairment of glucose transport in adipocytes (79) and skeletal muscles (90) of type 2 diabetic Goto-Kakizaki (GK) rats, the reversal of this defect, and in fact actual enhancement, in insulin-stimulated glucose transport in rat adipocytes following thiazolidinedione treatment (79) correlate with alterations in PKC-ζ/λ, rather than PKBα, activation. In addition, improvement in insulin signaling in skeletal muscles and adipocytes of diabetic GK rats following insulin-sensitization by treatment with thiazolidinediones or intensive insulin treatment or following short-term fasting and reduction of blood glucose is attended by increases in the activation of PKC-ζ/λ, rather than PKBα (unpublished observations). We have also observed excellent correlation between alterations in glucose transport and PKC-ζ/λ activity in a number of other physiological and pathological conditions in several additional rodent models of insulin resistance. We therefore believe that alterations in PKC-ζ/λ/ι activation will prove to be important in determining the effectiveness of insulin in the regulation of glucose transport in humans. As discussed above, this does not imply that PKB is not required for insulin-stimulated glucose transport. In this regard there has been no report of a situation in which PKB activation is specifically compromised, i.e., without concomitant inhibition of PKC-ζ/λ activation, and which is attended by a state of

impaired activation of glucose transport by insulin that can be corrected by restoration of PKB activation. Nevertheless, regardless of whether PKC-ζ/λ/ι or PKB-α/β/γ is more important for glucose transport, it seems clear that the combination of PKC-ζ/λ/λ and PKB-α/β/γ serves as a powerful, integrated mechanism for insulin regulation of glucose transport, subsequent storage of glucose in glycogen, and thus overall glucose metabolism. This being the case, it is likely that we are on a threshold that will lead to a better understanding of the proximate causes of clinical insulin resistance, and such understanding should ultimately allow us to devise more effective treatments for type 2 diabetes mellitus and associated states of insulin resistance.

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