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REVIEW

# Protein Kinase Cζ and Glucose Uptake

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**Abstract**—Protein kinase Cζ (PKCζ) is a member of the PKC family, serving downstream of insulin receptor and phosphatidylinositol (PI) 3-kinase. Many evidences suggest that PKCζ plays a very important role in activating glucose transport response. Not only insulin but also glucose and exercise can activate PKCζ through diverse pathways. PKCζ activation and activity are impaired with insulin resistance in muscle and adipose tissues of type II diabetes individuals, but heightened in liver tissue, wherein it also increases lipid synthesis mediated by SREBP-1c (sterol-regulatory element-binding protein). Many studies have focused on linkage between PKCζ and GLUT4 translocation and activation. Exploring the molecular mechanisms and pathways by which PKCζ mediates glucose transport will highlight the insulin-signaling pathway.

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Type II diabetes is a common chronic disease occurring in about 10% of our general population. Insulin resistance, manifested by a reduction in glucose uptake in skeletal muscle following insulin stimulation, is a key feature of the disease. Despite intense research efforts, the molecular mechanism of insulin resistance remains poorly understood. Insulin stimulates glucose uptake into skeletal muscle tissue mainly through GLUT4 translocation from intracellular pools to the plasma membrane [1, 2]. Although several molecules have been found to play important roles in the insulin signal pathway, the precise steps linking GLUT4 translocation with insulin stimulation have not been entirely elucidated. Experimental studies suggest that insulin stimulates glucose transport through insulin receptor-mediated tyrosine phosphorylation of insulin receptor substrate (IRS)-1 or other intermediates that activate phosphatidylinositol (PI) 3-kinase (PI3K), which through increase in phosphatidylinositol-3,4,5-triphosphate ($PIP_3$) activates downstream effectors protein kinase B (PKB/Akt) [3-6] and atypical protein kinases C (aPKCs) ζ and λ/τ [7-11]. The activation of aPKCs by insulin in skeletal muscles is defective in type II diabetic patients, monkeys, and rodents, and this defect in aPKCs activation seems to contribute significantly to the diminution in insulin-stimulated glucose disposal and muscle-dependent insulin resistance seen in these diabetic states [7, 8, 12-14].

*Abbreviations*: DAG) diacylglycerol; GEF) GLUT4-activating factor; IRS) insulin receptor substrate; PI) phosphatidylinositol; PI3K) phosphatidylinositol-3-kinase; PDK-1) PI3-kinase-dependent protein kinase-1; $PIP_3$) phosphatidylinositol-3,4,5-triphosphate; PKCζ) protein kinase Cζ; PKB) protein kinase B; aPKC) atypical protein kinase C; PH) pleckstrin homology; PS) pseudosubstrate; VAMP-2) vesicle-associated membrane protein-2.

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## PKCζ STRUCTURE

Protein kinase C zeta (PKCζ) is one isotype of the PKC family [7, 8] that constitutes a group of multifunctional Ser/Thr protein kinases involved in many kinds of functions in cell life. The PKC family is divided into three groups according to their activation mode: conventional isoforms (α, $\beta_1$, $\beta_2$, and γ) are dependent on $Ca^{2+}$ and diacylglycerol (DAG) for stimulation of activity, novel isoforms (δ, ε, θ, and η) are dependent on DAG, and the atypical isoforms (ζ, λ/τ) that are independent of $Ca^{2+}$ and DAG but are activated by phosphatidic acid and $PIP_3$ [15, 16].

PKCζ shares 72% identity with PKCλ, another atypical member [17]. PKCζ consists of four functional domains and motifs including PB1 domain, pseudosubstrate (PS) sequence, and a C1 domain with a single Cys-

rich zinc-finger motif in the N-terminus, and a kinase domain in the C-terminus. The kinase domain of PKCζ is conserved among the other PKC isotypes that belong to the conventional and novel subfamilies, containing an ATP-binding region, an activation loop, and a hydrophobic motif and a turn motif. The ATP-binding region contains a key residue, Lys281, which is very important for PKCζ activity. The residues Thr410 and Thr560, located in the activation loop and turn motif separately, are crucial sites when PKCζ is phosphorylated.

In contrast, the N-terminus (regulatory domain) of PKCζ is clearly different from those of the other members of the PKC super family. The C1 domain has one zinc-finger motif whereas others have two, both of which are essential for interaction with and activation by second messenger DAG and phorbol-diester tumor promoters. PKCζ lacks the characteristic C2 domain that is present in the conventional isoforms. These important structural differences may explain why the atypical PKCs are insensitive to $Ca^{2+}$, DAG, and phorbol esters, which are potent activators of other isoforms. The PB1 domain recognizes the OPCA motif. The PS sequence is a short peptide that resembles a substrate sequence except for Ala occupying the position of Thr or Ser as a phosphoryl-group acceptor. The PS sequence is assumed to block the substrate-binding cavity of the kinase domain as an auto-inhibition mechanism.

## PKCζ ACTIVATION

Normally, PKCζ can be activated by two steps: release of the PS sequence from its auto-inhibition binding site in the kinase domain and the phosphorylation of Thr410 inside the activation loop and Thr560 inside the turn motif [18]. $PIP_3$, produced by PI3-kinase after this enzyme is activated by insulin signal, is thought to be very important for activating PKCζ. Although there is no identified $PIP_3$ binding site in PKCζ, the activation of PKCζ seems partly dependent on the presence of the PS sequence in the N-terminus because insulin can provoke PKCζ activity through phosphorylation-independent and conformational-dependent relief of pseudosubstrate auto-inhibition [19]. On the other hand, PI3-kinase-dependent protein kinase-1 (PDK-1) can be activated after directly binding to $PIP_3$ through its pleckstrin homology (PH) domain and translocated to the plasma membrane [20]. Then PDK-1 will activate PKCζ by phosphorylating the T-loop residue Thr410 after allosteric relief of PS-dependent auto-inhibition. During this process, the hydrophobic motif of PKCζ acts as a docking site that enable the recruitment of PDK-1 to PKCζ. This is essential for its activation in cells [21]. After Thr410 is phosphorylated, another important residue, Thr560, located in the turn motif undergoes autophosphorylation [19]. Whether Thr560 of PKCζ is phosphorylated by itself or other protein kinases remains to be resolved.

## PKCζ PLAYS AN IMPORTANT ROLE IN THE INSULIN-SIGNALING PATHWAY

Some research works have suggested that PKCζ is necessary for insulin-stimulated glucose uptake. Even though there are more than 10 PKC family members, it seems that PKCζ is involved in insulin-stimulated glucose uptake, but not the DAG-sensitive isoforms [7].

Overexpression of wild-type PKCζ or constitutively active PKCζ has an insulin-like effect on glucose transport during *in vitro* incubation of different kinds of cell lines: 3T3-L1 cell [9], preadipocyte-derived human adipocytes [22], and L6 skeletal muscle cell [7]. There is an enhancement of glucose uptake after recombinant human PKCζ is delivered into rat skeletal muscle by adenovirus [23]. Use of PKCζ specific inhibitor, the cell-permeable myristoylated PKCζ pseudosubstrate peptide, which has no effect on inhibition on PKB [11, 24], significantly inhibits insulin-induced glucose transport in L6 myotubes [7] and preadipocyte-derived human adipocytes [22].

PKB, also known as Akt, can also be activated downstream from PI3-kinase by $PIP_3$ and PDK-1. Even PKB is reported to contribute to glucose uptake too, but there is still controversy about its contribution to glucose transport, and some reports show that PKB is not important in the resistance to insulin action in adipocytes and muscle tissues [10, 25, 26]. In our unpublished data, we also get the result that PS sequence can nearly eliminate the insulin-induced GLUT4 translocation and glucose uptake, which is as same as the inhibition effect of wortmannin on PI3-kinase. The inhibition effect of wortmannin and PS not only show the PI3-kinase–PKCζ pathway is very important in the insulin-signaling pathway, but also reveals PKCζ may be the main molecule to inherit the insulin signal from PI3-kinase and transfer it further downstream.

Despite the general agreement that PI3-kinase activity is necessary for insulin stimulated PKCζ activation and following glucose uptake, its activity seems insufficient and additional signals are required. For example, activation PI3-kinase by platelet-derived growth factor (PDGF) or interleukin-4, or through engagement of integrin receptors does not stimulate glucose uptake [27]. In addition, two naturally occurring insulin receptor mutations were fully capable of activating PI3-kinase, yet were unable to mediate insulin action [28]. Moreover, addition of a $PIP_3$ analog has no effect on glucose transport [29]. Thiazolidinedione and resiglitazone treatments enhance insulin effects on PKCζ activation and glucose transport in adipocytes of non-diabetic and Goto–Kakizaki type II diabetic rats without increasing PI3-

kinase and PKB activation [30]. Recently another PI3-kinase-independent pathway has been found in 3T3-L1 adipocytes [31]. The translocation of phosphorylated Cb1 (cannabinoid receptor) recruited additional signaling proteins to the lipid raft, resulting in the activation of the G protein TC10. This molecular switch appears to provide a second signal to the Glut4 protein that functions in parallel with the activation of the PI3-kinase-dependent signaling pathway [32].

Though these two pathways transfer insulin signal in parallel at the beginning, they have a convergent point downstream from PKCζ, and TC10 may regulate PKCζ activity [33]. In muscle cells, there is another Rho family member, Rac1, instead of TC10 in adipocytes, which plays an important role in glucose uptake [34]. Whether there is the same set of proteins regulating Rac1 activity in muscle cells as they do in adipocytes is not known. But evidence shows that Rac1 activity is controlled downstream from PI3-kinase [34, 35]. Both TC10 and Rac1 are shown to regulate actin remodeling [34, 36, 37]. On the other hand, PKCζ has also been reported to play the central role in maintaining cell polarity in yeast and mammalian cells by forming a quaternary complex with adaptor protein par6 and par3, and GTP-binding Rac/Cdc42 [38, 39]. We also found that PKCζ could affect actin remodeling caused by Rac1 in muscle cells (unpublished observation). It is conceivable that PKCζ activity cannot only be regulated by PI3-kinase but also be modified by other molecules that mediate insulin signal.

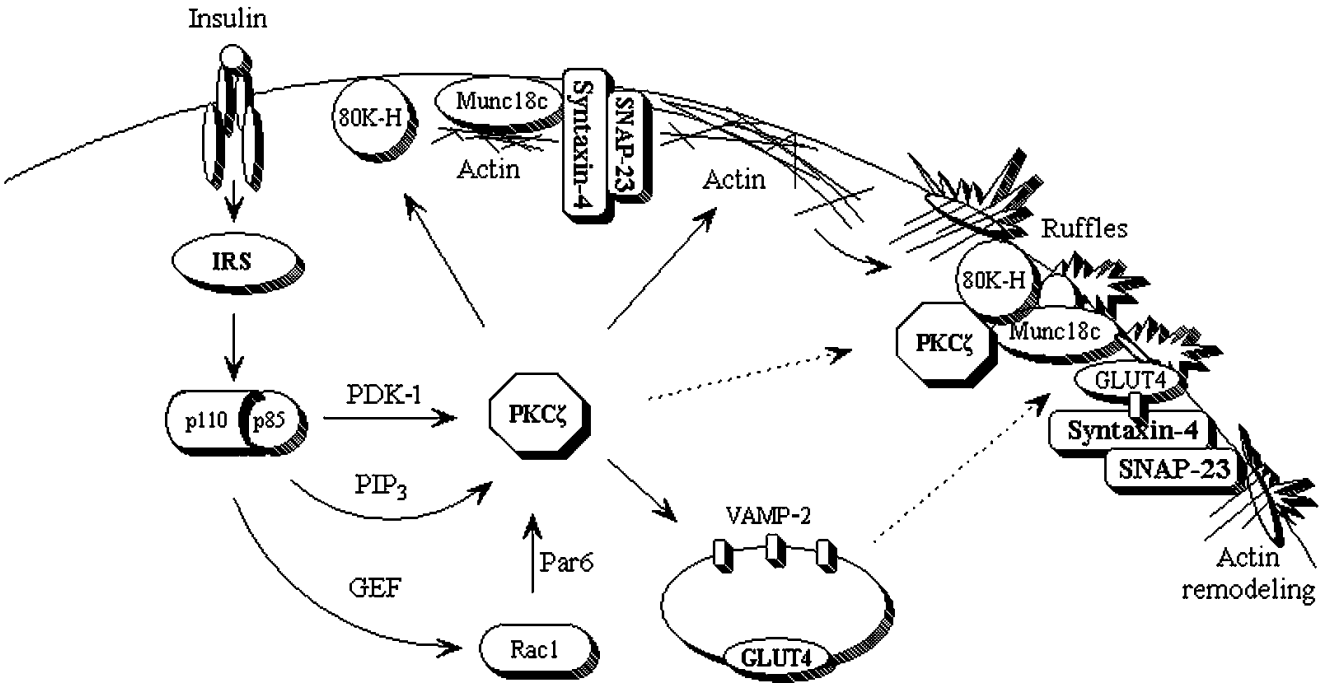
## PKCζ IS REQUIRED FOR GLUCOSE TRANSPORT DURING THE ACTION OF NON-INSULIN FACTORS

Like insulin, exercise increases the translocation of GLUT4 glucose transporters to the plasma membrane in skeletal muscle; however, these exercise-regulated glucose transporters may largely exist in functional pools different from those mobilized by insulin [40]. Exercise activates GLUT4 translocation/glucose transport in skeletal muscle by a mechanism that does not involve the activation of PI3-kinase [41]. Both exercise and AMPK (AMP-activated protein kinase) activators, AICAR and DNP, are found to activate ERK (extracellular signal-regulated kinase) and aPKCs in rodent skeletal muscle preparations and studies of AICAR action further suggest that glucose transport is activated by sequential activation of PYK2 (proline-rich tyrosine kinase-2), GRB2 (growth factor receptor-bound protein-2), SOS (guanine exchange factor), G-proteins of RAS family, serine/threonine protein kinases coded by *c-raf* genes (RAF), mitogen-activated protein kinase (MEK1), extracellular signal-regulated kinase (ERK), phospholipase D (PLD), and aPKCs [42]. The study of the effects of acute exercise on different PKC isoforms in human skeletal muscle also shows that exercise can induce PKCζ redistribution and increase PKCζ expression together with its activity [43]. Moreover, PKCζ activity increases too in exercise endurance-trained human skeletal muscle [44]. So, PKCζ is a candidate PKC isoform that may play a role in the regulation of exercise-related changes in metabolic and gene-regulatory responses. Up to now, the signaling events that increase glucose transport in response to exercise are not fully elucidated, exercise-induced glucose uptake in skeletal muscle is mediated by an insulin-independent mechanism, and PKCζ has been certified to participate in this process, though in an unknown manner.

Under normal condition, insulin controls glucose uptake by translocating GLUT4 and other glucose transporters to the plasma membrane in muscle and adipose tissues by a mechanism that appears to require PKCζ operating downstream of PI3-kinase. In diabetes mellitus (type I), insulin-stimulated glucose uptake is diminished, but with hyperglycemia, uptake is maintained by uncertain mechanisms. Recently, Bandyopadhyay and colleagues reported that glucose acutely activated PKC-ζ/λ in rat adipocytes and rat skeletal muscle preparations by a mechanism that was independent of PI3-kinase but, interestingly, dependent on the apparently sequential activation of the dantrolene-sensitive, non-receptor proline-rich tyrosine kinase-2; components of the extracellular signal-regulated kinase (ERK) pathway including GRB2, SOS, RAS, RAF, MEK1, and ERK1/2; and phospholipase D, thus yielding increases in phosphatidic acid, a known activator of PKC-ζ/λ. This activation of PKC-ζ/λ, moreover, appeared to be required for glucose-induced increases in GLUT4 translocation and glucose transport in adipocytes and muscle cells [45]. This finding reveals a novel pathway for activating PKC-ζ/λ and glucose transport. Compared with the results of Chen et al., we find that both exercise and glucose can activate PKCζ through the ERK pathway to promote glucose transport.

## POSSIBLE MECHANISM OF PKCζ TO MEDIATE GLUCOSE TRANSPORT

PKCζ expression and activity are positively related to glucose uptake, and PKCζ is one key factor in insulin and non-insulin signaling pathways. It is necessary to explore the mechanism of how PKCζ transfers insulin signal downstream and affects glucose uptake. Recent studies have suggested that the incorporation of insulin-responsive GLUT4 containing vesicles into the plasma membrane of muscle and adipose cells involves the v-SNARE proteins (family of membrane-associated proteins), vesicle-associated membrane protein 2 (VAMP2) [46], and the t-SNARE proteins—syntaxin-4 [47] and synaptosome-associated 23-kD protein (SNAP-23) [48]. PKCζ




Possible model of insulin signaling via PKCζ to promote GLUT4 translocation. The insulin signaling to PI3K may be bifurcated into Rac1 and PKCζ, and Rac1 may also activate the latter. PKCζ may simultaneously play two roles in relaying the upstream signals inducing GLUT4 translocation and stimulating actin remodeling. The actin remodeling and resulting plasma membrane ruffles may facilitate GLUT4 translocation. Its fusion to plasma membrane may be modulated by PKCζ via many PKCζ interacting proteins, such as 80K-H, Munc18c, v-SNARE (VAMP2), and t-SNAREs (SNAP23 and syntaxin-4)

cannot only phosphorylate VAMP2 to promote GLUT4 translocation [49]; it also interacts with protein kinase C substrate 80K-H to release the clamp action of Munc18c (syntaxin-binding protein) [50] on syntaxin-4, allowing VAMP2 to bind t-SNARE proteins [51].

In the liver of type II diabetes, although with insulin resistance, this signaling, insulin receptor (IR)/IRS-2–PI3K–PKCζ, is maintained normal, unlike its impairment in muscle and adipose tissues, but the maintained activation of PKCζ functions with increasing lipid synthesis mediated by SREBP-1c (sterol-regulatory element-binding protein), without its operation in muscle and adipose tissues [52]. The different expressing profiles of insulin signal molecules among muscle, adipose, and liver tissues may explain the different functions of PKCζ activation. It is also suggested that PKCζ exerting its specific functions requires other signaling association in specialized temporal and spatial states.

If it is true that PKCζ can interact with myotilin directly (unpublished data), PKCζ may be associated with actin remodeling. Myotilin is a novel sarcomeric protein with two Ig-like domains, which are encoded by a candidate gene for limb-girdle muscular dystrophy [53]. Myotilin can bind F-actin and α-actinin directly to prevent filament disassembly induced by latrunculin A [54]. Another new Z-disc protein, γ-filamin, can provide a link between the plasma membrane and myofibrils because it binds directly to γ- and δ-sarcoglycans and indirectly to α-actinin via calsarcin family protein FATZ and myotilin [55]. And actin remodeling is necessary to recruit GLUT4, PI3-kinase, and vesicle-associated membrane protein VAMP2, which is a precondition for glucose uptake [35, 56].

Combined with our observation and the results of others [36, 37, 57-59], we may propose the following possible model of insulin signaling transfer via PKCζ to promote GLUT4 translocation to the plasma membrane (figure): a portion of intracellular PKCζ molecules tether to the GLUT4 vesicles in the low density microsome fraction in basal state. Upon binding to its receptor, insulin activates PI3-kinase, which in turn activates (i) PKCζ through PDK1 and PI3K product $PIP_3$ and (ii) Rac1 through GLUT4-activating factor GEF (muscle cell) or TC10 through Cb1 (fat cell). Then the activated Rac1 or TC10 leads to actin remodeling through PKCζ. The reorganized actin filaments concentrate into the projections at the dorsal surface of the muscle cell and recruit GLUT4 compartments to their target membrane, then the vesicles fuse with the membrane.

In order to dissect the precise role of PKCζ in glucose uptake, it is necessary to investigate the sequential processes of GLUT4 vesicle formation, sorting, and translocation, unmasking its domain and fusion to plasma membrane acquiring its activity. We have found that

PKCζ directly induces formation of GLUT4 storage vesicles (GSVs) in L6 myotubes. How PKCζ facilitates GSVs formation and what other signals participate in the process need further research.

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