Membrane Localization of 3-Phosphoinositide-dependent Protein Kinase-1 Stimulates Activities of Akt and Atypical Protein Kinase C but Does Not Stimulate Glucose Transport and Glycogen Synthesis in 3T3-L1 Adipocytes*

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It is reported that 3-phosphoinositide-dependent protein kinase-1 (PDK-1) is activated in a phosphatidylinositol 3,4,5-trisphosphate-dependent manner and phosphorylates Akt, p70S6 kinase, and atypical protein kinase C (PKC), but its function on insulin signaling is still unclear. We cloned a full-length pdk-1 cDNA from a human brain cDNA library, and the adenovirus to overexpress wild type PDK-1 (PDK-1WT) or membrane-targeted PDK-1 (PDK-1-CAAX) was constructed. Overexpressed PDK-1WT existed mainly at cytosol, and PDK-1CAAX was located at the plasma membrane. In 3T3-L1 adipocytes, insulin induced mobility shift of PDK-1 protein, but overexpressed PDK-1WT and CAAX were shifted at the basal state. Insulin stimulated tyrosine phosphorylation of PDK-1WT, but PDK-1CAAX was already tyrosine-phosphorylated at the basal state. Overexpression of PDK-1WT led to a full activation of PKCα without insulin stimulation but showed only the minimum effects to stimulate phosphorylation of Akt and GSK-3. In contrast, the overexpression of PDK-1CAAX caused phosphorylation of Akt and GSK-3 more strongly without insulin stimulation. However, PDK-1CAAX did not affect 2-deoxyglucose uptake and inhibited glycogen synthesis, surprisingly. Finally, PDK-1CAAX expression inhibited insulin-induced ERK1/2 phosphorylation in a dose-dependent manner. Taken together, the translocation of PDK-1 from cytosol to the plasma membrane is critical for Akt and GSK-3 activation. On the other hand, only atypical PKC and Akt activation was insufficient for stimulation of glucose transport, and constitutive activation of Akt-GSK-3 pathway may inhibit glycogen synthesis and MAPK cascade in 3T3-L1 adipocytes.

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The abbreviations used are: Glut4, glucose transporter; PI, phosphatidylinositol; PDK-1, 3-phosphoinositide-dependent protein kinase-1; PKC, protein kinase C; ERK, extracellular signal-regulated kinase; DME medium, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; PBS, phosphate-buffered saline; MAPK, mitogen-activated protein kinase; GSK, glycogen synthase kinase-3; MBP, myelin basic protein; PDK-1WT, wild type PDK-1; PDK-1CAAX, membrane-targeted PDK-1; E1, ubiquitin-activating enzyme.

The overexpression of either the N-terminal Src homology 2 domain of the p85 subunit of phosphatidylinositol (PI) 3-kinase or Δp85, which was not able to bind insulin receptor and insulin receptor substrates, inhibited both the insulin-stimulated translocation of Glut4 and glucose transport (1–3). The membrane-targeted p110 subunit of PI 3-kinase, which is constitutively active, stimulated glucose transport without insulin stimulation (4). Thus, PI 3-kinase pathway plays an important role on this effect of insulin.

Akt and atypical protein kinase C (PKC), such as PKCα and PKCζ, are known to be downstream molecules of PI 3-kinase. It was reported that membrane-targeted Akt activated glucose transport without insulin stimulation (5). Thus, Akt activation looks sufficient for glucose transport. On the other hand, another group showed that a dominant negative PKCα inhibited insulin-stimulated glucose transport without affecting Akt activity (6). Thus, the molecular mechanism and candidate signaling molecule(s) for insulin-stimulated glucose transport is/are still unclear.

Recently, 3-phosphoinositide-dependent protein kinase-1 (PDK-1) was cloned from rabbit skeletal muscle extracts (7) or sheep brain cytosol (8) as a protein kinase that phosphorylated Akt at Thr308 and increased its activity in the presence of phosphatidylinositol 3,4,5-trisphosphate. p70S6 kinase, which is another downstream molecule of PI 3-kinase, is also phosphorylated at Thr246 and Thr412 by PDK-1 in vivo and in vitro (9–11). Moreover, PDK-1 directly phosphorylates the activation loop of PKCζ (12, 13), and PKCζ/βII (14). Thus, PDK-1 mediates signaling pathways between PI 3-kinase and its downstream molecules, and it is possible that PDK-1 plays an important role on insulin-stimulated glucose transport. In fact, it was reported that the overexpression of wild type PDK-1 by the electroporation method provoked the increases in the activity of cotransfected hemagglutinin-tagged PKCζ and concomitantly enhanced hemagglutinin-tagged Glut4 translocation in rat adipocytes (15). However, very recently, it was reported that the overexpression of constitutively active mutant PDK-1Ala280-Val but not wild type PDK-1 was able to phosphorylate Akt at Thr308 to the same extent as that stimulated by insulin (16). Furthermore, PDK-1Ala280-Val localized in the cytosol and at the plasma membrane, and PDK-1-Ala280-
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Val lacking the pleckstrin homology domain, which localized predominantly in the cytosol, showed much weaker effect to phosphorylate Akt. Thus, the roles of PDK-1 on insulin signal transduction are still unclear.

In this study, we overexpressed wild type and membrane targeted PDK-1 in 3T3-L1 adipocytes by adenovirus-mediated gene transfer and examined the role of PDK-1 on insulin signaling. Overexpression of wild type PDK-1 stimulated atypical PKC activity but small effects for Akt phosphorylation. On the other hand, a membrane-targeted PDK-1 enhanced the phosphorylation of Akt and GSK-3 at the basal condition but inhibiting glycogen synthesis and insulin-stimulated ERK phosphorylation. Moreover, neither wild type nor membrane-targeted PDK-1 enhances glucose transport in 3T3-L1 adipocytes. This finding suggests that PDK-1 stimulates atypical PKC and Akt by different mechanisms, and localization of PDK-1 is important for regulation of downstream signaling but another mechanism is still needed for total activation.

EXPERIMENTAL PROCEDURES

Materials—Human insulin was provided by Eli Lilly, Inc. (Indianapolis, IN). Anti-PDK-1 antibody was purchased from Upstate Biotechnology, Incorporated (Lake Placid, NY). Anti-phosphospecific PKCα, -Akt, and -ERK1/2 antibodies were from Cell Signaling Technology, Inc. (Beverly, MA). Phosphotyrosine antibody (PY20) was from Transduction Laboratories (Lexington, KY). Horseradish peroxidase-linked anti-rabbit and anti-mouse antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Horseradish peroxidase-linked anti-actin antibody was from Chemicon (Temecula, CA). Anti-PKCα, -Akt, and -fetal calf serum (FCS) were obtained from Invitrogen. All radioisotopes were obtained from PerkinElmer Life Sciences. Kodak X-Omat AR was obtained from Eastman Kodak Co. All other reagents and chemicals were purchased from Sigma.

Isolation of the pdk-1 cDNA—The partial nucleotide sequence containing open reading frame of human pdk-1 cDNA (GenBank™ accession number AP017986) was amplified from total RNA of HepG2 cells by reverse transcription-PCR reaction with use of the primers 5’-gacctgagcgcacccagcactggagacgagcctg-3’ and -tactccgagagcctg-3’, and the fragments were subcloned into pCR2.1 vector (Invitrogen). The 1.6-kb fragments obtained were used as a probe for standard plaque excision. After purification of primers 5’/H11032, H9261 and the fragments were subcloned into pCR2.1 vector (Invitrogen). The fragment containing full-length human pdk-1 cDNA, in vitro excision was performed using a helper phage (ExAssist™, Stratagene). Finally, full-length cDNA subcloned into pBluescript was isolated.

After confirmation of the sequence, a FLAG epitope (MDYLDLDIDKK) was added by another PCR reaction using the following primers 5’-caagtgagcgcacccagcactggagacgagcctg-3’ and -tacctccgagagcctg-3’, and the fragments were subcloned into pCR2.1 (Invitrogen). The 1.6-kb fragments obtained were used as a probe for standard plaque hybridization with a human brain cDNA library (Catalog no. 9572253, Stratagene, La Jolla, CA). After purification of 2 plague (Stratagene) containing full-length human pdk-1 cDNA, in vitro excision was performed using a helper phage (ExAssist™, Stratagene). Finally, full-length cDNA subcloned into pBluescript was isolated.

Cell Treatment—3T3-L1 adipocytes were infected at a multiplicity of infection of 10–40 plaque-forming units/cell for 16 h with stocks of either a control recombinant adenovirus, Ad5-ctrl, containing the cytomegalovirus promoter, or the adenovirus (Ad5-ctrl) gene function of AdEasy is provided E1 (at least in cells lacking the E1 region of adenovirus) but fully infectious. The recombinant adenovirus Ad5-PDK-1CAAX was cultured in DME high glucose medium containing 10% FCS, 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% FCS in a 5% CO2 environment.

Cell Culture—3T3-L1 adipocytes, which were provided by Dr. J. M. Olefsky, were grown and maintained in DME high glucose medium (Invitrogen) containing 50 units/ml penicillin, 50 μg/ml streptomycin, and 10% FCS in a 10% CO2 environment. The cells were allowed to grow until 2 days postconfluence and then differentiated by the addition of the same medium containing isobutylmethylxanthine (500 μM), dexamethasone (25 μM), and insulin (4 μg/ml) for 3 days and the medium containing insulin for 3 additional days. The medium was then changed every 3 days until the cells were fully differentiated, typically after 15 days. Prior to experimentation, the adipocytes were trypsinized and resuspended in the appropriate culture dishes.

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units/ml apotinin, 1 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride, and 50 mM NaF (pH 7.5), for 30 min at 4°C. The cell lysates were centrifuged to remove insoluble materials. For Western blot analysis, whole cell lysates (20 μg protein/lane) were denatured by boiling in Laemmli sample buffer containing 100 mM dithiothreitol and resolved by SDS-PAGE. Cells were transferred to nitrocellulose by electroblotting in Towbin buffer containing 20% methanol. For immunoblotting, membranes were blocked and probed with specified antibodies. Blots were then incubated with horseradish peroxidase-linked secondary antibody followed by chemiluminesence detection according to the manufacturer’s instructions (Pierce).

In Vitro Kinase Assay—Kinase activity was measured by a solid-phase kinase assay as described previously with some modification (21). 3T3-L1 adipocytes were infected as described above, starved for 16 h and then medium was replaced with DME medium containing 1% serum and 2% heat-inactivated serum for 56 h. The cells were serum-starved for 16 h, and then medium was replaced with a buffer containing 20 mM HEPES (pH 7.5), 150 mM NaCl, 10% glycerol, 20 mM NaF, 0.2 mM Na3VO4, and 0.1% Triton X-100 and once with kinase buffer (20 mM HEPES (pH 7.5), 10 mM MgCl2, 0.2 mM vanadate, and 1 mM dithiothreitol). The immunocomplexes were then incubated with 30 μl of kinase buffer containing 50 μM unlabeled ATP, 50 μM of myelin basic protein, and 10 μCi of [γ-32P]ATP at 30°C for 10 min. The reaction was terminated by the addition of 15 μl of 3× Laemmli sample buffer and boiling at 100°C for 5 min. Phosphorylated proteins were resolved by 15% SDS-PAGE followed by autoradiography. The relative kinase activities were quantified by Instant-Imager.

2-Deoxyglucose Transport—The procedure for glucose transport was a modification of the methods described by Klip et al. (22). Differentiated 3T3-L1 adipocytes were infected with Ad5-PDK-1-WT or Ad5-ctrl at 40 m.o.i. as described above and grown in medium containing heat-inactivated serum (2%) for 72 h. Serum- and glucose-deprived cells were incubated in minimum Eagle’s medium-α in the absence (basal) or presence of the indicated concentration of insulin for 1 h at 37°C. Glucose uptake was determined in duplicate or triplicate at each point after subtraction of the initial 10 μl of substrate (2-3H)deoxyglucose or l-[3H]glucose (0.1 μCi/ml), final concentration (0.01 mM/liter)) to provide a concentration at which cell membrane transport was rate-limiting. The value for l-glucose was subtracted to correct each sample for the contributions of diffusion and trapping.

Glycogen Synthesis—Glycogen synthesis was measured as described previously (23). 3T3-L1 adipocytes were infected with Ad5-PDK-1-WT or Ad5-PDK-1CAAX at 50 m.o.i. for 16 h and grown in medium containing 2% heat-inactivated serum for 56 h. The cells were serum-starved for 16 h, and then medium was replaced with DME medium containing 1% bovine serum albumin. The cells were incubated with [1-14C]glucose (0.4 μCi/ml) and 100 ng/ml insulin for 2 h in CO2 incubator, washed with PBS three times, and lysed with 2 N NaOH at 55°C. Synthesized [1-14C]glycogen was precipitated with cold glycogen in 66% ethanol and washed. The radioactivity was measured.

Statistics—The values are expressed as mean ± S.E. unless otherwise stated. The step-down method by Tukey-Welsch was used to determine the significance of any differences among more than four groups. p < 0.05 was considered significant.

RESULTS

Effect of Co-expression of PDK-1 on Akt Phosphorylation in SF-9 Cells—Recombinant His-tagged Akt-1 baculovirus was injected to SF-9 cells with or without the following infection of baculovirus including His-3-phosphoinositide-dependent protein kinase-1 (PDK-1) at the indicated multiplicity of infection. As shown in Fig. 1, overexpressed PDK-1 was autophosphorylated and phosphorylated Akt. Thus, our PDK-1 has kinase activity to phosphorylate Akt.

Expression of PDK-1 and PDK-1CAAX in Rat Primary Cultured Hepocytes or 3T3-L1 Adipocytes—Isolated primary cultured hepatocytes were infected with recombinant adenovirus expressing wild type PDK-1 and CAAX at 40 m.o.i. for 16 h. Following 56-h incubation, the cells were incubated for 16 h and stimulated with insulin or without 100 ng/ml insulin for 30 min. B, the cells were lysed and analyzed by SDS-PAGE followed by Western blotting with anti-PDK-1 antibody. C, the cell lysates were immunoprecipitated by anti-PDK-1 antibody and immunoblotted by phosphotyrosine antibody. Each Western blot is a representative of three independent experiments.

Differentiated 3T3-L1 adipocytes were infected with Ad5-PDK-1-WT or Ad5-PDK-1CAAX at 40 m.o.i. for 16 h. Following a 56-h incubation, the cells were starved for 16 h and stimulated with 100 ng/ml insulin for 30 min. The cells were lysed and analyzed by SDS-PAGE followed by Western blotting with anti-PDK-1 antibody (Fig. 2B). Overexpressed PDK-1-WT showed a mobility shift at the basal condition and shifted more by insulin stimulation. PDK-1CAAX was highly shifted at the basal condition, and insulin stimulation had no additive effect. Insulin stimulation led to tyrosine phospho-
rylation of PDK-1WT, but PDK-1CAAX was already tyrosine-phosphorylated at the basal condition (Fig. 2C). We were not able to see any phosphorylation bands in control cells, probably because of a small amount of endogenous PDK-1 protein (data not shown).

In Vitro Kinase Activity of Overexpressed PDK-1 in 3T3-L1 Adipocytes—Cell lysates from 3T3-L1 adipocytes infected with Ad5-PDK-1WT or Ad5-PDK-1CAAX at 40 m.o.i. were immunoprecipitated with anti-PDK-1 antibody, and in vitro kinase activity was measured using MBP as substrate (Fig. 3). Insulin stimulated PDK-1 activity ~1.3-fold in uninfected 3T3-L1 adipocytes. PDK-1WT overexpression itself increased PDK-1 activity ~6-fold at the basal condition, and insulin had no additive effect. PDK-1CAAX expression also elevated kinase activity ~2.4-fold at the basal condition, and insulin had no additive effect but it showed lower activity than PDK-1WT.

Effects of PDK-1 Expression on Atypical-PKC Phosphorylation in 3T3-L1 Adipocytes—Differentiated 3T3-L1 adipocytes were infected with Ad5-PDK-1WT or Ad5-PDK-1CAAX at 40 m.o.i. and stimulated with 100 ng/ml insulin for 10 min. The cells then were lysed, and Western blotting was performed by anti-phosphospecific PKCζ antibody. As shown in Fig. 4A, insulin stimulated the phosphorylation of PKCζ/λ, and overexpression of PDK-1WT or PDK-1CAAX caused its phosphorylation without insulin stimulation. We observed the identical findings by in vitro kinase assay using MBP as substrate. When basal activity was adjusted to 100%, PKCζ activity was stimulated up to 132 ± 7.8% by insulin in control cells and up to 140 ± 6.5% and 145 ± 16.8% by PDK-1WT and PDK-1CAAX expression, respectively. Insulin had no further effects on PDK-1 activity in these cells.

Effects of PDK-1 Expression on Akt and GSK-3 Phosphorylation in 3T3-L1 Adipocytes—PDK-1WT or PDK-1CAAX expressing 3T3-L1 adipocytes were stimulated with or without insulin for 30 min, lysed, and analyzed by Western blotting with phosphospecific Akt or GSK-3 antibody. In Fig. 4B, PDK-1CAAX expression stimulated Akt phosphorylation on both Thr308 and Ser473 at the basal condition weakly but significantly. This increased phosphorylation of Akt led the phosphorylation of GSK-3 up to the insulin-stimulated level at the basal condition (Fig. 4C). PDK-1WT expression caused small phosphorylation of Akt and GSK-3 at the basal condition. PDK-1CAAX and PDK-1WT overexpression caused the phosphorylation of p70S6 kinase at basal level as well as Akt (data not shown).

Effects of PDK-1 Expression on Glycogen Synthesis and 2-Deoxy-glucose Uptake in 3T3-L1 Adipocytes—We next measured the effect of PDK-1WT and PDK-1CAAX on glycogen synthesis in 3T3-L1 adipocytes. Surprisingly, PDK-1CAAX inhibited glycogen synthesis >50% both at the basal and insulin-stimulated conditions (Fig. 5). PDK-1WT also inhibited glycogen synthesis, but this effect was weaker than PDK-1CAAX. On the other hand, neither PDK-1WT nor PDK-1CAAX overexpression affected both Glut4 translocation from cytosol to plasma membrane and glucose uptake both at the basal and insulin-stimulated conditions (Fig. 6, A and B).

Effects of PDK-1 Expression on MAPK Phosphorylation in 3T3-L1 Adipocytes—PDK-1WT or CAAX expressing 3T3-L1 adipocytes were stimulated with insulin for 10 min, lysed, and subjected to SDS-PAGE followed by Western blotting with anti-phosphospecific ERK1/2 antibody (Fig. 7). PDK-1WT expression had no significant effect on ERK1/2 phosphorylation in 3T3-L1 adipocytes. Interestingly, PDK-1CAAX expression inhibited the insulin-stimulated ERK1/2 phosphorylation in a dose-dependent manner.

DISCUSSION

PDK-1 was originally cloned as Akt kinase, and several lines of evidence demonstrated that PDK-1 played an important role to mediate signal transduction in the PI 3-kinase pathway. We cloned a full-length pdk-1 cDNA from a human brain cDNA library and overexpressed PDK-1WT or PDK-1CAAX by adenovirus-mediated gene transfer. Our cloned PDK-1 was auto-phosphorylated and phosphorylated Akt in Sf-9 cells in the baculovirus system (Fig. 1). Moreover, overexpression of PDK-1WT showed elevated kinase activity in 3T3-L1 adipocytes (Fig. 3). Thus, our cloned PDK-1 appeared to be an intact enzyme. However, surprisingly, the overexpression of PDK-1WT did not significantly enhance the phosphorylation of Akt and GSK-3 at the basal condition (Fig. 4). It was previously suggested that PDK-1 was constitutively active in cells and...
that phosphorylation and activation of its downstream substrates was mediated mainly by translocation and conformational changes in its substrates (24, 25). Furthermore, PDK-1 purified from cells was constitutively active and treatment of cells with mitogen did not stimulate PDK-1 activity in vitro (26). Moreover, the translocation of PDK-1 to the plasma membrane was also important in allowing PDK-1 to activate Akt (27). However, it was recently reported that overexpression of the constitutively active mutant PDK-1Ala280-Val but not wild type PDK-1 was able to phosphorylate Akt at Thr308 to the same extent as that stimulated by insulin (16). PDK-1 Ala280-Val localized in the cytosol and at the plasma membrane, and PDK-1Ala280-Val lacking the pleckstrin homology domain, which localized predominantly in the cytosol, showed much weaker effect to phosphorylate Akt. In this study, the overexpression of PDK-1WT showed a high level of kinase activity at the basal condition (Fig. 3), so it appeared constitutively active. However, insulin stimulation or co-expression of membrane-targeted p110 (p110CAAX) (4) caused a mobility shift of PDK-1WT (data not shown). Furthermore, insulin-stimulated tyrosine phosphorylation of PDK-1WT (Fig. 2). It was reported recently (28) that phosphorylation of PDK-1 on Tyr373/376 is important for PDK-1 activity. Thus, this additional phosphorylation by the PI 3-kinase pathway may be important for activation of its downstream molecules. In fact, overexpressed PDK-1CAAX was mobility-shifted more, tyrosine-phosphorylated, and able to phosphorylate Akt and GSK-3 without stimulation more strongly than PDK-1WT (Figs. 2 and 4). On the other hand, the amount of the insulin-stimulated tyrosine-phosphorylated PDK-1WT was the same as PDK-1CAAX, but insulin-stimulated mobility shift of PDK-1WT was not so great as that of PDK-1CAAX. Thus, only the phosphorylation of tyrosine residues is not able to explain mobility shift, and phosphorylation of other residues may occur by membrane targeting. Further study is still needed to clarify this mechanism. PDK-1CAAX-expressing cells had higher activity than control cells at the basal condition, but it was much lower than that of PDK-1WT (Fig. 3). The expression level of PDK-1WT and PDK-1CAAX was comparable, but kinase activity per protein was 1, 0.9, and 0.25 in endogenous PDK-1, PDK-1WT, and PDK-1CAAX, respectively. This means that increased kinase activity in PDK-1WT cells was the result of increased protein mass, but membrane-targeted PDK-1 had lower activity. Because we measured kinase activity in vitro by using MBP as substrate, the attached CAAX signal might interfere with the affinity between PDK-1 and MBP. Another possibility is that membrane localization might be affected by negative regulator

FIG. 5. Effect of PDK-1 expression on glycogen synthesis in 3T3-L1 adipocytes. Differentiated 3T3-L1 adipocytes infected with Ad5-PDK-1WT or CAAX at 40 m.o.i. were stimulated with or without insulin at the indicated concentrations for 60 min. Glucose incorporation into glycogen was measured as described under “Experimental Procedures.” The graph shows the mean ± S.E. of four independent experiments, and the values are expressed as a percentage of the basal activity (100%) observed in unstimulated and uninfected cells. *, the difference from the insulin-stimulated values of each concentration in the cells with control virus at p < 0.01.

FIG. 6. Effects of PDK-1 expression on Glut4 translocation and 2-deoxy-glucose uptake in 3T3-L1 adipocytes. Differentiated 3T3-L1 adipocytes infected with Ad5-PDK-1 or CAAX at 40 m.o.i. were stimulated with or without insulin at the indicated concentrations for 60 min. Each fraction was separated by ultracentrifugation, and Western blot was performed by anti-Glut4 antibody (A). PM, plasma membrane; LDM, low density microsome. 2-Deoxy-glucose uptake was measured as described under “Experimental Procedures” (B). The graph shows the mean ± S.E. of five independent experiments.

FIG. 7. Effects of PDK-1 expression on MAPK phosphorylation in 3T3-L1 adipocytes. PDK-1WT or CAAX expressing 3T3-L1 adipocytes were stimulated with insulin for 10 min, lysed, and subjected to SDS-PAGE followed by Western blotting with anti-phosphospecific MAPK antibody (upper panel). The membrane then was stripped and rebotted with anti-Erk1 antibody (lower panel).
for kinase activity of PDK-1. However, at least it supports the fact that membrane localization is important because PDK-1CAAX has lower kinase activity than PDK-1WT but stimulates the phosphorylation of Akt and GSK-3 more strongly at the basal condition. PDK-1WT showed small effects for its downstream molecules at the basal condition. It is possible that a part of overexpressing PDK-1WT exists near the plasma membrane by chance, and it behaves like PDK-1CAAX. On the other hand, atypical PKC was fully activated by PDK-1WT overexpression alone at the basal conditions and showed no further stimulation by insulin (Fig. 4). These data suggest that PDK-1 stimulates Akt, GSK-3, and atypical PKC by different mechanisms. Localization of PDK-1 is important for activation of Akt and GSK-3 but not for atypical PKC.

The ability of PDK-1CAAX to phosphorylate Akt at the basal state was much weaker than insulin stimulation. It is known that Akt translocates to plasma membrane in a phosphatidylinositol 3,4,5-trisphosphate-dependent manner and gets phosphorylation by PDK-1. When PDK-1CAAX is overexpressed, PI 3-kinase is not activated. Thus, it is possible that because Akt does not translocate to plasma membrane, PDK-1CAAX is not able to interact with Akt, even though PDK-1CAAX is enough to phosphorylate Akt.

It was reported that the membrane-targeted Akt and the constitutively active Akt mutant stimulated glucose transport without insulin stimulation (5). Another study (6) showed that a dominant negative PKCα inhibited the insulin-stimulated glucose transport without affecting Akt activity. Thus, the molecular mechanism of insulin-stimulated glucose transport is still unclear. In this study, the overexpression of PDK-1WT stimulated atypical PKC activity but did not affect glucose transport (Fig. 6), so it is probable that atypical PKC activation alone is insufficient for stimulation of glucose transport. It was recently reported that overexpression of atypical PKC isoform-specific interacting protein, which specifically interacts with the atypical PKC isoforms PKCα and PKCγ, inhibits insulin stimulation of glucose uptake partially with complete inhibition of PKCα activity (29). This finding also suggests that other pathways exist for insulin-stimulated glucose transport with the exception of atypical PKC. PDK-1CAAX showed full activation of atypical PKC and partial activation of Akt but did not stimulate glucose transport. Akt activation is not so great; thus, it may not be enough for the stimulation of glucose transport. Another explanation is that both atypical PKC and Akt activation are still not enough for stimulation of glucose transport.

PDK-1CAAX fully stimulated GSK-3 phosphorylation but inhibited glycogen synthesis (Figs. 4 and 5). We previously reported that membrane-targeted PI 3-kinase (p110CAAX) constitutively activated Akt but inhibited glycogen synthesis (4). Thus, constitutive activation of Akt-GSK-3 pathway may inhibit glycogen synthesis because of desensitization. It is possible that activated GSK-3 stimulates glycogen synthesis, and increased glycogen content may provide a feedback signal to diminish synthesis rate. To supporting this idea, PDK-1WT also stimulated Akt-GSK-3 pathway; however, it was weak, so glycogen synthesis was partially inhibited in the cells expressing PDK-1WT.

A previous study reported that the expression of a dominant-negative Δδ5 PI 3-kinase subunit, kinase-inactive PDK-1, and kinase-inactive PKCα inhibited insulin-induced ERK activation in rat adipocytes (30), suggesting that PI 3-kinase-PDK-1/ atypical PKC pathway is important to activate MAPK cascade. However, in this study, overexpressed PDK-1WT did not enhance ERK phosphorylation at both the basal and insulin-stimulated conditions (Fig. 7). Even though it stimulated PKCζ.

Moreover PDK-1CAAX inhibited the insulin-stimulated ERK phosphorylation. Similar to these findings, we also previously reported that p110CAAX did not stimulate ERK phosphorylation at the basal and inhibited insulin-stimulated ERK phosphorylation, even though a full activation of PI 3-kinase cascade (4). Because it is reported that Akt inhibits Raf activation (31–33), constitutive activation of Akt may decrease insulin-stimulated ERK phosphorylation in the PDK-1CAAX-expressing cells. Taken together, the activation of the PI 3-kinase/ PDK-1 pathway alone is insufficient for ERK activation, and constitutive activation of this pathway inhibits insulin-stimulated ERK phosphorylation.

In summary, wild type PDK-1 overexpression is sufficient for phosphorylation of atypical PKC, but membrane localization is needed for Akt and GSK-3 activation. Moreover, the activation of both atypical PKC and Akt alone is insufficient for glucose transport in 3T3-L1 adipocytes.

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Membrane Localization of 3-Phosphoinositide-dependent Protein Kinase-1 Stimulates Activities of Akt and Atypical Protein Kinase C but Does Not Stimulate Glucose Transport and Glycogen Synthesis in 3T3-L1 Adipocytes

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