Role of PKC isoforms in glucose transport in 3T3-L1 adipocytes: insignificance of atypical PKC

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Tsuru, Masatoshi, Hideki Katagiri, Tomoichiro Asano, Tetsuya Yamada, Shigeo Ohno, Takehide Oghara, and Yoshitomo Oka. Role of PKC isoforms in glucose transport in 3T3-L1 adipocytes: insignificance of atypical PKC. Am J Physiol Endocrinol Metab 283: E338–E345, 2002.—To elucidate the involvement of protein kinase C (PKC) isoforms in insulin-induced and phorbol ester-induced glucose transport, we expressed several PKC isoforms, conventional PKC-α, novel PKC-δ, and atypical PKC isoforms of PKC-λ and PKC-ζ, and their mutants in 3T3-L1 adipocytes using an adenovirus-mediated gene transduction system. Endogenous expression and the activities of PKC-α and PKC-λ/ζ, but not of PKC-δ, were detected in 3T3-L1 adipocytes. Overexpression of each wild-type PKC isoform induced a large amount of PKC activity in 3T3-L1 adipocytes. Phorbol 12-myristate 13-acetate (PMA) activated PKC-α and exogenous PKC-δ but not atypical PKC-λ/ζ. Insulin also activated the overexpressed PKC-δ but not PKC-α. Expression of the wild-type PKC-α or PKC-δ resulted in significant increases in glucose transport activity in the basal and PMA-stimulated states. Dominant-negative PKC-α expression, which inhibited the PMA activation of PKC-α, decreased in PMA-stimulated glucose transport. Glucose transport activity in the insulin-stimulated state was increased by the expression of PKC-δ but not of PKC-α. These findings demonstrate that both conventional and novel PKC isoforms are involved in PMA-stimulated glucose transport and that other novel PKC isoforms could participate in PMA-stimulated and insulin-stimulated glucose transport. Atypical PKC-λ/ζ was not significantly activated by insulin, and expression of the wild-type, constitutively active, and dominant-negative mutants of atypical PKC did not affect either basal or insulin-stimulated glucose transport. Thus atypical PKC enzymes do not play a major role in insulin-stimulated glucose transport in 3T3-L1 adipocytes.

gluose transport; insulin; phorbol 12-myristate 13-acetate

REGULATION OF GLUCOSE HOMEOSTASIS is one of the most important actions of insulin. Insulin stimulates the translocation of GLUT4 glucose transporters from intracellular storage sites to the plasma membrane in muscle and adipose tissue, resulting in increased glucose uptake. Many intensive studies have focused on the cellular and molecular mechanisms responsible for these trafficking events (15, 39).

The involvement of protein kinase C (PKC) in glucose transport activation was originally recognized in studies using pharmacological agents. Phorbol 12-myristate 13-acetate (PMA), an activator of conventional and novel PKC, stimulates glucose transport activity (27) by inducing translocation of both GLUT1 and GLUT4 glucose transporters (1). In addition, insulin- and PMA-stimulated glucose transport activity was inhibited by staurosporine in isolated rat adipocytes (33, 41). These results suggest that PKC participates in activating glucose transport in adipocytes.

Several lines of evidence have indicated that phosphatidylinositol 3-kinase (PI 3-kinase) activation is important in insulin-stimulated glucose transport. The PI 3-kinase pharmacological inhibitors, such as wortmannin (37) and LY-294002 (12), and expression of the dominant-negative mutants of PI 3-kinase (18, 22, 25) reportedly markedly block insulin-stimulated glucose transport and GLUT4 translocation in rat and 3T3-L1 adipocytes. Furthermore, overexpression of wild-type PI 3-kinase tagged with the GLUT2 COOH-terminal domain (16) or the constitutively active mutant of PI 3-kinase (29) promoted glucose transport activity and GLUT4 translocation. These findings suggest a central role for PI 3-kinase in insulin-stimulated glucose transport.

Recently, several pathways have been reported to be activated by growth factor stimulation downstream from PI 3-kinase. Atypical PKC, consisting of PKC-ζ and PKC-λ, which are not activated by diacylglycerol or phorbol ester, is one of such effectors of PI 3-kinase. Atypical PKC enzymes are activated in vitro in the presence of the products of PI 3-kinase and phosphati-

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dyinositol trisphosphate (32, 43), and are also activated by growth factor stimulation (2). Furthermore, two groups of investigators have reported that PKC-ζ or PKC-λ played a major role in glucose transport activation by insulin in 3T3-L1 adipocytes (8, 26), L6 myocytes (6), and rat (42) and human (5) adipocytes, although they hypothesized that different atypical PKC isoforms were involved. In contrast, it was also reported that activation of PKC-δ, a novel PKC, but not an atypical PKC, is a major signal in insulin-induced glucose transport (10). Thus the necessity of activating atypical PKC in insulin-induced glucose transport is controversial.

In the present study, to elucidate the involvement of conventional, novel, and atypical PKCs in insulin-induced and PMA-induced glucose transport, we examined the effects of overexpressing several PKC isoforms and their mutants (PKC-α as a conventional PKC isoform, PKC-δ as a novel PKC isoform, and PKC-λ and PKC-ζ as atypical PKC isoforms). 3T3-L1 adipocytes were transfected with these isoforms using an adenovirus-mediated gene transduction system.

MATERIALS AND METHODS

Antibodies. The rabbit polyclonal anti-PKC-α antibody, anti-PKC-δ antibody, and anti-PKC-ζ antibody, which also reacts with the λ isoform, were purchased from Santa Cruz Biotechnology.

Cell culture. 3T3-L1 fibroblasts were maintained in DMEM containing 10% donor calf serum (GIBCO) in an atmosphere of 10% CO₂ at 37°C. Two days after the fibroblasts had reached confluence, differentiation was induced by treating cells with DMEM containing 0.5 mM 3-isobutyl 1-methylxanthine, 4 μM dexamethasone, and 10% FBS for 48 h. Cells were incubated with DMEM supplemented with 10% FBS every other day for the following 4–10 days. More than 90% of the cells expressed the adipocyte phenotype (23).

Expression constructs. The cDNAs encoding the entire rabbit PKC-α (35), its dominant-negative mutant (7) mouse PKC-δ (36), its dominant-negative mutant (19) mouse PKC-ζ (17), its dominant-negative mutant (9) mouse PKC-λ (2), and its dominant-negative (2) and constitutively active (26) mutants were kindly provided by Dr. S. Ohno. Recombinant adenoviruses containing each PKC gene were constructed by homologous recombination between the expression cosmid cassette and the parental virus genome, as described previously (22, 30, 34).

Gene transduction. 3T3-L1 adipocytes were incubated with DMEM containing the adenovirus for 1 h at 37°C, and the growth medium was then added. Experiments were performed 60 h after the infection. Recombinant adenoviruses were applied at a multiplicity of infection (MOI) of 200–300 plaque-forming units (pfu)/cell, and 3T3-L1 adipocytes infected with the Adex1Calacz virus (21), which encodes Escherichia coli lacZ, were used as a control, since Adex1Calacz gene expression was observed in >90% of 3T3-L1 adipocytes at an MOI of 200–300 pfu/cell on postinfection day 3 but did not affect glucose transport activity compared with noninfected cells, as reported previously (22, 23).

PKC activity assay. PKC activity was assayed using a peptide substrate, as previously described (8). 3T3-L1 cells cultured in 12-well plates were incubated with or without 1 μM insulin or 1.6 μM PMA for 10 min. Cells were homogenized in buffer containing 20 mM Tris·HCl (pH 7.5), 0.25 mM sucrose, 1.2 mM EGTA, 20 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM sodium vanadate, 1 mM sodium pyrophosphate, 1 mM NaF, 1% Triton X-100, 0.5% Nonidet P-40, and 150 mM NaCl and then cleared of insoluble substances by centrifugation. PKC-α and PKC-δ were immunoprecipitated by incubation with polyclonal anti-PKC-α antibody or anti-PKC-δ antibody, respectively, for 16 h at 4°C. The immunoprecipitates were suspended in reaction buffer containing 50 mM Tris·HCl (pH 7.5), 1 mM NaHCO₃, 5 mM MgCl₂, and 1 mM PMSF and were then assayed for the ability to phosphorylate a PKC peptide substrate, as previously described (8). 3T3-L1 cells were applied at a multiplicity of infection (MOI) of 200 plaque-forming units (pfu)/cell, and 3T3-L1 adipocytes were infected with the Adex1Calacz virus (21), which encodes lacZ, were used as a control, since Adex1CAlacZ was quantitated using an image analyzer BAS2000 (Fujix).

Glucose transport assay. 3T3-L1 adipocytes in 12-well plates were serum starved for 3 h in DMEM containing 0.2% BSA, followed by a 45-min glucose-free incubation in Krebs-Ringer phosphate buffer. Cells were then incubated with or without 1 μM insulin or 1.6 μM PMA for 15 min, and 0.1 mM 2-deoxy-D-[³H]glucose uptake was measured as described previously (22, 23).

RESULTS

Conventional PKC, PKC-α, has some involvement in PMA-induced, but not in insulin-induced, glucose transport. First, we expressed the wild-type and dominant-negative mutants of a conventional PKC isoform, PKC-α, to increase and suppress PKC-α activity, respectively, using an adenovirus-mediated gene transduction system. Immunoblotting with anti-PKC-α antibody showed that successful expression of wild-type and the mutated PKC-α was achieved in 3T3-L1 adipocytes by infection with the recombinant adenoviruses (Fig. 1A). PKC-α activity was measured in the immunoprecipitates with anti-PKC-α antibody (Fig. 1B). Consistent with the results obtained from the immunoblotting study, overexpression of the wild-type PKC-α produced an 18-fold increase in PKC-α activity in the basal state compared with that in control 3T3-L1 adipocytes that were infected with adenovirus recombined with the lacZ gene. Stimulation with insulin did not affect the endogenous or exogenously expressed PKC-α activity. In contrast, PMA increased both endogenous and exogenously expressed PKC-α activity by ~2.5-fold and 1.6-fold, respectively (Fig. 1B). Thus exogenous PKC-α was functionally expressed. In addition, expression of the dominant-negative mutant of PKC-α completely inhibited PKC-α activity by PMA while having no effect on endogenous PKC-α activity in the basal- and insulin-stimulated states (Fig. 1B). Thus expression of the mutant PKC-α resulted in exerting a dominant-negative effect on PKC-α activation by PMA.
To examine the effects of PKC-α activity on insulin- and PMA-induced glucose transport, we measured 2-deoxyglucose uptake in 3T3-L1 adipocytes overexpressing wild-type and dominant-negative PKC-α with or without insulin or PMA stimulation (Fig. 2). Insulin and PMA stimulated 2-deoxyglucose uptake by ~7.0- and 1.7-fold, respectively, in control (lacZ-expressing) 3T3-L1 adipocytes. Expression of the wild-type PKC-α in 3T3-L1 adipocytes produced a significant increase in glucose transport activity to ~1.9- and 2.2-fold in the basal and PMA-stimulated states, respectively. In contrast, expression of the dominant-negative mutant of PKC-α did not affect basal glucose transport activity, but a significant decrease was observed in the PMA-stimulated state. Neither the wild-type nor the dominant-negative mutant of PKC-α had an effect on insulin-stimulated glucose transport activity. These findings demonstrate that 1) PKC-α activation stimulates glucose transport activity, 2) PKC-α activation is involved in the PMA-stimulated glucose transport, although this effect is partial, and 3) PKC-α activation is not involved in the insulin-stimulated glucose transport activity.

Exogenously expressed PKC-δ, a novel PKC enzyme, in 3T3-L1 adipocytes and measured PKC-δ activity and glucose transport activity in the presence or absence of insulin or PMA. Endogenous PKC-δ expression was very low, almost undetectable, in control 3T3-L1 adipocytes, whereas the exogenous expressions of the wild-type and dominant-negative mutants of PKC-δ were clearly detected, as shown by immunoblotting with anti-PKC-δ antibody (Fig. 3A). PKC-δ activity was measured in the immunoprecipitates with anti-PKC-δ antibody (Fig. 3B). Consistent with the results of the immunoblotting study, endogenous PKC-δ activity was almost undetectable in control 3T3-L1 adipocytes in either the basal, the insulin-stimulated, or the PMA-stimulated state. Overexpression of PKC-δ induced a large amount of PKC-δ activity in 3T3-L1 adipocytes. Insulin and PMA stimulated this activity by 1.3- and 2.4-fold, respectively. In contrast, expression of the dominant-negative mutant of PKC-δ did not lead to PKC-δ activity in either the basal, insulin-stimulated, or PMA-stimulated states (Fig. 3B).

We further assayed the 2-deoxyglucose uptake in 3T3-L1 adipocytes overexpressing the wild-type or dominant-negative PKC-δ (Fig. 4). Expression of the wild-type PKC-δ induced significant increases in glucose transport activity of ~2.0-, 1.5-, and 3.2-fold in the basal, insulin-, and PMA-stimulated states, respectively, compared with that in control 3T3-L1 adipocytes. In contrast, expression of the dominant-negative mutant of PKC-δ did not affect basal, insulin-stimulated, or PMA-stimulated glucose transport activity (Fig. 4). These findings demonstrate that a novel PKC could participate in PMA-induced and insulin-induced glucose transport activation, although endogenous expression of PKC-δ is below the detection limits.

Fig. 1. Protein kinase C (PKC)-α expression levels and activities in the absence or presence of insulin or phorbol 12-myristate 13-acetate (PMA). A: expression levels of PKC-α proteins. Lysates from 3T3-L1 adipocytes (control) and from those expressing lacZ (control), wild-type (WT) PKC-α, or the dominant negative (DN) mutant of PKC-α were immunblotted with anti-PKC-α antibody. B: control 3T3-L1 adipocytes and 3T3-L1 adipocytes expressing PKC-α WT or PKC-α DN were incubated with or without 1 μM insulin or 1.6 μM PMA for 10 min. Lysates were immunoprecipitated with anti-PKC-α antibody, and PKC-α activities in the immunoprecipitates were assayed, as described in MATERIALS AND METHODS. Values presented as means ± SD of 3 separate experiments.

Fig. 2. Insulin- or PMA-stimulated 2-deoxy-D-glucose uptake in control and PKC-α WT or PKC-α DN overexpressing 3T3-L1 adipocytes. Uptake of 2-deoxy-D-glucose after incubation with or without 1 μM insulin or 1.6 μM PMA for 15 min was assayed in control 3T3-L1 adipocytes and 3T3-L1 adipocytes expressing PKC-α WT or PKC-α DN for 4 min. Data presented as means ± SD of 3 separate experiments.
Atypical PKC enzymes do not play a major role in insulin-stimulated glucose transport. Seemingly conflicting results were reported by two research groups who found that different isoforms of atypical PKC enzymes, PKC-\(\zeta\) and PKC-\(\lambda\), are mainly involved in insulin-stimulated glucose transport. To determine which isoform plays the major role in glucose transport activation by insulin, we exogenously expressed the wild type or dominant-negative mutant of PKC-\(\zeta\) or PKC-\(\lambda\) in 3T3-L1 adipocytes, followed by measurement of atypical PKC activity and glucose transport activity with or without insulin or PMA stimulation.

First, to assess whether endogenous PKC-\(\zeta/\lambda\) was activated by PMA and insulin, the endogenous enzymes were immunoprecipitated from control 3T3-L1 adipocytes with a polyclonal anti-PKC-\(\zeta/\lambda\) antibody that also reacts with the PKC-\(\lambda\) isoform. Immunoprecipitates from control 3T3-L1 adipocytes showed substantial activity above the background measured in irrelevant IgG immunoprecipitates. Immunoblotting study revealed a faint but detectable band of endogenous atypical PKCs (Figs. 5A and 6A). As expected, PMA stimulation of control 3T3-L1 adipocytes did not alter atypical PKC-\(\zeta/\lambda\) activities. In addition, contrary to previous reports, insulin stimulation did not activate the endogenous atypical PKCs (Figs. 5B and 6B).

Exogenously overexpressed wild-type or the dominant-negative mutant of PKC-\(\zeta\) or PKC-\(\lambda\) was clearly detected by immunoblotting with anti-PKC-\(\zeta\) antibody (Figs. 5A and 6A). Overexpression of atypical PKC-\(\zeta\) or PKC-\(\lambda\) resulted in increases in atypical PKC activity of ~10.4- and 4.1-fold, respectively, but atypical PKC was not activated by PMA. Insulin treatment did not significantly increase exogenously expressed atypical PKC activity.

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Exogenously overexpressed wild-type or the dominant-negative mutant of PKC-\(\zeta\) or PKC-\(\lambda\) was clearly detected by immunoblotting with anti-PKC-\(\zeta\) antibody (Figs. 5A and 6A). Overexpression of atypical PKC-\(\zeta\) or PKC-\(\lambda\) resulted in increases in atypical PKC activity of ~10.4- and 4.1-fold, respectively, but atypical PKC was not activated by PMA. Insulin treatment did not significantly increase exogenously expressed atypical PKC activity.
PKC and glucose transport

PKC activities (Figs. 5B and 6B). In addition, expression of dominant-negative mutants of PKC-ζ or PKC-λ did not significantly alter atypical PKC activity in either the basal, insulin-stimulated, or PMA-stimulated state (Figs. 5B and 6B).

2-Deoxyglucose uptake was measured in 3T3-L1 adipocytes overexpressing the wild-type or dominant-negative PKC-ζ (Fig. 7) or PKC-λ (Fig. 8) in the presence or absence of insulin or PMA. As expected, exogenous expression of these proteins did not influence PMA-stimulated glucose transport. Unexpectedly, overexpression of the wild-type PKC-ζ or PKC-λ did not increase either basal or insulin-stimulated glucose transport activity. In addition, expression of dominant-negative mutants of these atypical PKCs did not influence insulin-stimulated glucose transport activity.

Because glucose transport activity was not affected by overexpression of wild-type atypical PKC-λ, we performed additional experiments using a constitutively active mutant of PKC-λ to further increase atypical PKC activity. Expression of constitutively active PKC-λ increased atypical PKC activity to ~33-fold (Fig. 9B), which was consistent with its expression level (Fig. 9A). However, expression of constitutively active PKC did not affect either basal or insulin-stimulated glucose transport activity (Fig. 9C). Thus endogenous atypical PKC activity was not increased by insulin treatment, and no evidence indicating involvement of atypical PKC in insulin-induced signaling stimulating glucose transport was obtained in experiments focusing on expression of wild-type, dominant-negative, and constitutively active atypical PKC. These data indicate that atypical PKC enzymes do not play a major role in insulin-stimulated glucose transport.

DISCUSSION

We expressed the wild-type and dominant-negative mutants of each PKC isozyme, PKC-α as a conventional PKC isoform, PKC-δ as a novel PKC isoform, and PKC-ζ and PKC-λ representing atypical PKC isoforms. The present results show clearly that conventional PKC participates, to some extent, in PMA-stimulated but not in insulin-stimulated glucose transport activity. It was reported that stable expression of the wild-type or constitutively active forms of PKC-α, PKC-β1, and PKC-β2 failed to influence basal glucose transport in 3T3-L1 fibroblasts and adipocytes (8). However,
no detectable increase in endogenous PKC-δ activity, even in the PMA-stimulated state. In primary cultures of rat skeletal muscle, PKC-δ reportedly mediates insulin-stimulated glucose transport (10) and regulates insulin receptor activity and routing (11). In 3T3-L1 adipocytes, although no information is available as to what novel PKC isozyme(s) is expressed, our exogenously expressed PKC-δ results suggest that another novel PKC isozyme(s), if present, could respond to PMA and contribute to PMA-stimulated glucose transport activity.

Insulin treatment did not activate endogenously or exogenously expressed PKC-α, whereas insulin activated exogenously expressed PKC-δ. This is consistent with reports that novel PKC is activated downstream from PI 3-kinase (31). In accordance with these results, the average value of insulin-stimulated glucose transport activity was greater (1.4-fold) in adipocytes overexpressing PKC-δ than in control adipocytes, although the difference did not reach statistical significance in four independent experiments. These findings suggest that conventional PKC is not involved in insulin-stimulated glucose transport but that novel PKC isoforms, if expressed in 3T3-L1 adipocytes, may contribute, to a limited extent, to insulin-stimulated glucose transport activity.

Two research groups have reported atypical PKC to be involved in insulin-stimulated glucose transport in adipocytes, although the two groups proposed the involvement of different atypical PKC isoforms. Stan-daert et al. (42) reported that inhibition of PKC-ζ activity by the PKC-ζ pseudosubstrate or Ro31–8220 paralleled inhibition of insulin-stimulated glucose transport in rat adipocytes. They also reported that expression of wild-type or constitutively active PKC-ζ stimulated translocation of coexpressed GLUT4, which was tagged with myc and that expression of dominant-negative PKC-ζ partially inhibited the translocation of tagged GLUT4 in rat adipocytes. They thus suggest PKC-ζ to be a downstream effector of PI 3-kinase through insulin-stimulated GLUT4 translocation. Kotani et al. (26) reported, however, that overexpression of the constitutively active mutant of PKC-λ activated glucose transport activity in 3T3-L1 adipocytes. In addition, they showed that overexpression of the dominant-negative mutant of PKC-λ partially inhibited glucose transport activity, suggesting that PKC-λ lies in the insulin signaling pathway responsible for regulating glucose uptake. In their study, overexpression of constitutively active PKC-λ induced an ~600-fold increase in PKC-λ activity, whereas insulin stimulated endogenous PKC-λ activity by at most threefold. Despite this huge, nonphysiological increase in PKC-λ activity with overexpression of the constitutively active mutant, glucose transport activity was rather smaller than that caused by insulin in control (noninfected) 3T3-L1 adipocytes. On the other hand, in the present study, a modest increase in atypical PKC activity, close to the physiological condition, was achieved. Expression of the wild-type PKC-ζ, PKC-λ, and constitutively active PKC-λ produced increases in atypical PKC ac-

in the present study, a small increase in glucose transport activity in the basal state was observed in PKC-α-overexpressing adipocytes. The PKC enzyme was transiently expressed using an adenovirus-mediated gene transduction system, and PKC activity and glucose transport activity were measured 2 days after the transfection; i.e., the period of expression of these PKC enzymes was much shorter than that of stable expression. Some unknown mechanisms compensating for increased PKC activities may operate in cells overexpressing conventional PKC enzymes, especially in stable transfectants. The discrepancy between the previous and present results may therefore be attributable to different periods of expression.

The results obtained for exogenously expressed PKC-δ appeared to be very similar to those obtained for PKC-α. However, the activity of endogenous PKC-δ was below the detectable level because of its extremely low expression level in 3T3-L1 adipocytes. There was

Fig. 9. Effects of expression of constitutively active PKC-λ on atypical PKC activity and glucose transport activity. A: expression levels of PKC-λ proteins. Lysates from control 3T3-L1 adipocytes and from those expressing constitutively active (CA) PKC-λ were immunoblotted with anti-atypical PKC antibody. B: control 3T3-L1 adipocytes and 3T3-L1 adipocytes expressing PKC-λ CA were incubated with or without 1 μM insulin for 10 min. Lysates were immunoprecipitated with anti-atypical PKC antibody, and PKC activities in the immunoprecipitates were assayed as described in MATERIALS AND METHODS. Values presented as means ± SD of 3 separate experiments. C: uptake of 2-deoxy-D-glucose after incubation with or without 1 μM insulin or 1.6 μM PMA for 15 min was assayed in control 3T3-L1 adipocytes and 3T3-L1 adipocytes expressing PKC-λ CA for 4 min. Data presented as means ± SD of 3 separate experiments.
tivity to ~10-, 4-, and 33-fold, respectively, but these procedures did not affect glucose transport activity. Thus, under physiological conditions, an increase in atypical PKC activity is not sufficient for glucose transport activation. Furthermore, we detected no measurable activation of atypical PKC-\(\zeta\) by insulin, whereas insulin fully activated (i.e., an ~9-fold increase) glucose transport activity. It is also noteworthy that exogenously expressed wild-type PKC-\(\zeta\) was not significantly activated by insulin. On the contrary, when wild-type PKC-\(\lambda\) was overexpressed in Chinese hamster ovary cells using an adenovirus-mediated gene transduction system, PKC-\(\lambda\) was efficiently activated by insulin (data not shown), indicating that the adenovirus-mediated gene transduction system and our assay system for atypical PKC activity worked well and that insulin does not activate atypical PKC in 3T3-L1 adipocytes. Expression of dominant-negative mutants of atypical PKC enzymes did not influence insulin-stimulated glucose transport activity in the present study. In addition, we also observed that Go6983 (100 nM), which reportedly inhibits the activities of several PKC enzymes, including PKC-\(\zeta\), did not inhibit basal or insulin-stimulated glucose transport activity in 3T3-L1 adipocytes (data not shown). Taken together, these findings clearly show that activations of atypical PKC enzymes, PKC-\(\lambda\) and PKC-\(\zeta\), do not play a major role in insulin signaling through glucose transport activation under physiological conditions. Recently, similar results were also reported in L6 myocytes, i.e., insulin did not significantly stimulate either endogenous atypical PKC-\(\zeta\)/PKC-\(\lambda\) or transfected hemagglutinin epitope-tagged PKC-\(\zeta\) (44). Indeed, we observed that overexpression of constitutively active atypical PKC-\(\lambda\) in undifferentiated L6 myoblasts using recombinant adenovirus increased basal glucose transport activity without altering basal or insulin-stimulated glucose transport activity in differentiated L6 myocytes (unpublished observation). Thus atypical PKC activation by insulin might depend on ambient factors such as the differentiation state of the cells, and activation of comitant stimuli may also be important.

Regarding the mechanism whereby insulin stimulates glucose transport activity, 3-phosphoinositide-dependent protein kinase 1 (PDK1) and protein kinase B (PKB) have received considerable attention as signals downstream from PI 3-kinase activation. Several studies using the dominant-negative mutants of PKB were reported, but the effects on insulin-stimulated glucose transport and GLUT4 translocation were different among these mutants, such as the kinase-inactive mutant (14), the phosphorylation-deficient mutant (24), and the mutant having both mutations (44). It was recently reported that PDK1, which phosphorylates PKB and leads to its activation (3), also activates serine/threonine kinases, including atypical PKC (28), p70 (4, 40) and p90 (20) S6 kinase, cAMP-dependent protein kinase (13), and serum and glucocorticoid-inducible kinase (38). It is possible that each PKB mutant binds to PDK1 and affects PDK1 to activate these pathways in a different manner. Similarly, stable and long-term expression and extremely high levels of expression of atypical PKC or its mutants may also induce a feedback effect on upstream kinases, such as PDK1. The discrepancies among several reports may have arisen from the difference in periods and levels of expression of exogenous proteins. In the present study, by use of the adenovirus gene transduction system, transient physiological expression was achieved. Taken together, our results strongly suggest that atypical PKC isoforms do not play a major role in insulin-stimulated glucose transport under physiological conditions in 3T3-L1 adipocytes.

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