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PKC-ζ Mediates Insulin Effects on Glucose Transport in Cultured Preadipocyte-Derived Human Adipocytes

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Insulin-stimulated glucose transport is impaired in the early phases of type 2 diabetes mellitus. Studies in rodent cells suggest that atypical PKC (aPKC) isoforms (ζ, λ, and ι) and PKB, and their upstream activators, PI3K and 3-phosphoinositide-dependent protein kinase-1 (PDK-1), play important roles in insulin-stimulated glucose transport. However, there is no information on requirements for aPKCs, PKB, or PDK-1 during insulin action in human cell types. Presently, by using preadipocyte-derived adipocytes, we were able to employ adenoviral gene transfer methods to critically examine these requirements in a human cell type. These adipocytes were found to contain PKC-ζ, rather than PKC-λ/ι, as their major aPKC. Expression of kinase-inactive forms of PDK-1, PKC-ζ, and PKC-λ (which functions interchangeably with PKC-ζ) as well as chemical inhibitors of PI 3-kinase and PKC-ζ/λ, wortmannin and the cell-permeable myristoylated PKC-ζ pseudosubstrate, respectively, effectively inhibited insulin-stimulated glucose transport. In contrast, expression of a kinase-inactive, activation-resistant, triple alanine mutant form of PKB-α had little or no effect, and expression of wild-type and constitutively active PKC-ζ or PKC-λ increased glucose transport. Our findings provide convincing evidence that aPKCs and upstream activators, PI 3-kinase and PDK-1, play important roles in insulin-stimulated glucose transport in preadipocyte-derived human adipocytes. (*J Clin Endocrinol Metab* 87: 716–723, 2002)

TRANSPORT ACROSS THE plasma membrane is the initial and rate-limiting step for glucose disposal in muscle and adipose tissue, and the activation of this transport process by insulin is defective in type 2 diabetes mellitus and obesity. Insulin stimulates glucose transport by increasing the translocation of GLUT4 glucose transporters to the plasma membrane by a mechanism that is postulated to sequentially require PI3K activation, increases in D3-PO_4 polyphosphoinositides (in particular, PI-3,4,5-$(PO_4)_3$) and subsequent action of 3-phosphoinositide-dependent protein kinase-1 (PDK-1), and activation of atypical PKCs (aPKC-ζ, -λ, and/or -ι) (1–7) and/or PKB (PKB-α and -β or Akt1 and -2) (8–11). In support of a requirement for aPKCs, plasmid-mediated stable expression of kinase-inactive PKC-ζ in 3T3/L1 adipocytes (1) and L6 myotubes (5), plasmid-mediated transient expression of kinase-inactive PKC-ζ and PKC-λ in rat adipocytes (3), and adenovirus-mediated expression of kinase-inactive PKC-λ in 3T3/L1 adipocytes (4) and L6 myotubes (7) have been found to inhibit insulin-stimulated GLUT4 translocation and/or glucose transport in these cell types. In support of a requirement for PKB, plasmid-mediated expression of a kinase-inactive form of PKB-α that is also activation resistant by virtue of additional alanine substitutions at Thr^{308} and Ser^{473} phosphorylation sites, a so-called triple alanine, or AAA, mutant, has been reported to largely inhibit insulin-stimulated epitope-tagged GLUT4 translocation in L6 myoblasts (10). Microinjection of antibodies has also provided evidence for requirements for aPKC-λ (12) and PKB-β (11) in 3T3/L1 adipocytes. On the other hand, plasmid-mediated transient expression of a kinase-inactive form of PKB-α had only a modest 20% inhibitory effect on insulin-stimulated epitope-tagged GLUT4 translocation in rat adipocytes (13), and adenovirus-mediated expression of an activation-resistant, double alanine mutant (T308A/S473A) form of PKB-α had no significant effect on insulin-stimulated glucose transport and GLUT4 translocation in 3T3L1 adipocytes despite inhibiting the activation of both PKB-α and PKB-β (14).

In contrast to rat- and mouse-derived cultured L6 and 3T3/L1 cells, which contain primarily PKC-ζ (7) and PKC-λ (4), respectively, there are no reported studies of expression of dominant negative mutants to examine requirements for aPKCs during insulin-stimulated glucose transport in human cells, which reportedly contain PKC-ι, an isoform that is 98% identical to PKC-λ (15), and, as shown here, PKC-ζ. Similarly, there are no reported studies of expression of dominant negative mutants to examine requirements for PDK-1 and PKB during insulin-stimulated glucose transport in human cells. However, such expression studies are possible only with cells that are suitable for plasmid transfections or viral infections. In this regard, unlike 3T3/L1 adipocytes and L6 myotubes, we have not been able to use the more definitive adenoviral gene transfer methods for studies of insulin-stimulated glucose transport in freshly prepared rodent adipocytes (in our experience, these adenovirus-infected fresh adipocytes are "leaky"), and although not yet reported, the same problem is likely to be encountered with freshly prepared human adipocytes. Also, we have found it difficult to

Abbreviations: AAA, Triple alanine; aPKC, atypical PKC; KRP, Krebs-Ringer phosphate; MOI, multiplicity of infection; PDK-1, 3-phosphoinositide-dependent protein kinase-1.

satisfactorily express proteins with at least certain plasmid/transfection methods in human adipocytes. To circumvent these problems, we presently found that we could satisfactorily use adenoviral gene transfer methods in primary cultures of human adipocytes that had been prepared by differentiation of preadipocytes obtained during liposuction procedures in healthy nonobese women (16, 17). With this approach, the expression of kinase-inactive forms of PDK-1, PKC-ζ, and PKC-λ each largely inhibited insulin-stimulated glucose transport as well as aPKC activation in these cultured human adipocytes; in contrast, the expression of the kinase-inactive, activation-resistant, triple alanine mutant form of PKB-α (*i.e.* PKB-α-AAA) had little or no effect on insulin-stimulated glucose transport in these cells despite inhibiting the activation of PKB-α and PKB-β.

Subjects and Methods

As described previously (16, 17), in each study sc adipose tissue was obtained by the Zen-Bio Corp. from a group of approximately six healthy, nondiabetic, nonobese (body mass index, ~25) women (mean age, 35–38 yr) undergoing elective cosmetic liposuction procedures. After digestion with collagenase, preadipocytes were harvested, cultured to confluence in either T-75 flasks for PKC/PKB enzyme activation studies or 24-well plates for glucose transport studies, passaged up to, but not more than, five times, and finally differentiated to mature lipid-laden adipocytes by incubation in DMEM/F-10 medium (1:1) with 3% FBS (Sigma, St. Louis, MO), insulin (Life Technologies, Inc., Gaithersburg, MD), isobutylmethylxanthine (Sigma), and dexamethasone (Sigma), using methods comparable to those used for differentiation of mouse-derived 3T3/L1 adipocytes from 3T3/L1 fibroblasts. As assessed histologically, greater than 80% differentiation to adipocytes was achieved (16, 17). Institutional review board approval and informed consent for use of the adipose tissue were given, and adipocytes were obtained after liposuction were obtained by Zen-Bio, Inc.

After differentiation, insulin, isobutylmethylxanthine, and dexamethasone were removed, and adipocytes were incubated in DMEM/F-10 medium containing 3% FBS and infected with adenovirus alone or adenovirus encoding wild-type, constitutively active, or kinase-inactive forms of PKC-λ, PKC-ζ, or PDK-1 or a kinase-inactive, activation-resistant, triple alanine mutant form of PKB-α (PKB-α-AAA), as previously described (4, 7, 14). After 48 h to allow time for expression, the medium was removed, and cells were incubated for 3–4 h in serum-free DMEM containing 4% BSA (Sigma), equilibrated for 30 min in glucose-free Krebs-Ringer phosphate (KRP) medium containing 1% BSA, and then incubated with or without 50 nM insulin and other agents, as described in the text. After incubation, as previously described (1–3, 5–7), cell lysates were examined for immunoprecipitable aPKC, PKB-α, or PKB-β activity; uptake of [^{3}H]2-deoxyglucose; or levels of immunoreactive proteins.

In addition to human adipocytes, we conducted comparable adenoviral gene transfer studies in cultured 3T3/L1 adipocytes and L6 myotubes using methods previously described (4, 7, 14). As described in the text, these studies served as starting points and provided important information for conducting essentially comparable studies in more difficult to obtain human adipocytes.

Adenoviruses encoding wild-type, constitutively active, and kinase-inactive forms of PKC-λ have been described previously (4) and were provided by Dr. Masato Kasuga. Note that the constitutively active PKC-λ is truncated and missing the first 135 amino acids that contain the autoinhibitory pseudosubstrate sequence, and therefore migrates faster on SDS-PAGE. Adenoviruses encoding wild-type and/or kinase-inactive forms of PKC-ζ and PDK-1, were constructed using plasmid cDNA inserts described previously (1–3, 5–7) and Adeno-X Expression kits obtained from CLONTECH Laboratories, Inc. (Palo Alto, CA). Adenovirus encoding kinase-inactive, activation-resistant PKB-α-AAA (K179A/T308A/S473A) was constructed with a plasmid described previously (18). All adenoviral cDNA inserts were sequenced to verify the continued presence of their mutations.

Other materials used included cell-permeable myristoylated PKC-ζ pseudosubstrate (Biosource Technologies, Inc., Camarillo, CA), wortmannin (Sigma), rabbit polyclonal anti-C-terminal PKC-ζ/λ/ι antiserum (Santa Cruz Biotechnology, Inc., Santa Cruz, CA; used for PKC-ζ/λ/ι enzyme assays; note that PKC-ζ, -λ, and -ι have nearly identical C-termini that are recognized by this antiserum), mouse monoclonal anti-PKC-λ antibodies that recognize both PKC-λ and PKC-ι (Transduction Laboratories, Inc., Lexington, KY; used for Western analyses), rabbit polyclonal isoform-specific anti-N-terminal PKC-ζ antiserum (supplied by Dr. Todd Sacktor; used for Western analyses), sheep polyclonal anti-PKB-α and anti-PKB-β antisera (Upstate Biotechnology, Inc., Lake Placid, NY; used for PKB-α and PKB-β enzyme assays), rabbit polyclonal anti-GLUT1 antiserum (provided by Dr. Ian Simpson), and mouse monoclonal anti-GLUT4 antibodies (Biogenesis, Bournemouth, UK).

Results

Studies in 3T3/L1 adipocytes and L6 myotubes

For practical reasons of availability and expense, we initially used cultured rodent cells to characterize the actions of adenoviral constructs. The effects of adenoviral transfer of cDNAs encoding wild-type, constitutively acting, and kinase-inactive forms of PKC-λ on insulin-stimulated aPKC activity and glucose transport in mouse-derived 3T3/L1 adipocytes (4) and rat-derived L6 myotubes (7) have been described previously. Presently, we observed similar effects of adenovirally transferred wild-type and kinase-inactive forms of PKC-ζ in 3T3/L1 adipocytes and L6 myotubes. As shown in Fig. 1, expression of kinase-inactive PKC-ζ inhibited insulin-stimulated, but not basal, 2-deoxyglucose uptake in both L6 myotubes and 3T3/L1 adipocytes (note that virus alone had little or no effect). Inhibitory effects on insulin-stimulated glucose transport were near maximal at 10 multiplicity of infection (MOI; or viral plaque-forming units per cell) kinase-inactive PKC-ζ in both cell types, and this concentration of adenovirus encoding kinase-inactive PKC-ζ was similar to that found to be maximally or near maximally effective in studies of expression of adenovirally transferred kinase-inactive PKC-λ in these cells (4, 7) (Bandyopadhyay, G., M. P. Sajan, Y. Kanoh, M. L. Standaert, and R. V. Farese, unpublished observations). Moreover, 10 MOI kinase-inactive PKC-ζ and 10 MOI kinase-inactive PKC-λ effectively inhibited insulin- induced activation of aPKCs in both cell types (7) (Fig. 2 and Bandyopadhyay, G., M. P. Sajan, Y. Kanoh, M. L. Standaert, and R. V. Farese, unpublished observations). Accordingly, this viral concentration was used, and it proved to be effective in studies of human adipocytes, as described below.

In contrast to findings with kinase-inactive PKC-ζ, expression of wild-type PKC-ζ provoked increases in basal 2-deoxyglucose uptake, presumably reflecting enzyme concentration-dependent autoactivation of PKC-ζ/λ seen with overexpression of wild-type PKC-ζ (1–3, 5–7). Further increases in glucose uptake were observed upon the addition of insulin to incubations of 3T3/L1 adipocytes and, to a lesser extent, L6 myotubes (Fig. 1), most likely reflecting added insulin-induced increases in PKC-ζ/λ activity (1–3, 5–7).

Note that rat-derived L6 myotubes contain primarily PKC-ζ (7), and mouse-derived 3T3/L1 adipocytes contain primarily PKC-λ (4). Accordingly, the fact that kinase-inactive forms of both PKC-ζ (present results) and PKC-λ (4, 7) inhibit insulin-stimulated aPKC enzyme activity and glucose transport in both of these cell types is in keeping with our

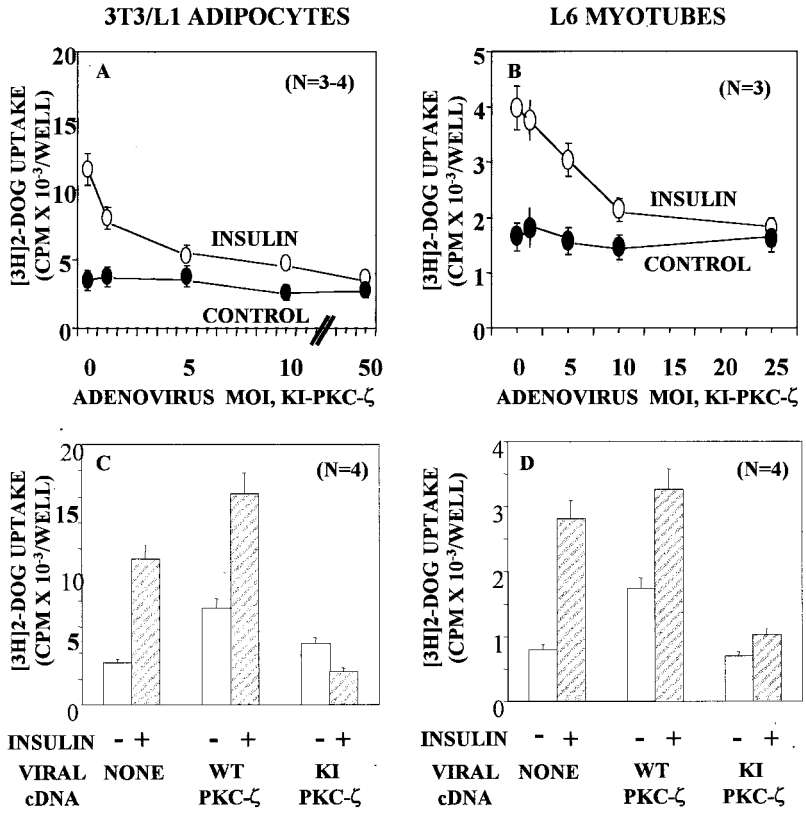

FIG. 1. Effects of adenovirus alone and adenovirus encoding wild-type (WT) and kinase-inactive (KI) PKC-ζ on basal and insulin-stimulated 2-deoxyglucose uptake in 3T3/L1 adipocytes (*left*) and L6 myotubes (*right*). Cells were infected with increasing amounts (as MOI or plaque-forming units per cell) of adenovirus in the *upper panels* (A and B) and 10 MOI adenovirus in the *lower panels* (C and D). After 48 h to allow time for expression, cells were incubated in glucose-free KRP medium with or without 100 nM insulin for 30 min, after which, uptake of [^{3}H]2-deoxyglucose (DOG) was measured over 5 min. Values are the mean ± SE of (n) determinations.

previous findings suggesting that these aPKCs can function interchangeably for insulin-stimulated glucose transport (6), most likely because of homology in functionally important domains of these aPKCs.

As with kinase-inactive PKC-ζ, adenovirus-mediated expression of kinase-inactive PDK-1, at a virus concentration of 10 MOI, effectively inhibited insulin-induced activation of both aPKCs and glucose transport in both 3T3/L1 adipocytes and L6 myotubes (Fig. 2). As expected, this concentration of kinase-inactive PDK-1 also inhibited PKB activation (Fig. 2).

In contrast to kinase-inactive forms of aPKCs and PDK-1, adenovirus-mediated expression of kinase-inactive, activation-resistant PKB-α-AAA had only a small (~20–25% at most), if any (presently not significant), effect on insulin-stimulated glucose transport in both 3T3/L1 adipocytes and L6 myotubes (Fig. 2), despite inhibiting insulin-induced activation of PKB-α (Fig. 2). Note that a relatively high concentration of 150 MOI adenovirus expressing PKB-α was needed to effectively inhibit PKB-α activation (Fig. 2), and this concentration of virus also inhibited the activation of PKB-β as well as PKB-α (data not shown). Also note that 1) virus-mediated PKB-α expression did not inhibit PKC-ζ activation by insulin (data not shown, but this may also be surmised from the lack of effect of PKB-α-AAA on insulin-stimulated glucose transport), and likewise, virus-mediated expression of aPKC does not inhibit the activation of PKB by insulin (4, 7).

Studies in preadipocyte-derived human adipocytes

Similar to findings in 3T3/L1 adipocytes and L6 myotubes, insulin provoked acute increases in the activity of total immunoprecipitable aPKC in cultured human adipocytes, and these increases were largely inhibited by expression of kinase-inactive PKC-ζ, kinase-inactive PKC-λ, and kinase-inactive PDK-1 (Fig. 3).

Note that we were able to detect the presence of endogenous PKC-ζ, but no significant endogenous PKC-λ/ι, in cultured human adipocytes (Fig. 4). On the other hand, immunoreactive PKC-λ was readily detected in adipocytes that had been infected with adenovirus expressing wild-type, constitutively acting, and kinase-inactive PKC-λ (Fig. 4). Accordingly, it may be surmised that PKC-ζ is the major aPKC in cultured human adipocytes.

Also, similar to findings in 3T3/L1 adipocytes and L6 myotubes, we found in cultured human adipocytes that 1) as expected, 100 nM wortmannin completely blocked insulin-stimulated 2-deoxyglucose uptake (data not shown), indicating dependence on PI 3-kinase; 2) adenovirally mediated expression of wild-type forms of both PKC-λ and PKC-ζ provoked increases in basal 2-deoxyglucose uptake (Fig. 5); 3) constitutively active PKC-λ provoked insulin-like effects on 2-deoxyglucose uptake (Fig. 5); and 4) kinase-inactive forms of both PKC-λ and PKC-ζ inhibited insulin-stimulated 2-deoxyglucose uptake (Figs. 5 and 6).

The inhibitory effects of kinase-inactive PKC-ζ on insulin-stimulated glucose transport in cultured human adipocytes could not be explained by alterations in the levels of GLUT4 or GLUT1. In fact, similar to findings in L6 myotubes (7), adenovirally mediated expression of kinase-inactive PKC-ζ, if anything, led to increases in GLUT4 levels in cultured human adipocytes (Fig. 5). As discussed previously, this may

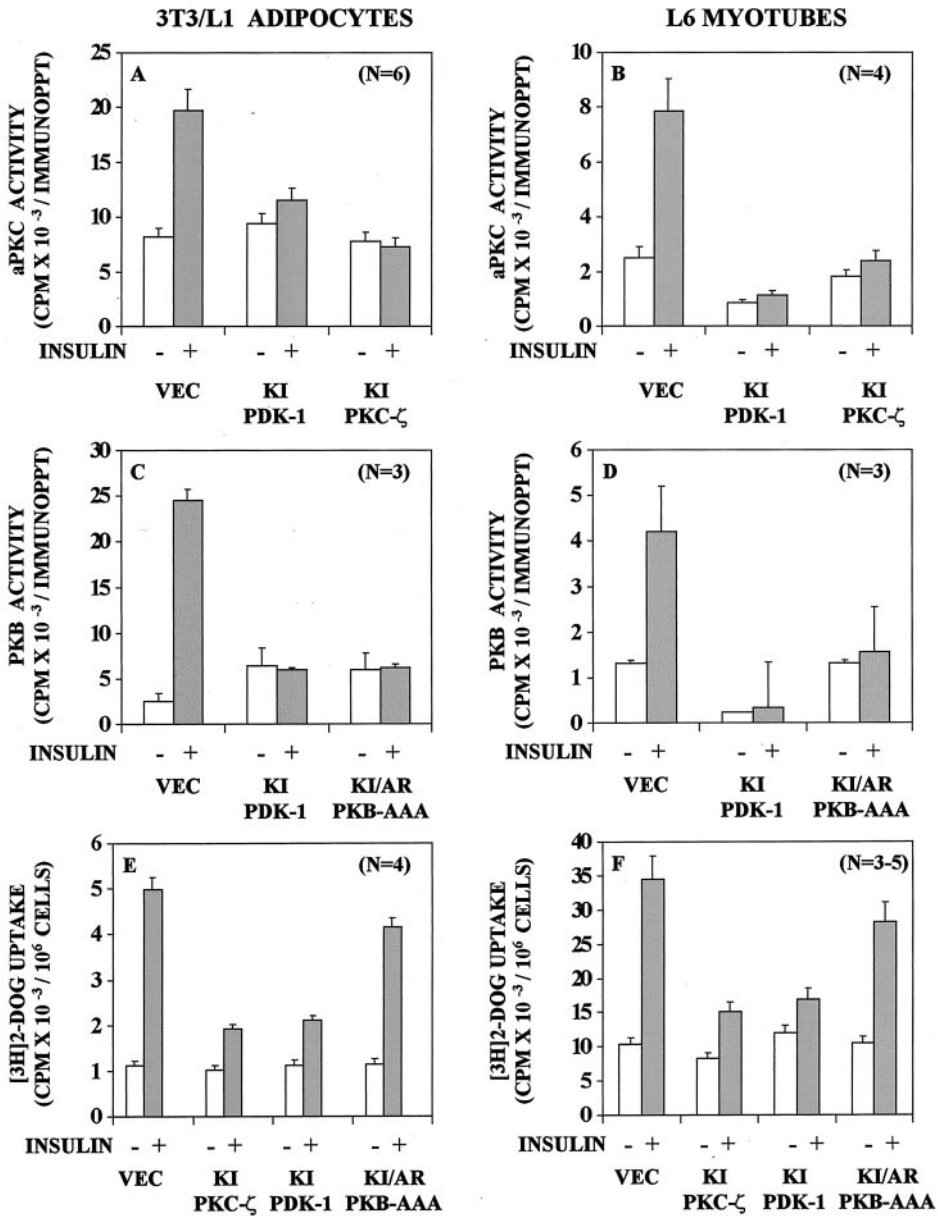

FIG. 2. Effects of adenovirally mediated expression of kinase-inactive (KI) PKC-ζ, KI-PDK-1, and KI/activation-resistant (KI/AR) PKB-α-AAA on insulin-induced increases in activity of immunoprecipitable aPKC (A and B) and PKB-α (C and D) and in glucose transport (E and F) in 3T3/L1 adipocytes (*left*, A, C, and E) and L6 myotubes (*right*, B, D, and F). Cells were infected with 10 MOI adenovirus encoding KI-PKC-ζ or KI-PDK-1 or with 150 MOI adenovirus encoding KI/AR PKB-α-AAA. After 48 h, adipocytes were incubated in glucose-free KRP medium with or without 100 nM insulin for 10 min in enzyme activation studies or for 30 min in glucose transport studies, after which cell lysates were assayed for immunoprecipitable aPKC or PKB-α activity, or [^{3}H]2-deoxyglucose (DOG) uptake into intact cells was measured over 5 min, as described in *Subjects and Methods*. Values are the mean ± SE of (n) determinations.

be a compensatory mechanism for restoring glucose transport in cells expressing kinase-inactive PKC-ζ.

Complementing the adenoviral gene transfer studies, the aPKC inhibitor, the cell-permeable myristoylated PKC-ζ pseudosubstrate, inhibited insulin-stimulated glucose transport in cultured human adipocytes (Fig. 5). It may be noted that this pseudosubstrate inhibits aPKCs, but not PKB (3, 18).

In contrast to kinase-inactive PKC-ζ and kinase-inactive PKC-λ, kinase-inactive, activation-resistant PKB-α-AAA had only a relatively small (~20–25% at most), if any, effect on insulin-stimulated glucose transport in cultured human adipocytes (Fig. 6) despite effectively inhibiting the activation of total cellular PKB-α (Fig. 3).

In addition to kinase-inactive forms of PKC-ζ and PKC-λ, kinase-inactive PDK-1 inhibited insulin-stimulated glucose transport in human adipocytes (Fig. 6).

Discussion

The present findings provided the first evidence in a human cell type that 1) insulin activates aPKCs; 2) this activation of aPKCs by insulin is dependent on PI 3-kinase and PDK-1; and 3), most importantly, aPKCs and PDK-1 are required for insulin-stimulated glucose transport. Except for differences in the abundance of specific aPKC isoforms, the present findings on aPKC activation and its requirement for insulin-stimulated glucose transport in cultured human adipocytes are similar to findings in 3T3/L1 adipocytes (2, 4), rat adipocytes (1–3, 5, 6, 19, 20), and L6 myotubes (5, 7). On the other hand, note that this is the first report in which adenoviruses were used to transfer cDNAs encoding PKC-ζ, PDK-1, and the triple alanine mutant form of PKB into any cell type. Also note that although we have documented that

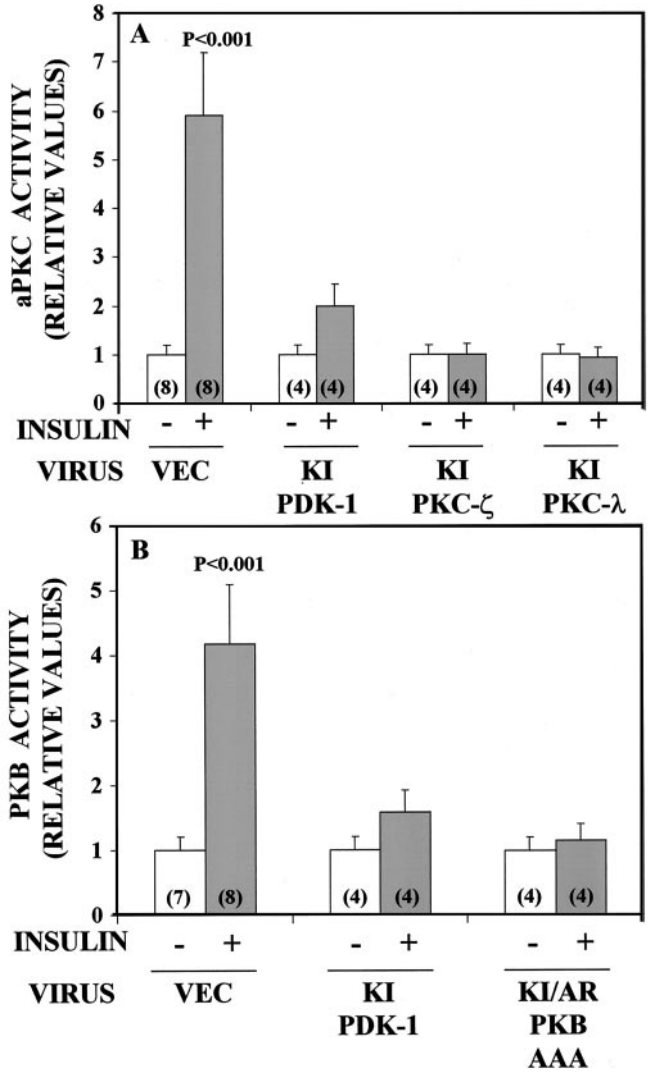

FIG. 3. Effects of adenovirus alone (VEC) and adenovirus encoding kinase-inactive (KI) PKC-ζ, KI-PKC-λ, KI-PDK-1, or KI/activation-resistant (KI/AR) PKB-α-AAA triple alanine mutant on basal and insulin-stimulated aPKC (A) or PKB-α (B) enzyme activity in cultured human adipocytes. Cells were infected with 10 MOI adenovirus encoding KI-PKC-ζ, KI-PKC-λ, or KI-PDK-1, or 150 MOI adenovirus encoding KI/AR PKB-α-AAA. After 48 h to allow time for expression (see Fig. 4 where equal amounts of cell lysates were resolved by SDS-PAGE and blotted with indicated antibodies), cells were incubated in glucose-free KRP medium with or without 50 nM insulin for 10 min, after which cell lysates were subjected to immunoprecipitation and kinase assays. Values are the mean ± SE of (n) determinations.

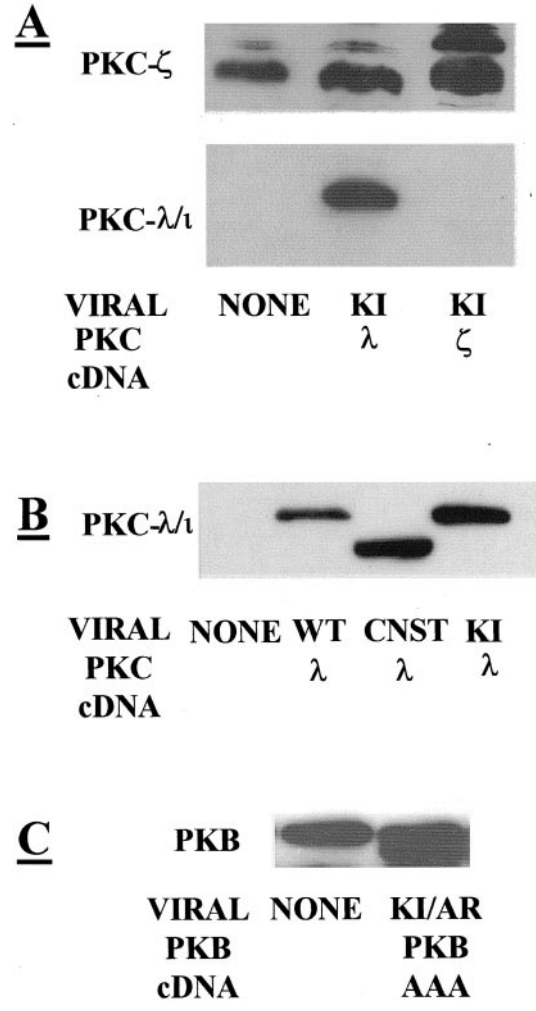

FIG. 4. Adenovirus-mediated expression of kinase-inactive (KI) PKC-ζ, KI-PKC-λ, and KI/activation-resistant (KI/AR) PKB-α-AAA in cultured human adipocytes. Cells were infected with adenoviruses encoding KI-PKC-ζ (10 MOI), KI-PKC-λ (10 MOI), wild-type (WT) PKC-λ (10 MOI), constitutively active (CNST) PKC-λ (10 MOI) or KI/AR PKB-α-AAA (150 MOI) 48 h before harvesting, lysate preparation, and Western analyses, using isoform-specific rabbit polyclonal anti-N-terminal-PKC-ζ antiserum, isoform-specific mouse monoclonal anti-PKC-λ/ι antibodies, and sheep polyclonal anti-PKB-α antiserum. Note that, as expected, the truncated form of expressed constitutively active PKC-λ migrates faster than 75-kDa expressed wild-type (WT) and kinase-inactive PKC-λ. Also note that there is little or no endogenous PKC-λ/ι, PKC-ζ migrates as a doublet at 75 and 80 kDa, and, although not shown, a weakly reactive band of immunoreactive PKBβ could also be discerned in human adipocytes.

insulin rapidly activates aPKCs in human skeletal muscles during hyperinsulinemic-euglycemic clamp studies (unpublished observations), similar to that observed in rodent skeletal muscles *in vivo* (21), it has not been possible to use adenoviral gene transfer methods to examine requirements for aPKCs during insulin-stimulated glucose transport in human or, for that matter, rat skeletal muscles. Nevertheless, it seems reasonable to suggest that findings in adenoviral gene transfer studies in cultured preadipocyte-derived human adipocytes will also be applicable to human skeletal muscles.

The present findings support our previous suggestion (6, 7) that the aPKCs can function interchangeably during insulin-stimulated glucose transport in a variety of cell types. Accordingly, we presently found that kinase-inactive forms of both PKC-ζ, [which is approximately 70% homologous to PKC-λ (22) and PKC-ι (15)] and PKC-λ [which is 98% homologous to PKC-ι (15, 22)] are capable of largely or fully inhibiting the effects of insulin on 2-deoxyglucose uptake in cultured human adipocytes, which contain primarily PKC-ζ. This interchangeability most likely reflects the fact that these PKCs share a high degree of homology in functionally important domains, including the autoinhibitory pseudosub-

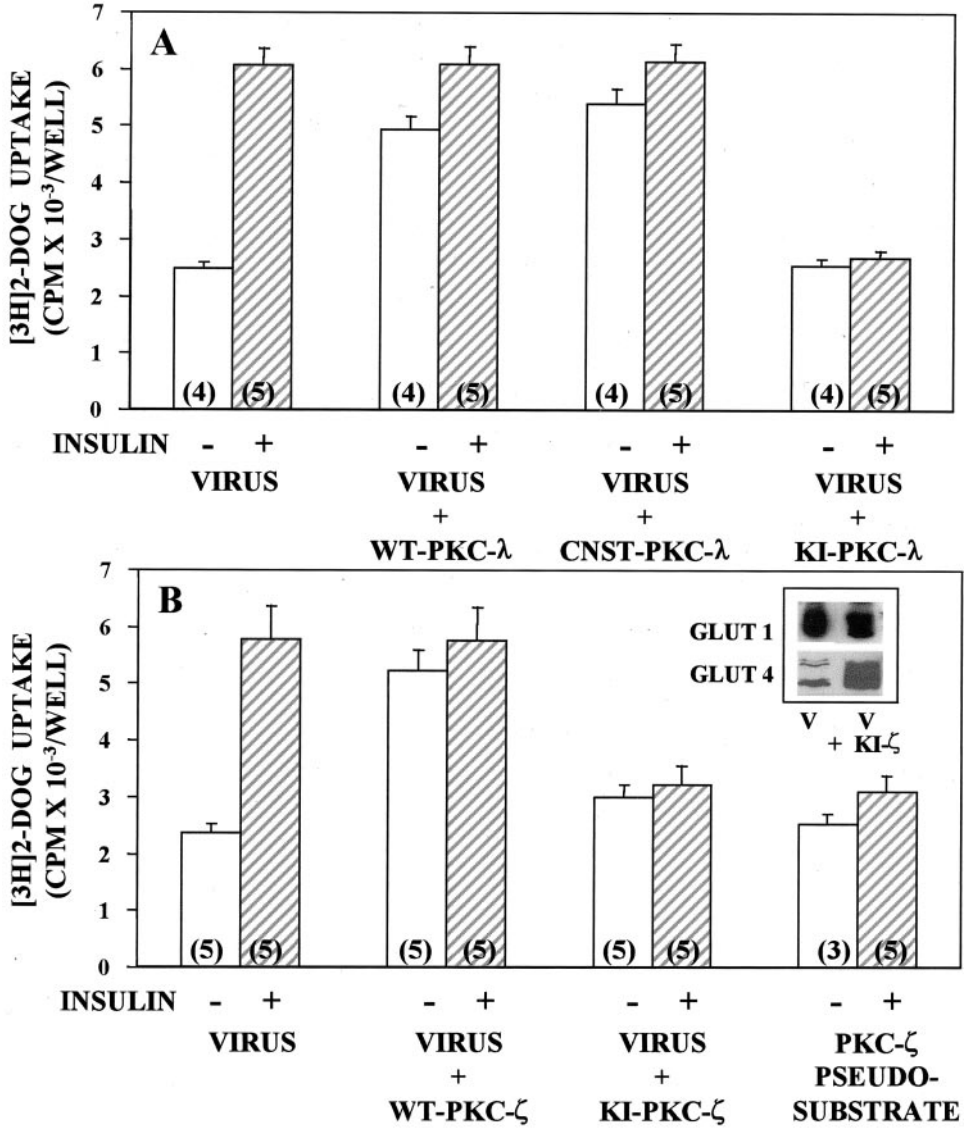

FIG. 5. Effects of adenovirus alone (VIRUS) and adenovirus encoding wild-type (WT), constitutively active (CNST), and kinase-inactive (KI) PKC-λ (A) or PKC-ζ (B) on basal and insulin-stimulated 2-deoxyglucose uptake in cultured human adipocytes. Cells were infected with 10 MOI of the indicated adenovirus, and after 48 h to allow time for expression, cells were incubated in glucose-free KRP medium with or without 50 nM insulin for 30 min, following which, uptake of [^{3}H]2-deoxyglucose was measured over 5 min. The *inset* in B shows levels of immunoreactive GLUT1 and GLUT4 in cells infected with adenovirus alone (V) and or adenovirus encoding KI-PKC-ζ (KI-ζ). B also shows effects of the cell-permeable PKC-ζ pseudosubstrate (50 μM) on basal and insulin-stimulated [^{3}H]2-deoxyglucose uptake in cultured human adipocytes. Values are the mean ± SE of (n) determinations.

strate sequence, and stimulatory threonine activation loop phosphorylation and autophosphorylation sites, all of which are responsive to increases in PI3K-dependent PI-3,4,5-$(PO_4)_3$ and enhanced action of PDK-1 (20). This interchangeability also suggests that each of these aPKCs is capable of phosphorylating a common substrate(s) that is required for insulin-stimulated glucose transport. Given the physiological importance of insulin-stimulated glucose transport throughout mammalian species, it is perhaps not surprising that the functional aspects of the aPKCs have survived through the evolutionary process.

That we were able to infect GLUT4-containing cultured human adipocytes with an adenovirus insert-free vector without significantly altering basal or insulin-stimulated glucose transport is noteworthy. In this respect, the presently used cultured human adipocyte system is similar to cultures of 3T3/L1L1 adipocytes and L6 myotubes, but different from freshly prepared rat adipocytes, which in our hands have not been useful for adenovirus-mediated gene transfer studies. Accordingly, the presently used cultured human adipocyte system proved to be particularly useful for applying adenoviral gene transfer methods to express signaling factors that are postulated to be important during insulin stimulation of glucose transport. Obviously, it is of utmost importance to be able to use such state of the art adenoviral gene transfer methods to critically evaluate the mechanism that insulin uses to control the initial rate-limiting step for glucose utilization in human cells, which is defective in type 2 diabetes mellitus.

It was surprising to find substantial amounts of PKC-ζ, but little or no immunoreactive PKC-λ/ι (PKC-λ and PKC-ι are 98% homologous and are similarly recognized by the presently used mouse monoclonal anti-PKC-λ antibody) in cultured human adipocytes. Accordingly, the present findings are likely to be relevant to human skeletal muscles, in which PKC-ζ similarly appears to be the major aPKC (Beeson, M., G. Bandyopadhyay, M. P. Sajan, Y. Kanoh, M. L. Standaert, and R. V. Farese, unpublished observations).

Finally, we were able to observe only a relatively small, if any, inhibitory effect of kinase-inactive, activation-resistant

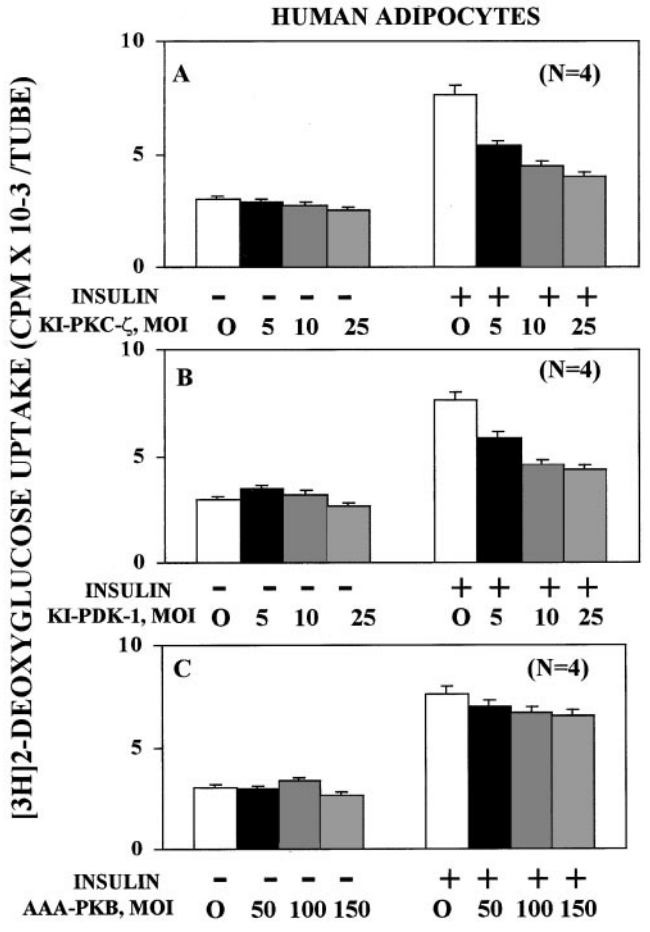

FIG. 6. Effects of increasing amounts of adenovirus encoding kinase-inactive (KI) PKC-ζ (A), KI-PDK-1 (B) or KI/activation/resistant (KI/AR) PKB-α-AAA (C) on basal (*left*) and insulin-stimulated (*right*) [^{3}H]2-deoxyglucose uptake in cultured human adipocytes. Cells were infected with the indicated MOI of adenovirus, and after 48 h to allow time for expression, cells were incubated in glucose-free KRP medium for 30 min with or without 50 nM insulin, following which, uptake of [^{3}H]2-deoxyglucose was measured over 5 min. Values are the mean ± SE of (n) determinations.

PKB-α-AAA on insulin-stimulated glucose transport in cultured human adipocytes. In this respect note that in addition to PKB-α, we were able to document the presence of at least some immunoreactive PKB-β in cultured human adipocytes. However, our findings in 3T3/L1 adipocytes suggested that the kinase-inactive activation-resistant PKB-α-AAA mutant inhibited endogenous PKB-β, as well as PKB-α. Also note that a plasmid expressing an identical or similar kinase-inactive activation-resistant mutant form of PKB-α-AAA was used to demonstrate a requirement for PKB during insulin-stimulated translocation of epitope-tagged GLUT4 in L6 myoblasts (10).

In summary, we found that insulin rapidly activated aPKCs in cultured preadipocyte-derived human adipocytes, and using adenoviral gene transfer methods, expression of wild-type and constitutively active PKC-ζ and PKC-λ increased glucose transport, whereas expression of kinase-inactive forms of PKC-ζ, PKC-λ, and PDK-1, but not kinase-inactive, activation-resistant PKB-α-AAA, markedly inhibited insulin-stimulated glucose transport in these cells. We conclude that PDK-1 and PKC-ζ are required for and contribute importantly to insulin stimulated-glucose transport in cultured preadipocyte-derived human adipocytes. However, as a caveat, it is possible that some of our findings in the presently used preadipocyte-derived adipocytes may not be applicable to mature human adipocytes.

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