Recent data have implicated the serine/threonine protein kinase Akt/protein kinase B (PKB) in a diverse array of physiological pathways, raising the question of how biological specificity is maintained. Partial clarification derived from the observation that mice deficient in either of the two isoforms, Akt1/PKBα or Akt2/PKBβ, demonstrate distinct abnormalities, i.e. reduced organismal size or insulin resistance, respectively. However, the question still persists as to whether these divergent phenotypes are due exclusively to tissue-specific differences in isoform expression or distinct capacities for signaling intrinsic to the two proteins. Here we show that Akt2/PKBβ+/− adipocytes derived from immortalized mouse embryo fibroblasts display significantly reduced insulin-stimulated hexose uptake, clearly established mouse embryo fibroblasts display significantly reduced organ-

ismal size or insulin resistance, respectively. Moreover, in adipocytes differentiated from primary fibroblasts or immortalized mouse embryo fibroblasts, and brown preadipocytes the absence of Akt2/PKBβ resulted in reduction of insulin- induced hexose uptake and glucose transporter 4 (GLUT4) translocation, whereas Akt1/PKBα was dispensable for this effect. Most importantly, hexose uptake and GLUT4 translocation were completely restored after re-expression of Akt2/PKBβ in Akt2/PKBβ+/− adipocytes, but overexpression of Akt1/PKBα at comparable levels was ineffective at rescuing insulin action to normal. These results show that the Akt1/PKBα and Akt2/ PKBβ isoforms are uniquely adapted to preferentially transmit distinct biological signals, and this property is likely to contribute significantly to the ability of Akt/ PKB to play a role in diverse processes.

A critical problem in signal transduction research is understanding how a limited number of protein kinases or other signaling molecules can couple to a vast array of physiological actions and yet retain specificity. For example, the serine/threonine protein kinase Akt/PKB1 has been implicated in anti-apoptosis, and angiogenesis, yet agonists that activate Akt/PKB are capable of regulating single responses in a time- and cell-specific manner (1). Although tissue-specific expression of protein kinases might contribute to specificity, the virtually ubiquitous distribution of many critical signaling molecules including Akt/PKB makes this an unlikely general mechanism. The existence of gene families has been often invoked as a strategy to confer specificity in signaling, but in fact there are few examples of well defined differences in the signaling capabilities of homologous isoforms. The Akt/PKB sub-family consists of three closely related species, Akt1/PKBα, Akt2/PKBβ, and Akt3/PKBγ (2). All three gene products are similar in structure and size and are regulated by similar mechanisms involving the 3'-phosphoinositide-dependent stimulation (1). However, no differences in substrate specificity have been established, and there are few convincing examples in which unique downstream pathways are modulated by the three enzymes (3).

Akt/PKB has been strongly implicated in the insulin-promoted storage of nutrients in muscle and adipose tissue. An impairment in insulin ability to maintain normal glucose homeostasis, a condition termed insulin resistance, predisposes to the development of type 2 diabetes, hypertension, and cardiovascular disease (4). Some of the strongest data suggest differences in the roles of Akt/PKB isoforms in these processes. For example, the levels of Akt2/PKBβ increase during the differentiation of cells into insulin-responsive adipocytes, whereas the expression of Akt1/PKBα declines, consistent with a role for Akt2/PKBβ in adipocyte cell function (5, 6). Akt1/PKBα exists primarily in the cytosolic fraction, whereas significant amounts of Akt2/PKBβ reside in a membrane fraction that also contains the intracellular compartment housing the insulin-responsive GLUT4 glucose transporter (7). Furthermore, Akt2/PKBβ can phosphorylate component proteins in this GLUT4-positive compartment (8). However, interpretation of these biochemical data is confounded by the relatively higher level of expression of Akt2/PKBβ in adipocytes compared with Akt1/PKBα. Nonetheless, based on these data, it was proposed that the Akt2/ PKBβ isoform might be more important in mediating the insulin effect on such metabolic functions as glucose uptake and GLUT4 translocation.

Confirmation of the importance and distinct role of Akt2/ PKBβ in the physiological response to insulin derived from animal studies, in which it was shown that mice lacking Akt2/ PKBβ displayed insulin resistance and a diabetes-like syndrome (9). On the other hand, mice lacking Akt1/PKBα demonstrated normal glucose homeostasis but were small throughout life (10, 11). Thus, Akt1/PKBα seems to be involved predominantly in control of growth/proliferation, whereas Akt2/PKBβ regulates cellular metabolism. Nonetheless, there are several ambiguities in such in vivo studies that preclude a mechanistic interpretation. First, because all the cells were
lacking in Akt/PKB, it is unclear whether the insulin resistance in liver and muscle represents a cell autonomous defect. This problem is of particular concern when studying integrative metabolism, since distant effects produced by non-endocrine organs have been well established (12, 13). Second, and more importantly, the in vivo experiments leave unresolved whether isoform-specific phenotypes relate to differences in the level and distribution of the Akt/PKB forms or to distinct capacities for signaling intrinsic to each of the proteins. To clarify these issues, we have established hormone-responsive cell lines lacking either Akt1/PKBα or Akt2/PKBβ. We show here that insulin-induced glucose uptake and GLUT4 translocation are preferentially mediated by Akt2/PKBβ.

EXPERIMENTAL PROCEDURES

Cell Lines—Primary mouse fibroblasts were established from E13.5 embryos. Embryos were dissected from pregnant Akt1+/PKBα−/−/Akt2+/PKBβ−/− females that had been bred to Akt1+/PKBα−/−/Akt2+/PKBβ−/− males. The yolk sacs, heads, and internal organs were isolated and used for genotyping by reverse transcription-PCR. Carcasses were utilized for the preparation of immortalized fibroblasts by continuous culturing for 30 passages as described previously (14). Brown adipose cell lines were generated by a modification of a previously described protocol (15). Briefly, interscapular brown adipose tissue was isolated from 6-week-old mice, minced, and subjected to collagenase digestion (1 mg/ml in isolation buffer containing 0.123 M NaCl, 5 mM KCl, 1.3 mM CaCl2, 5 mM glucose, 100 mM Hepes-OH pH 7.3, and 4% bovine serum albumin). Digested tissue was overlaid onto a fetal bovine serum cushion for 10 min. The upper phase consisting of precursor cells was washed with serum-free Dulbecco’s modified Eagle’s medium and grown in a humidified atmosphere of 7.5% CO2. After reaching 80% confluence, cells were selected and used at passages 1-3 (5). The precursors were maintained in culture medium for 72 h and then subjected to selection with puromycin (10 μg/ml) for at least 2 weeks. Retroviral Infection and Adipocyte Differentiation—Generation of retrovirus was as described previously with slight modifications (16). Briefly, ecotropic BOSC cells (a gift from Warren S. Pear, University of Pennsylvania) were transiently transfected with pVSV G and pCgp pan-rtretroviral packaging constructs and retroviral vectors pMIGR-Akt/PKB, pMIGR-C/EBPα, or pMIGR-SV40T (17). Cell-free viral supernatants were harvested at 24 h and used to infect immortalized mouse embryo fibroblast (MEF) cells. retroviral vectors in the PLVX vector system were cotransfected with the pWP53/NeoRE-1 plasmid (18). DD cells were infected with a retrovirus encoding C/EBPα, a gift from Dr. Mitchell A. Lazar at University of Pennsylvania. Cells were incubated with viral supernatant for 2 days followed by selection in 10 μg/ml puromycin. Pre-brown adipocytes were immortalized by infection with puromycin-resistant pBABE vector encod- ing SV40 large T antigen as described above. Clones of resistant cells were pooled and used for all experiments. Adipocyte differentiation was induced by treatment with 2 μg/ml insulin, 0.4 μg/ml dexamethasone, 0.5 mM isobutylmethylxanthine, and 0.1 mg/ml Toglirazone (Bi- OMOL) as described (16). Brown adipocytes were differentiated as described previously (15). Experimental procedures were performed 8–10 days after initiation of differentiation.

Immunocytochemistry—Differentiated cells were serum-starved for 12 h before exposure to 100 nM insulin for 20 min. Cells were fixed with 4% paraformaldehyde (EM Science), permeabilized by 0.2% Triton X-100, and incubated with the indicated primary antibody for 1 h at room temperature. Cells were washed with 0.2% Triton X-100, and incubated with the indicated secondary antibody for 1 h. Cells were washed and mounted with DABCO (3,3-diaminobenzidine) and coverslips were mounted using a Fluoromount-G (Southern Biotechnology Associates, Birmingham, AL). Nuclei were counterstained with DAPI. Digital images were analyzed using Metamorph version 4.6 software (Universal Imaging Corp., West Chester, PA). To create a mask for identification of areas of interest, we used caveolin immunostaining. Eight randomly selected fields of view were analyzed per experiment. All experiments were performed at least three times and presented as the means ± S.E. of the mean (S.E.) of triplicates.

Western Blotting and General Reagents—Cells were lysed in 20 mM Tris-HCl, pH 7.3, 1 mM EDTA, 10 mM NaF, and 10% glycerol. Cell lysates (40 μg) were submitted to SDS-polyacrylamide gel electrophoresis on 7–18% gradient polyacryl- amide gel under reduced conditions. Proteins were transferred to poly- vinylidene difluoride membranes, which were subjected to immuno- blotting using the indicated primary antibodies and horseradish peroxidase-conjugated secondary antibodies. Western blots were devel- oped using enhanced chemiluminescence (Amersham Biosciences). Anti-C/EBPα and anti-hemagglutinin were from Santa Cruz, anti-pan caveolin antibody was from BD Transduction Laboratory, and anti- insulin receptor, anti-IRS-1, anti-IRS-2, and anti-phosphatidylinositol 3-kinase p85 antibodies were purchased from Upstate Biotechnologies. Anti-UCP-1 was obtained from Oncogene Science. Anti-aP2 antibody was a generous gift of Dr. David A. Bernlohr (University of Minnesota). All other high quality reagents were purchased from Sigma-Aldrich unless indicated elsewhere.

RESULTS

Defects in GLUT4 Translocation in Akt2/PKBβ-deficient Fat Cells—Previously, we have shown that mice lacking Akt2/PKBβ display a diabetes-like syndrome (9). However, it re- mains unclear whether the phenotype derives from a systemic failure in the knockout animals or a cell autonomous inability of insulin to signal to glucose uptake and GLUT4 translocation in target cells. As an initial approach to this problem we measured glucose uptake into adipocytes isolated from Akt2+/PKBβ−/− mice. As shown in Fig. 1, Akt2/PKBβ−/− adipose cells took up less glucose than wild type cells at all insulin concentrations tested. This indicates that a loss in Akt2/PKBβ−/− impairs glucose uptake in freshly isolated adipocytes, as it does in skeletal muscle (9).

To further resolve whether this was a cell-autonomous de- fault, we established MEF cells from each combination of geno- types, i.e. wild type (WT), Akt1+/PKBα−/− (1KO), Akt2+/PKBβ−/− (2KO), and Akt1+/PKBα−/−Akt2+/PKBβ−/− (DKO). Expression of both Akt1/PKBα and Akt2/PKBβ is undetect- able in the expected knockout cell lines, as shown in Fig. 2A. Primary MEF cells were induced to differentiate into adipocytes solely by treatment with differentiation mixture as de- scribed under “Experimental Procedures.” Using the protocol described under “Experimental Procedures,” about 10–20% of WT and 2KO cells differentiated into fat cells, whereas the differentiation of 1KO cells was less than 1% (data not shown).
DKO primary fibroblasts appeared completely incapable of differentiation. Nonetheless, even in 1KO cells, adipocytes were unambiguously identified by the appearance of fat droplets and, more definitively, significant amounts of caveolin, whose expression dramatically increased during adipogenesis, and then assessed for insulin-dependent GLUT4 translocation by confocal microscopy (19). Stimulation with insulin of either WT or 1KO cells, but not 2KO adipocytes, resulted in the translocation of GLUT4 to the plasma membrane (Fig. 2B). These results, although non-quantitative, suggest that Akt1/PKB preferentially regulates adipogenesis, whereas Akt2/PKB is more critical to GLUT4 translocation.

**Defects in Deoxyglucose Uptake in Adipocytes Derived from Immortalized MEF Cells**—To achieve a higher efficiency of differentiation, we first generated spontaneously immortalized fibroblasts by continuous passage of MEFs. As expected, insulin stimulated a modest, 2-fold increase in deoxyglucose in these cells (Fig. 3). Although there was a trend for decreased insulin-stimulated transport in all of the mutant cell lines, none of these achieved statistical significance (p > 0.05).

To assess the consequences of Akt/PKB loss on deoxyglucose uptake in adipocytes, we expressed C/EBPα, a known adipogenic transcription factor, in immortalized fibroblast cells (20, 21). As assessed either by accumulation of fat droplets or the induction of adipose-specific markers, expression of C/EBPα induced differentiation only in WT and 2KO cells about 90–95% but not in 1KO and DKO cells, consistent with the data presented above using primary cells (Fig. 4, A and B). Defects in differentiation of 1KO and DKO cells were not due to differences in C/EBPα expression, since the levels of C/EBPα among the cell lines were quite similar (Fig. 4B). As shown in Fig. 4C, insulin-induced hexose uptake into 2KO adipocytes was only 60% of that in WT cells.

**Rescue of Hexose Uptake and GLUT4 Translocation in 2KO Cells by Re-expression of Akt2/PKB but Not by Akt1/PKBa**—The demonstration that only 2KO fat cells displayed reduced hexose uptake and GLUT4 translocation after exposure to insulin still left unresolved whether this isoform specificity was caused by properties intrinsic to the kinase or simply to cell context. To resolve this issue we assessed both hexose uptake and GLUT4 translocation in 2KO cells expressing retrovirally transduced Akt1/PKBα or Akt2/PKBβ at equivalent levels (Fig. 5A). Expression was monitored by GFP fluorescence, as both Akt/PKB and GFP are translated from a single mRNA containing an intervening internal ribosome entry site. As shown in Fig. 5B, both Akt1/PKBα and Akt2/PKBβ were expressed appropriately in GFP-positive cells. Expression of HA-Akt2/PKBβ was about 2–3-fold higher than that of endogenous Akt2/PKBβ, and HA-Akt1/PKBα levels exceeded those of HA-Akt2/PKBβ by about 2-fold (Fig. 5C). Infection with a retrovirus encoding HA-Akt1/PKBα elevated amounts of this isoform about 3- to 4-fold (Fig. 5C). Expression of Akt2/PKBβ but not Akt1/PKBα restored hormone-dependent hexose uptake completely (Fig. 6A). In a further effort to more quantitatively measure GLUT4 translocation, we utilized the plasma membrane sheet assay (18). Insulin-stimulated translocation of GLUT4 was about 6–7-fold in WT cells, which is similar to that reported previously in 3T3-L1 adipocytes (Fig. 6, B and C) (16). However, in 2KO cells insulin stimulated only a 4-fold increase in GLUT4 translocation to plasma membrane. Re-expression of Akt2/PKBβ in 2KO cells completely restored insulin-dependent GLUT4 translocation, whereas expression of Akt1/PKBα to –2-fold more than Akt2/PKBβ was without effect. It is striking...
that the diminution in insulin-stimulated hexose uptake precisely paralleled that of GLUT4 translocation (Fig. 6). These results indicate that Akt2/PKB2 is more effective than Akt1/PKB1 in conveying the signal for accelerated glucose uptake in adipose cells, and this is likely to be mediated exclusively by translocation of GLUT4.

Hexose Uptake and GLUT4 Translocation in Akt2/PKB2/Brown Adipose Cells—To study an alternative, physiologically relevant in vitro model of insulin action, we generated brown adipose precursor cells from wild type and Akt KO mice (15, 22). The levels of expression of either Akt1/PKB1 or Akt2/PKB2 were verified by Western blotting with specific antibodies against each isoform, providing further confirmation of the genotype (Fig. 7A). In contrast to differentiation induced by forced expression of C/EBPα, spontaneous differentiation of brown preadipocytes proceeded equally well for WT, Akt1/PKB1−/−, and Akt2/PKB2−/− exposed to differentiation mixture (Fig. 7B). As above, adipose conversion was assessed by accumulation of lipid droplets as well as Western blot of several adipose marker proteins and the brown fat cell specific marker protein, UCP-1. In addition, expression of insulin receptor, IRS-1 and IRS-2, and the p85 regulatory subunit of PI 3-kinase were unchanged in the KO lines (Fig. 7C). Most importantly, we measured insulin-induced hexose uptake in these brown adipocytes. As shown in Fig. 7D, complete loss of Akt2/PKB2 caused a significant reduction in insulin-induced hexose uptake, whereas a deficiency of Akt1/PKB1 produced only a marginal reduction. GLUT4 translocation precisely paralleled hexose uptake, with a significant reduction only in Akt2/PKB2−/− cells (Fig. 7E).

Effect of Akt/PKB on GLUT1 Translocation—In cultured adipocytes, although GLUT4 displays the greatest “fold” stimulation in response to insulin, the GLUT1 glucose transporter also translocates to the cell surface (23). Because it has been suggested that different signaling pathways regulate the two carriers (24), we assessed the effect of Akt/PKB loss on GLUT1.

Fig. 4. Hexose uptake in wild type and Akt2/PKB2-deficient fat cells derived from immortalized fibroblasts. Fibroblasts were immortalized and induced to differentiate into adipocytes by overexpression of the transcription factor C/EBPα. A, nine days after the initiation of differentiation, cells were stained with Oil Red-O, and images were obtained. B, Western blot analysis of fibroblast cell extracts by using antisera directed against C/EBPα (upper panel), the fatty acid binding protein aP2 (middle panel), or the insulin-response glucose transporter GLUT4 (lower panel). C, hexose uptake was measured in adipocytes as described under “Experimental Procedures.” Values are the mean ± S.E. of three independent experiments. The asterisk (*) indicates values different from that of wild type cells, p < 0.005.

Fig. 5. Retrovirus-mediated expression of Akt1/PKB1 and Akt2/PKB2 in Akt2/PKB2−/− cells. A, schematic representation of the retroviral Akt1/PKB1 and Akt2/PKB2 constructs. An HA epitope was introduced on the amino terminus of both Akt1/PKB1 and Akt2/PKB2. LTR, long terminal repeat; MCS, multi-cloning site; IRES, internal ribosome entry site. B, expression of either Akt1/PKB1 or Akt2/PKB2 was assessed in adipocytes by confocal microscopy, visualizing either GFP (green) or the HA epitope (red). Ab, antibody. C, cell lysates were probed with the indicated antibodies to verify expression level of endogenous and ectopically expressed Akt proteins.
translocation in brown adipocytes. As expected, GLUT1 was readily visualized at the plasma membrane and translocated 1.5–2-fold in response to insulin in WT cells. Interestingly, disruption of either the Akt1/PKB<sup>H9251</sup>/H11002 or Akt2/PKB<sup>H9252</sup>/H11002 gene did not affect the basal, surface level of GLUT1, but loss of Akt2/PKB<sup>H9252</sup> appeared to block the insulin-induced translocation of the transporter (Fig. 8).

**DISCUSSION**

Previously, we reported that Akt2/PKB<sup>H9252</sup>-deficient mice display moderate glucose intolerance due at least in part to insulin resistance (9). However, those studies left unresolved two important issues, first, whether the insulin resistance was because of a lack of Akt/PKB signaling in the target cell, i.e. if the defect was cell autonomous, and second, the mechanism underlying the apparent isoform specificity. In regard to the latter, it was possible that the marked differences in phenotype for the Akt2/PKB<sup>H9252</sup><sup>−/−</sup> mice compared with the Akt1/PKB<sup>H9251</sup><sup>−/−</sup> animals might well have been caused by tissue-specific patterns of expression as opposed signaling capabilities intrinsic to each of the isoforms (9–11). In the current study we answer each of these questions. By examining insulin-dependent physiological responses in cells immortalized from Akt/PKB knock-out mice, we show conclusively that Akt2/PKB<sup>H9252</sup> is required in a cell autonomous manner for maximal hexose uptake and GLUT4 translocation in adipocytes. Importantly, this is not due simply to the level of the kinase expression or the nature of the immortalized cell, as indicated by two critical observations. First, reintroduction Akt2/PKB<sup>H9252</sup>, but not Akt1/PKB<sup>H9251</sup> in the same cell lines completely restores hormone-responsive hexose flux and transporter translocation. Second, in precisely the same immortalized cell lines, adipogenesis is abrogated in Akt1/PKB<sup>H9251</sup> null cells but not Akt2/PKB<sup>H9252</sup>. The ability of each kinase isoform to preferentially signal to distinct biological responses in identical cell lines provides a convincing demonstration of the capacity of individual Akt members to link to specific downstream pathways.

Several previous studies have implicated differential signaling capabilities for pathways upstream of Akt/PKB. Cells from IRS1-null mice are defective in differentiation into brown fat cells (25). Interestingly, the lack of IRS1 selectively reduced Akt1/PKB<sub>o</sub> activation. However, IRS2 appeared to be more capable than IRS1 of conveying the insulin-dependent signal to GLUT4 translocation in immortalized murine brown adipocytes, reminiscent of our results, in which Akt2/PKB<sup>H9252</sup> is crucial for the same response (26). Whether the different IRS isoforms are capable of selectively activating distinct Akt/PKB proteins...
Akt2/PKB whereas knockdown of Akt2/PKB not significantly affect insulin-induced glucose uptake, (27, 28). Knockdown of Akt1/PKB did not result in complete loss of the ability of insulin to stimulate hexose uptake, but only a partial abrogation. This correlates well with the in vivo metabolic studies of the Akt2/PKBβ knockout mice, in which there was only a partial defect in peripheral insulin action (9). One possibility is that functional complementation is provided by other Akt/PKB isoforms still present in the adipocyte. In 3T3-L1 cells, there is significant amount of Akt1/PKBα after differentiation. Moreover, it has been reported that 3T3-L1 cells also contain significant Akt3/PKBγ, although this has been difficult to study due to the lack of high quality antisera (29). That Akt1/PKBα can provide part of the function of Akt2/PKBβ in the absence of the latter is suggested by the observation that mice null for Akt2/PKBβ and lacking one allele of Akt1/PKBα are more glucose-intolerant than those homozygous null for Akt2/PKBβ alone.2 If this interpretation is correct, it is striking that even when overexpressed, Akt1/PKBα is incapable of restoring insulin action to wild type levels (Fig. 6). This result suggests that Akt2/PKBβ is unique in its ability to confer some aspect of signaling to GLUT4 translocation. An alternative interpretation of these data is that a signaling pathway that functions completely independent of Akt/PKB confer the residual insulin-responsive hexose transport present in Akt2/PKBβ null adipocytes.

The molecular mechanism by which Akt1/PKBα and Akt2/PKBβ could dictate specificity is unknown. In rodents and humans, Akt1/PKBα, Akt2/PKBβ, and Akt3/PKBγ are encoded by distinct genes and share high sequence homology (2, 30).

1 H. Cho, M. Mizrahi, and M. J. Birnbaum, unpublished observations.

FIG. 7. Insulin-dependent hexose uptake and GLUT4 translocation in immortalized Akt2/PKBβ−/− brown adipocytes. A, lysates from SV40 T antigen-immortalized brown preadipocytes of the indicated genotypes were subjected to Western blotting for Akt1/PKBα and Akt2/PKBβ. B, established brown preadipocytes were induced to differentiate into adipocytes and stained with Oil Red-O as described under “Experimental Procedures.” C, cell lysates prepared from brown adipocytes (BAT) were immunoblotted with antibodies against the indicated proteins. Extract from 3T3-L1 adipocytes was included as a control (L1 adipocyte). IR, insulin receptor; p85 PI3K, the 85-kDa regulatory subunit of phosphatidylinositol 3′-kinase; UCP-1, uncoupling protein 1. D, brown adipocytes were assayed for insulin-induced hexose uptake as described under “Experimental Procedures.” Data are the mean ± S.E. of three independent experiments. The asterisks indicate values different from that of wild type cells. *, p < 0.005; **, p < 0.001. E, fully differentiated brown adipocytes were stimulated with insulin for 20 min, plasma membrane sheets were prepared as described under “Experimental Procedures,” and the plasma membrane was stained with anti-GLUT4 (red) and anti-caveolin (green).

FIG. 8. Glucose transporter translocation in brown adipocytes. Brown adipocytes of the indicated genotypes were stimulated with or without insulin, and plasma membrane sheets were prepared, then co-stained with either anti-GLUT4 (red) and anti-caveolin (green) (upper panels) or anti-GLUT1 (red) and anti-caveolin (green) (lower panels).
Nonetheless, the primary function of each of the Akt/PKB isoforms appears to be different. A plausible model to explain how each these closely related proteins maintain individual signaling capabilities is provided by the differential capacity of Akt1/PKBα to suppress c-Jun NH₂-terminal kinase activation and neuronal apoptosis (31). In this case specificity in conferred by the ability of Akt1/PKBα, but not Akt2/PKBβ, to bind via a non-catalytic domain to the scaffolding protein, c-Jun NH₂-terminal kinase-interacting protein. We think it likely that such localization motifs will be important for conferring specificity to signaling through Akt/PKB. It is also notable that Akt2/PKBβ can mediate insulin-induced GLUT1 translocation but not basal recycling in brown adipocytes (Fig. 8). Because it has been shown that GLUT4 exists in at least two intracellular compartments, i.e. insulin-responsive compartment and recycling compartment (32–34), these data suggest that Akt2/PKBβ contributes to the recruitment of insulin-responsive population of GLUT4 to the cell surface. Therefore, different subcellular localizations of Akt1/PKBα and Akt2/PKBβ may explain a different physiological response of Akt1/PKBα and Akt2/PKBβ. It is not clear at this time why our conclusions are different from Foran et al. (24), who find that overexpression of Akt/PKB led to the translocation of GLUT4 but not GLUT1. It is possible that the strategies of overexpression versus loss of function may contribute to these differences or, on the other hand, are due simply to the difficulty inherent in measuring the rather modest change of GLUT1 on the cell surface.

In summary, despite a high degree of sequence similarity, Akt1/PKBα and Akt2/PKBβ serve distinct cellular functions in regard to growth/differentiation and metabolism. Akt2/PKBβ is much more important for the control of glucose uptake through GLUT4 translocation to plasma membrane than Akt1/PKBα. Identification of specific target proteins for either Akt1/PKBα or Akt2/PKBβ will be key to understanding how both isoforms confer different physiological responses.

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