Akt1/PKBα Is Required for Normal Growth but Dispensable for Maintenance of Glucose Homeostasis in Mice*

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The serine-threonine kinase Akt, also known as protein kinase B (PKB), is an important effector for phosphatidylinositol 3-kinase signaling initiated by numerous growth factors and hormones. Akt2/PKBβ, one of three known mammalian isoforms of Akt/PKB, has been demonstrated recently to be required for at least some of the metabolic actions of insulin (Cho, H., Mu, J., Kim, J. K., Thorvaldsen, J. L., Chu, Q., Crenshaw, E. B., Kaestner, K. H., Bartolomei, M. S., Shulman, G. I., and Birnbaum, M. J. (2001) Science 292, 1728–1731). Here we show that mice deficient in another closely related isoform of the kinase, Akt1/PKBα, display a conspicuous impairment in organismal growth. Akt1−/− mice demonstrated defects in both fetal and postnatal growth, and these persisted into adulthood. However, in striking contrast to Akt2/PKBβ null mice, Akt1/PKBα-deficient mice are normal with regard to glucose tolerance and insulin-stimulated disposal of blood glucose. Thus, the characterization of the Akt1 knockout mice and its comparison to the previously reported Akt2 deficiency phenotype reveals the non-redundant functions of Akt1 and Akt2 genes with respect to organismal growth and insulin-regulated glucose metabolism.

Recent genetic analyses have emphasized the evolutionary conservation of insulin signaling as a generalized organismal response to nutritional abundance. Nonetheless, much uncertainty remains concerning how this signaling pathway diverges to allow independent regulation of such disparate biological outputs as metabolism, aging, and growth. The serine-threonine kinase Akt, also known as protein kinase B (PKB), represents an important mediator of insulin action in worms and flies. In Caenorhabditis elegans, mutations in Akt result in alterations in development and aging whereas in flies, Akt/PKB, as well as other components of the insulin signaling pathway, has been implicated as critical regulators of organism growth and longevity (1–6). In rodents and humans, the three isoforms Akt1/PKBα, Akt2/PKBβ, and Akt3/PKBγ, which share a high degree of sequence homology, are encoded by distinct genes (7, 8). Preliminary analyses of the three gene products support the notion that these isoforms have similar biochemical characteristics (7).

In mice, one of these Akt/PKB family members, Akt2/PKBβ, has been shown to be required for insulin to maintain normal glucose homeostasis (9). In the absence of Akt2/PKBβ, insulin-stimulated glucose uptake in muscle and fat was significantly reduced in association with reduction in whole body glucose disposal. However, the blockade in glucose uptake in response to insulin was incomplete, raising the possibility that other PI3-kinase-dependent effectors, including other Akt isoforms, might also signal to metabolism outputs. To determine whether the highly related Akt1/PKBα is also required for insulin-regulated glucose homeostasis in mice, we disrupted the c-Akt gene (hereafter referred as Akt1), which encodes Akt1/PKBα.

EXPERIMENTAL PROCEDURES

Generation of Akt1-targeted Mice—To map the Akt1 locus and derive DNA fragments for homologous recombination, we screened a mouse genomic BAC library by the polymerase chain reaction (PCR). The targeting vector was constructed by inserting a left arm fragment, which included exons 2 and 3, into KpnI and XbaI sites and a right arm fragment, which extended from within exon 8 to downstream of exon 11, into the Xbal site of pPNT (10). After transfection of the targeting vector into E14 embryonic stem (ES) cells, G418- and ganciclovir-resistant colonies were screened for homologous recombination by Southern blot analysis. ES cells carrying a recombinant allele were injected into C57BL/6 blastocysts, which were subsequently implanted into pseudo-pregnant CD-1 foster mothers. Resulting chimeric males were mated by the birth of agouti pups, which were screened for the targeted allele. For genotyping by PCR, the following primers were used in a single reaction: 851, 5′-AGCTCTTCCTCCACCAGTGTCTC-3′; 852, 5′-GCTCCATTAAGCACACCTTCAAGG-3′; 853, 5′-GTGGATGGGATGATGTGCGA-3′. For genotyping by Southern blotting, a PCR-amplified fragment (~400 base pairs) corresponding to sequence upstream of the left homologous recombination region (Fig. 1) was random labeled with [32P]dCTP.

Preparation of Embryonic Fibroblasts—After timed matings of heterozygous Akt1 parents, embryos were harvested at 13.5 days postcoitus. Embryos were dissected to remove the head and the visceral organs and were then finely minced and trypsinized before being plated in the presence of 10% fetal bovine serum in Dulbecco’s modified Eagle’s medium.

Metabolite Measurements—Glucometer Elite (Bayer) was used to determine glucose concentration from whole blood collected from the transversely sectioned tip of mouse tails. Sera were separated from whole blood for the determination of circulating insulin and free fatty acid concentration. For insulin levels, rat insulin enzyme-linked immunosorbent assay was performed by the Radioimmunoassay Core Facility at the Penn Center for Diabetes. NEFA C kit (Wako) was used to determine free fatty acid levels.

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The abbreviations used are: PKB, protein kinase B; PI 3-kinase, phosphatidylinositol 3-kinase; PCR, polymerase chain reaction; ES, embryonic stem; IRS, insulin receptor substrate; IGF, insulin-like growth factor; kb, kilobase.
RESULTS AND DISCUSSION

The targeting strategy for disruption of the Akt1 gene consisted of replacement of the coding exons 4, 5, 6, and 7 and the 5’ portion of exon 8 with the neomycin resistance gene (Fig. 1a). Exon 5 encodes the lysine residue necessary for the catalytic activity of Akt1/PKBα. The genotype of the ES cells and animals were easily distinguished by Southern blotting or PCR (Fig. 1, b and c). Mouse embryonic fibroblasts heterozygous and homozygous for the targeted allele were isolated and examined for the presence of Akt1/PKBα mRNA and protein by Northern and Western blot, respectively. Both Akt1 mRNA and protein were undetectable in mouse embryonic fibroblasts in which both alleles were targeted (Fig. 1, d and e). Thus, the targeted disruption resulted in a functionally null allele. Furthermore, we could not detect any compensatory increase in the expression of Akt2 or Akt3 in the Akt1−/− mouse embryonic fibroblasts, as assessed by Northern blot analysis (not shown).

When mice heterozygous for the targeted Akt1 allele were mated inter se, fewer than expected Akt1−/− mice were observed 2–3 weeks after birth (Table I). In contrast, Akt1−/− mice appeared with the expected mendelian frequency when 13.5-day-old embryos were examined. Thus, loss of expression of Akt1 resulted in partial lethality occurring some time between midembryonic development and the time of weaning. Careful monitoring of a small number of litters revealed that a significant number of Akt1−/− pups died within the first 3 days.

**Table I**

| Observed genotypic distribution of offspring from heterozygous matings |
|-----------------------------|----------------|----------------|
|                            | +/+  | +/-   | -/-  |
| P14-P21                    | 146  | 330   | 87   |
| 25.93%                      | 58.61% | 15.45% |
| E13.5                       | 22   | 54    | 21   |
| 23.68%                      | 55.67% | 21.65% |
of birth, suggesting that the lethality may have occurred during the early neonatal period (data not shown). In all cases, the surviving Akt1−/− pups continued to grow into adulthood and were fertile.

The surviving Akt1−/− animals were distinguishable from wild-type animals because of their smaller size. Examination of mice at birth revealed an ~20% reduction in body weight in Akt1/PKBα-deficient mice compared with wild-type mice (Fig. 2a), suggesting that reduction in size occurs during embryonic development. The decrease in body weight was evident throughout postnatal development regardless of sex and persisted into adulthood (Fig. 2b). At 14 months of age, wild type male mice were 37.7 ± 2.2 g whereas Akt1−/− male mice were 27.7 ± 2.0 g.

A role for Akt in the determination of cell and compartment size has been established in Drosophila melanogaster, but the present data provide the first indication as to the importance of this kinase in this regard to growth of a mammalian organism (2). In the fruit fly, the role of Akt in cell growth is relevant to its position in the insulin signal transduction pathway, as genetic manipulation of the fly insulin receptor, IRS ortholog Chico, or PI 3-kinase also results in similar alterations in growth (1, 11, 12). Experiments with mice in which signaling through the IGF-1 receptor has been reduced also led to reduction in body weight, although the relative contributions of cell size and cell number have not been established (13–15). Consistent with these data, mice rendered null for IRS-1 or IRS-2, two scaffolding proteins that serve as crucial substrates for the IGF-1 and insulin receptors, also demonstrate defects in growth (16–18). Because a conserved signaling pathway exists in which insulin or IGF-1 stimulates Akt activity via docking of PI 3-kinase to a tyrosine-phosphorylated IRS-1 or IRS-1, it is likely that these signaling proteins also represent intermediates in a pathway regulating cell and organismal growth. Interestingly, p70 S6 kinase, which is also activated by insulin but whose precise relationship with Akt remains unclear, also

![Graph](image)

**FIG. 2.** Akt1−/− mice demonstrate embryonic and postnatal defects in growth. a, birth weights. Newborns were weighed within 24 h of birth; data represent both male and female mice. Each filled circle and open circle represent weight measurements for individual wild-type and Akt1−/− newborn mice, respectively. Mean weights, indicated by bars, are 1.51 ± 0.058 g and 1.12 ± 0.036 g for wild-type and Akt1−/− newborns, respectively. p < 0.001 by t test. b, wild-type (filled circles) and Akt1−/− (open circles) mice from 24 litters derived from heterozygous matings were assessed for body weight at 3–8 weeks after birth. Left and right panels show growth curves for males and females, respectively. Values represent the mean ± S.D. p < 0.05 for weight comparisons between genotypes at all ages except for the values for 7-week-old females.

![Graph](image)

**FIG. 3.** In vivo assessment of glucose metabolism in wild-type and Akt1−/− mice. a, glucose tolerance test. Glucose (2 g/kg body weight) was administered by intraperitoneal injection to overnight fasted wild-type (filled squares) and Akt1−/− (open squares) mice as described (9). Blood was collected from the sectioned tip of the tail at the indicated times and assayed for glucose using a glucometer. Values at time 0 were from samples taken immediately after administration of glucose. b, insulin tolerance test. Porcine insulin (1 unit/kg body weight) was administered by intraperitoneal injection to overnight fasted mice, and glucose was assayed as above. For both experiments, n = 7–14 mice for each genotype. Data are presented as mean ± S.E.
appears to be important for normal growth of both flies and mice (19, 20).

Recently, we have shown thatAkt2/PKBβ is critical to the normal control of glucose homeostasis by insulin (9). Thus, it was important to also examine the contribution ofAkt1/PKBα to metabolism in vivo. As an initial step, we assessed adult mice for an alteration in whole body glucose metabolism by measuring the concentration of blood glucose. As shown in Table II, there was no change in blood glucose under either random-fed conditions or following a 15 h-fast. As a more sensitive measure of insulin resistance, we also measured circulating insulin levels, which also were unchanged in the Akt1−/− mice (Table II). Circulating free fatty acid levels at fed or fasting states were also indistinguishable between the Akt1−/− and wild-type mice, suggesting that lipid metabolism was unaffected by removal of Akt1/PKBα.

As a further evaluation of glucose metabolism, we challenged the mice with exogenous glucose and measured circulating levels of the sugar during the ensuing 2 h. The Akt1−/− mice responded as well as the control mice to the glucose load, indicating normal glucose tolerance (Fig. 5a). To more directly ascertain insulin responsiveness, the hormone was injected, and the resultant change in blood glucose was measured. Again, the Akt1−/− mice cleared glucose from circulation as efficiently as wild-type control mice (Fig. 5b). These analyses of whole body glucose metabolism indicate that Akt1/PKBα, in marked contrast to Akt2/PKBβ, is not a major effector for insulin-regulated glucose homeostasis.

Although the precise distribution of the Akt isoforms among different organs remains somewhat controversial, abundant evidence exists that Akt1/PKBα is expressed in classical insulin target tissues such as liver, muscle, and adipocytes (21–24). For this reason, it is surprising that mice rendered deficient in expression of either isoform. Akt1−/− is most important to the growth of the organism both in utero and after birth, whereas Akt2 is critical to insulin-dependent control of carbohydrate metabolism. The Akt1 knockout phenotype, in which mice are reduced in size at birth and remain small throughout life, is reminiscent of rodent models with altered expression in proximal components of insulin and IGF signaling and suggest that these hormones control growth through Akt1/PKBα (14, 16).

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Table II
Concentrations of circulating metabolites and insulin

|                      | Males                     | Females                   |
|----------------------|---------------------------|---------------------------|
|                      | +/+                       | --/-                      |                      |
| Fasting glucose (mg/dl) | 91.7 ± 2.19 (10)         | 91.4 ± 7.6 (9)            | 98.1 ± 3.1 (14)     |
| Fed glucose (mg/dl)   | 160.7 ± 9.7 (7)           | 180.9 ± 8.3 (7)           | 145.75 ± 2.6 (12)  |
| Fasting insulin (ng/ml)| 0.30 ± 0.059 (10)        | 0.28 ± 0.048 (9)          | 0.28 ± 0.022 (13)  |
| Fasting FFA (meq/liter)| 0.94 ± 0.076 (8)         | 0.93 ± 0.069 (7)          | ND                    |
| Fed FFA (meq/liter)   | 0.14 ± 0.034 (8)          | 0.14 ± 0.023 (7)          | ND                    |

Values are the mean ± S.D. obtained from 4–5-month-old mice. Values in parentheses denote number of mice tested. ND refers to values not determined. No comparisons between sex-matched genotypes revealed any statistically significant difference (p < 0.05) by t-test.