THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 29, Issue of July 16, pp. 20281–20286, 1999

Printed in U.S.A.

Regulation of *GLUT1* Gene Transcription by the Serine/Threonine Kinase Akt1*

(Received for publication, December 14, 1998, and in revised form, March 23, 1999)

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We used mouse hepatoma (Hepa1c1c7) cells to study the role of the serine/threonine kinase Akt in the induction of *GLUT1* gene expression. In order to selectively turn on the Akt kinase cascade, we expressed a hydroxytamoxifen-regulatable form of Akt (myristoylated Akt1 estrogen receptor chimera (MER-Akt1)) in the Hepa1c1c7 cells; we verified that hydroxytamoxifen stimulates MER-Akt1 activity to a similar extent as the activation of endogenous Akt by insulin. Our studies reveal that stimulation of MER-Akt1 by hydroxytamoxifen induces GLUT1 mRNA and protein accumulation to levels comparable to that induced by insulin; therefore, activation of the Akt cascade suffices to induce *GLUT1* gene expression in this cell system. Furthermore, expression of a kinase-inactive Akt mutant partially inhibits the response of the *GLUT1* gene to insulin. Additional studies reveal that the induction of GLUT1 mRNA by Akt and by insulin reflects increased mRNA synthesis and not decreased mRNA degradation. Our findings imply that the *GLUT1* gene responds to insulin at the transcriptional level and that Akt mediates a step in the activation of *GLUT1* gene expression in this system.

The members of a family of six membrane proteins, known as the glucose transporters (GLUT1–5 and GLUT7),[1] facilitate glucose uptake into mammalian cells (reviewed in Refs. 1 and 2). Glucose uptake is critical for maintaining intracellular ATP levels, and cells have evolved several strategies for regulating this process. For example, in fat and muscle, insulin stimulates the rapid translocation of the GLUT4 protein from an intracellular site to the plasma membrane, where it functions (3). In other tissues and cells, long term insulin treatment stimulates glucose uptake by increasing *GLUT1* gene expression, thereby providing more transporter proteins to the cell (4, 5). The *GLUT1* gene also responds to other stimuli. For example, hypoxia induces *GLUT1* gene expression (6–8); this response may help protect neurons from glucose starvation and death during brain ischemia (9). In addition, increased *GLUT1* gene expression is associated with oncogenic transformation of various cell types (10–12). These observations imply that the regulation of *GLUT1* gene expression is relatively complex and may involve several converging signaling pathways.

The *GLUT1* gene constitutes an interesting system for analyzing the mechanism by which insulin alters specific gene expression. Insulin binds to a cell surface receptor and activates an intrinsic receptor tyrosine kinase, a process that ultimately stimulates two major signaling cascades (13). One cascade leads to the activation of MAP kinase and the phosphorylation of transcription factors (14). The second cascade involves phosphatidylinositol (PI)-3 kinase (15) and numerous potential downstream effectors, including the serine/threonine kinase Akt (also called protein kinase B (PKB)) (16). Akt activity reflects contributions from three distinct isozymes (Akt1–3), which are regulated primarily by phosphorylation (16). Expression of a constitutively active Akt1 can mimic several nontranscriptional responses to insulin, including inhibition of glycogen synthase kinase-3, activation of the p70 ribosomal S6 kinase, stimulation of GLUT4 translocation and glucose uptake, stimulation of protein synthesis, and inhibition of apoptosis (13, 16).

It is less clear whether Akt can mediate transcriptional responses to insulin (17). Several reports provide conflicting data on this point (18–22). To further address this issue, we have asked here whether Akt can induce *GLUT1* gene transcription in mouse hepatoma (Hepa1c1c7) cells. We find that activation of the Akt1 kinase suffices to induce *GLUT1* transcription to an extent similar to that induced by insulin. Moreover, expression of an inactive Akt1 partially inhibits the ability of insulin to stimulate *GLUT1* gene expression. These results imply that Akt is a downstream effector of insulin action in inducing *GLUT1* gene transcription in this cell system.

EXPERIMENTAL PROCEDURES

Materials—Minimum Eagle's medium and G418 were from Life Technologies, Inc., and other cell culture media were from UCSF Cell Culture Facility (San Francisco, CA). Total RNA was isolated using the RNeasy kit from Qiagen (Chatsworth, CA). The reverse transcription polymerase chain reaction kit was from Stratagene (La Jolla, CA). The primers for the *GLUT1* and β-actin probes were from Operon Technologies Inc. (Alameda, CA), and the pGEM-T vector system was from Promega (Madison, WI). [γ-^{32}P]ATP (3000 Ci/mmol) was from NEN Life Science Products, and the random priming kit, [α-^{32}P]UTP (800 Ci/mmol), [α-^{32}P]dCTP (3000 Ci/mmol), and horseradish peroxidase-conjugated secondary antibody were from Amersham Pharmacia Biotech. The Akt-substrate peptide was synthesized in the Beckman PAN facility (Stanford, CA). Anti-GLUT1 polyclonal antiserum was from East Acres (Southbridge, MA), acrylamide was from National Diagnostics (Atlanta, GA), nitrocellulose and nylon membranes were from Schleicher & Schuell, insulin and anti-HA monoclonal antibody (12CA5) were from Roche Molecular Biochemicals, and anti-Akt1 antibodies (directed against the C terminus) were from Upstate Biochemicals (Lake Placid, NY). Anti-Akt1 and Akt3 antibodies directed against their respective pleckstrin homology domains were produced as described (23). Anti-Akt2 antibodies were a gift of Dr. Birnbaum (University of Pennsylvania) (24). Sephadex 10 microspin columns were from

* This work was supported in part by National Institutes of Health Grants DK 34926 (to R. A. R.) and ES08655 (to J. P. W.), an American Diabetes Association Mentor-Based Postdoctoral Fellowship (to K. N.), and a Feodor-Lynen Fellowship of the Alexander von Humboldt-Stiftung (to A. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: GLUT, glucose transporter; MER-Akt, myristoylated Akt estrogen receptor chimera; HA, hemagglutinin; PI, phosphatidylinositol.

Amersham Pharmacia Biotech, and Express-Hyb solution was from CLONTECH (Palo Alto, CA). Protein A-Sepharose was from Repligen (Cambridge, MA). Protein determination was performed using the BCA kit (Pierce). 4-Hydroxytamoxifen and all other chemicals were from Sigma.

Plasmid Constructs—The retroviral plasmids coding for HA-tagged MER-Akt1 and the inactive HA-tagged Akt1-S473A/T308A mutant were as described (20, 25), except that both constructs were expressed using the pWZL-neo retroviral vector (a gift from Dr. Garry P. Nolan, Stanford, CA).

Cell Culture—Hepa1c1c7 cells stably transfected with MER-Akt1-pWZL-neo, Akt1-S473A/T308A-pWZL-neo, or empty vector alone (pWZL-neo) were grown in 6-well Nunclon dishes (Nalge Nunc International, Roskilde, Denmark) in α-minimum Eagle's medium containing 10% (v/v) fetal calf serum, 100 μg/ml streptomycin, and 100 units/ml penicillin. After reaching confluency, the cells were put in serum-free α-minimum Eagle's medium supplemented with 0.1 mg/ml bovine serum albumin, 20 mM Hepes, pH 7.4, penicillin/streptomycin and treated. In some cases, cells were subjected to hypoxic conditions by placing them in a humidified automatic CO_2/O_2 incubator (Forma Scientific, model 3159) maintained at 37 °C and 1% O_2, 5% CO_2, and 94% N_2.

Retroviral Infection—Hepa1c1c7 cells were infected with MER-Akt1 pWZL-neo, Akt1-S473A/T308A-pWZL-neo, or empty vector alone (pWZL-neo) as described previously (25); selection was performed in 2 mg/ml G418.

Isolation of Membrane Proteins and Western Blots of GLUT1—Cells were scraped in 2 ml of buffer (50 mM Hepes, pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 1 mM dithiothreitol, 1 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride), homogenized by 10 push/pull cycles using a 23 gauge needle and a 3-ml syringe, and the membranes were spun down at 4 °C for 1 h at 100,000 $\times$ *g* in a tabletop microcentrifuge (Beckman). The membrane pellet was washed once in the same volume of buffer, resolubilized in buffer containing 0.1% (v/v) Triton X-100, and analyzed by SDS-PAGE. The proteins were transferred to nitrocellulose membranes (2 h, 300 mA tank blotting) and the transfer was verified by Ponceau red staining before blocking the membrane with 3% bovine serum albumin in Tris-buffered saline-Tween (0.1%). The membranes were then incubated for 2 h in the first antibody (usually 1:5000 in 3% bovine serum albumin/Tris-buffered saline-Tween), washed briefly and incubated for another 1 h in secondary antibody (1:5000 in 3% fat-free milk/Tris-buffered saline-Tween). After extensive washing (Tris-buffered saline-Tween) the signal was visualized by chemiluminescence using a system containing luminol and coumaric acid.

Akt Assays—The Akt kinase assay using GSK-3 peptide (GRPRTSSFAEG) as substrate was performed as described previously (25). In brief, cells were lysed in 400 μl of lysis buffer/well. Endogenous Akt1, Akt3, or expressed HA-tagged MER-Akt1 was immunoprecipitated from the lysates using protein A-Sepharose beads that were preabsorbed with anti-Akt1 or Akt3 PH-domain antiserum or monoclonal anti-HA antibody (12CA5). Nonspecific background was measured by incubating lysates with protein A-Sepharose beads that were preabsorbed with either normal rabbit serum or normal mouse IgG. Following the kinase reaction, the phosphorylated peptide was separated from free [γ^{32}-P]ATP on a 40% polyacrylamide gel containing 6 M urea. The phosphopeptide spots were excised and counted.

Generation of the GLUT1 and β-Actin Probes—cDNA from total Hepa1c1c7 cell RNA was made by reverse transcription using oligo-dT as primer. A 1479-base pair *GLUT1* fragment was amplified by polymerase chain reaction using the sequences 5′-ATG GAT CCC AGC AGC AAG AAG GTG A-3′ and 5′-TCA CAC TTG GGA GTC CGC CCC GAG A-3′ as primer; for β-actin, a 540-base pair spanning fragment was generated utilizing 5′-GTG GGC CGC TCT AGG CAC CAA-3′ and 5′-CTC TTT GAT GTC ACG CAC GAT TTC-3′, respectively. For the Northern blots, 20 ng of the *GLUT1* or actin cDNAs were random primed with 50 μCi of [γ^{32}-P]dCTP. For the nuclear run-on assays, the actin and *GLUT1*-polymerase chain reaction fragments were gel-purified and subcloned into the pGEM-T vector using the TA cloning kit from Promega.

Extraction of Total RNA and Northern Blots—Total RNA was extracted from Hepa1c1c7 cells and examined by agarose gel electrophoresis. The RNA concentration was determined spectrophotometrically (Beckman DU 640). Five μg of total RNA/lane were separated on a 1% (w/v) denaturing agarose/formaldehyde gel (26) and transferred overnight in 20× SSC onto nylon membranes. The membranes were air-dried and the bound RNA UV-cross-linked (1200 μJ in 1 min) using a Stratalinker (Stratagene, La Jolla, CA). The blots were probed overnight at 65 °C, visualized by autoradiography, and quantified using a PhosphorImager and the Imagequant software (Molecular Dynamics, Sunnyvale, CA).

Nuclear Run-on Assays—The assays were performed as described (7). In short, confluent Hepa1c1c7 cells (10-cm dishes) stably expressing MER-Akt1 were serum-starved overnight and treated with insulin, hydroxytamoxifen, or vehicle (ethanol) for another 16–18 h in serum-free medium. The cells from 1 plate were then washed once with ice-cold PBS and scraped into 8 ml of lysis buffer/plate (10 mM Tris, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% Nonidet P-40). Nuclei were spun down for 5 min at 1000 $\times$ *g*, resuspended in 100 μl of storage buffer (20 mM Tris, pH 8.1, 100 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 50% v/v glycerol), flash-frozen in liquid nitrogen, and stored at −80 °C. To perform transcriptional run-ons, 100 μl of the nuclear preparation were mixed with 50 μl of 3× transcription buffer (50 mM Tris, pH 8.1; 210 mM KCl; 3 mM $MgCl_2$; 3 mM dithiothreitol; 2.4 mM of each ATP, GTP, and CTP; 1 μCi/μl [α^{32}-P]UTP; and 200 units/ml RNAsin). The mixture was incubated at 30 °C for 30 min. Total RNA was isolated by treating the mixture with DNase I (10 units for 30 min at 37 °C) and then proteinase K (50 μg for 1 h at 37 °C) and then extracting with phenol/chloroform. Free unincorporated nucleotides were separated from the RNA with a Sephadex-10 spin column. The RNA was fragmented into smaller pieces (300–600 bases in length) by incubation in 200 mM NaOH for 5–10 min at 4 °C and precipitated with ethanol. Hybridization of the RNA (approximately 1×10^6 cpm/ml) to the filter bound *actin* and *GLUT1* probes in the pGEM-T vector (2 μg DNA/slot) was performed at 65 °C for 60 h in Express-Hyb solution. The filters were then washed at least four times at 65 °C in 2× SSC/0.1% SDS and two more times in 0.2× SSC/0.1% SDS before they were exposed to the film. The blots were visualized by autoradiography and quantified using a PhosphorImager and the Imagequant software (Molecular Dynamics).

RESULTS

Induction of Akt Activity and GLUT1 mRNA in Mouse Hepatoma Cells—We found that insulin produces substantial increases in both Akt activity and GLUT1 mRNA in mouse hepatoma (Hepa1c1c7) cells. Therefore, we used this cell system to explore the relationship between Akt activation and *GLUT1* gene expression. In Hepa 1c1c7 cells, insulin induced Akt1 activity with an EC_{50} of about 1 nM (Fig. 1*A*); induction was maximal following a 20-min exposure (data not shown). Insulin induced GLUT1 mRNA with an EC_{50} of about 10 nM (Fig. 1*B*); the response was maximal after about 18 h (data not shown). The approximately 10-fold higher concentration of insulin required for GLUT1 mRNA induction presumably results from the longer incubation time required for this latter response. For example, we observed that, at a starting concentration of 10 nM, >90% of the input insulin was degraded after 9 h of incubation with the cells (data not shown). Both responses exhibit sensitivities to insulin similar to those reported for other systems (5, 20, 27). These findings suggested that Akt activation might be linked mechanistically to *GLUT1* gene expression; therefore, we performed experiments designed to test this possibility.

Expression of a Regulatable Akt1—To identify a possible relationship between Akt activity and *GLUT1* gene expression, we infected Hepa1c1c7 cells with a retroviral vector encoding MER-Akt1, which is a hydroxytamoxifen-regulatable version of an epitope-tagged Akt1 (25). We verified expression of the protein by treating cells with hydroxytamoxifen, lysing them, immunoprecipitating the epitope-tagged MER-Akt1, and measuring its enzymatic activity. Our findings demonstrate that hydroxytamoxifen causes a rapid and sustained increase in Akt enzymatic activity in cells that express MER-Akt1 (Fig. 2*A*); however, the increase does not occur in uninfected cells (data not shown). The time course of MER-Akt1 activation by hydroxytamoxifen is only slightly slower then the time course of endogenous Akt1 activation by insulin (Fig. 2*B*). The maximal activity of hydroxytamoxifen-stimulated MER-Akt1 in the anti-HA antibody precipitates (about 70,000 cpm) (Fig. 2*A*) is about 3 times the maximal activity of insulin-stimulated endogenous Akt1 (Fig. 2*B*). Immunoblotting studies of total ly-

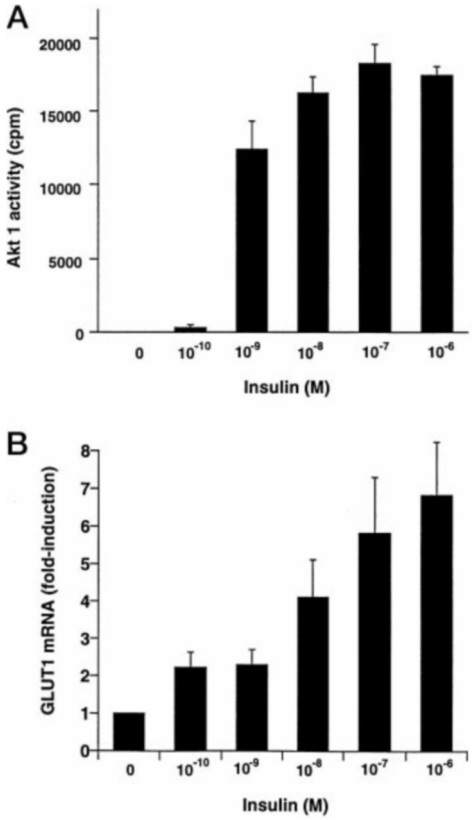

FIG. 1. **Insulin induces Akt1 activity and GLUT1 mRNA accumulation in a concentration-dependent fashion.** *A,* Akt1 activity. Hepa1c1c7 cells were serum-starved overnight, incubated for 30 min with the indicated insulin concentration, and lysed; endogenous Akt1 was immunoprecipitated and assayed for enzymatic activity using GSK3-peptide as substrate. *Bars* indicate the means (± S.E.) of three experiments. *B,* GLUT1 mRNA. Hepa1c1c7 cells were treated with the indicated concentration of insulin for 18 h and lysed; total RNA was isolated and assayed for GLUT1 and actin mRNA by Northern analysis. The autoradiograms from three experiments were scanned, normalized for the amounts of actin present, and expressed as the fold induction over the nontreated controls.

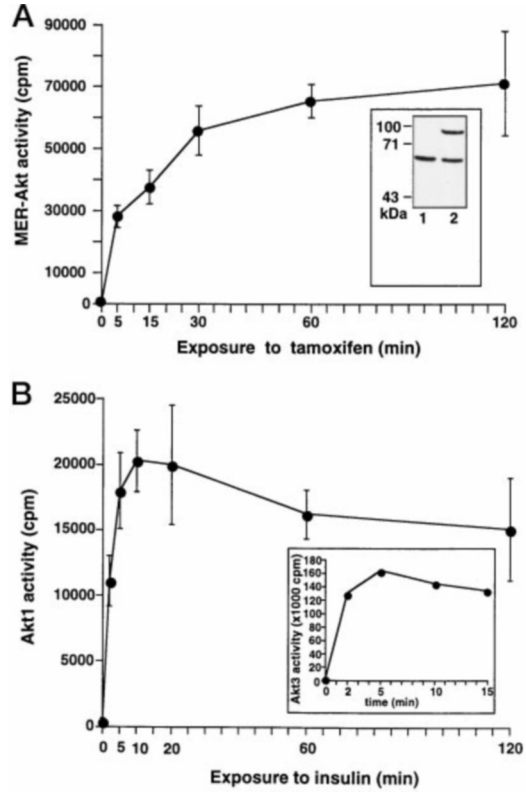

FIG. 2. **Time course of Akt activation.** *A,* activation of MER-Akt1 by hydroxytamoxifen. Cells were treated with 1 μM hydroxytamoxifen for the indicated periods of time and lysed, and the expressed HA-tagged MER-Akt1 was immunoprecipitated and assayed for enzyme activity. *Data points* indicate the means (± S.E.) of three experiments. The *inset* in *A* shows a Western blot of total cell lysate (10 μg of protein) obtained from cells infected with either empty retroviral vector (*lane 1*) or vector encoding MER-Akt1 (*lane 2*) probed with anti-Akt1 antibody. The higher molecular mass protein is the MER-Akt1, and the smaller protein is the endogenous Akt1. *B,* activation of endogenous Akt by insulin. Cells were treated with 1 μM insulin for the indicated periods of time and lysed, and endogenous Akt1 was immunoprecipitated and assayed for enzyme activity. *Data points* indicate the means (± S.E.) of three experiments. The *inset* in *B* shows the results of an assay for the insulin-stimulated endogenous Akt3.

sates indicate that the MER-Akt1 is expressed at a level comparable to that of the endogenous Akt1 (Fig. 2*A, inset*). Because Akt has three distinct isoforms, we also measured the activity of endogenous Akt2 and Akt3 in Hepa1c1c7 cells after stimulation with insulin. We observed that insulin stimulates endogenous Akt3 activity (Fig. 2*B, inset*) to a level that is about twice the maximal amount of hydroxytamoxifen-stimulated MER-Akt1 activity (Fig. 2*A*), whereas these cells contain only a low level of Akt2 activity (data not shown). These results indicate that the level of hydroxytamoxifen-stimulated MER-Akt1 activity in the infected cells is about half the level of insulin-stimulated total Akt activity in the parental cells. Therefore, the findings described below using cells containing hydroxytamoxifen-stimulated MER-Akt1 do not reflect artifacts related to overexpression of Akt enzyme activity.

To demonstrate a link between Akt activity and *GLUT1* gene expression, we asked whether hydroxytamoxifen induces GLUT1 mRNA accumulation in cells containing MER-Akt1. Our findings reveal that hydroxytamoxifen induces GLUT1 mRNA and GLUT1 protein to levels comparable to those that insulin induces in these cells (Fig. 3*A*). In control cells infected with an empty virus, hydroxytamoxifen does not induce GLUT1 mRNA or protein (Fig. 3*A*). We also measured the kinetics of hydroxytamoxifen-induced GLUT1 mRNA and protein accumulation; both responses reach plateaus after 24 h (Fig. 3, *B* and *C*). These kinetics are similar to those observed with insulin (data not shown). In addition, induction of GLUT1 mRNA and Akt1 activity both occur over the same concentration range of hydroxytamoxifen (Fig. 4). These findings, together with the observation that hydroxytamoxifen does not induce GLUT1 mRNA in cells that do not contain MER-Akt1, reveal that stimulation of Akt1 activity leads to an increase in GLUT1 mRNA. The simplest interpretation of these observations is that Akt1 regulates *GLUT1* gene expression.

Expression of an Enzymatically Inactive Akt1—To further examine the potential link between insulin, Akt, and *GLUT1* gene expression, we expressed an enzymatically inactive Akt1 mutant in Hepa1c1c7 cells. Immunoblotting studies indicate that the mutant Akt1 is expressed at levels that are comparable to those of the endogenous Akt1 protein (Fig. 5*A*). Induction experiments reveal that, in cells that express the mutant Akt1, the *GLUT1* gene exhibits about a 50% reduction in its response to insulin (Fig. 5*B*). In contrast, expression of the mutant Akt1 had no effect on the response of the *GLUT1* gene to hypoxia (data not shown). This selective inhibitory effect of the Akt mutant on the response of the *GLUT1* gene to insulin provides additional evidence that Akt is a component of the insulin-responsive signaling pathway that regulates *GLUT1* gene expression in Hepa1c1c7 cells.

Insulin and Akt Induce GLUT1 Transcription—In principle, insulin and Akt could induce GLUT1 mRNA accumulation by

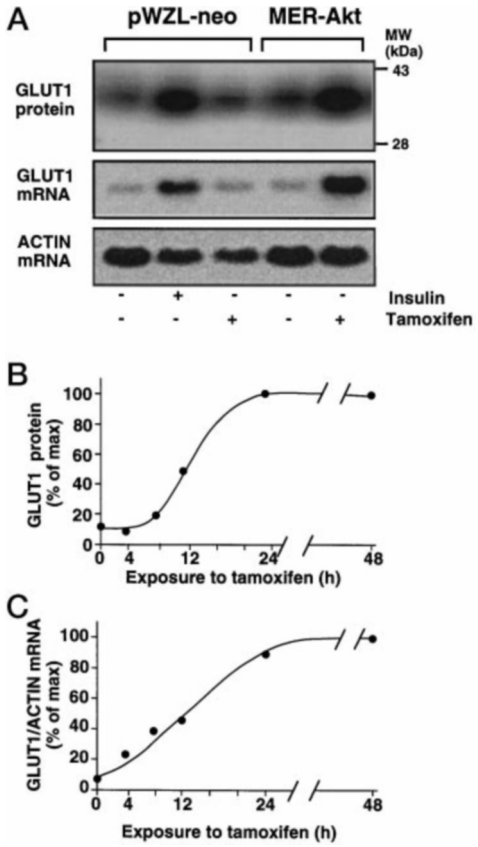

FIG. 3. **Insulin and Akt both induce Glut1 mRNA and protein in Hepa1c1c7 cells.** *A,* GLUT1 protein and mRNA induction by insulin and Akt. Cells infected with the empty retroviral vector (pWZL-neo) or cells stably expressing MER-Akt1 were incubated for 18 h with ethanol (as control), insulin (1 μM), or hydroxytamoxifen (1 μM). The cells were then lysed, and membrane proteins or total RNA was purified from the lysates. The *top panel* shows a Western blot of 10 μg of membrane protein probed with anti-Glut1-antibody. The *middle panel* shows an autoradiograph of a Northern blot probed for GLUT1 mRNA. The *bottom panel* shows a Northern blot probed for actin (as a control). *B,* time course of GLUT1 protein; *C,* time course of mRNA induction. Hepa cells expressing MER-Akt1 were stimulated with 1 μM hydroxytamoxifen for the times indicated, lysed, and analyzed for either GLUT1 protein or GLUT1 and actin mRNA, as described under "Experimental Procedures." The GLUT1 protein and mRNA levels (after normalization for the amounts of actin present) are expressed as the percentage of the highest values observed (at 24 and 48 h of treatment for the protein and mRNA, respectively). Results shown are representative of three experiments.

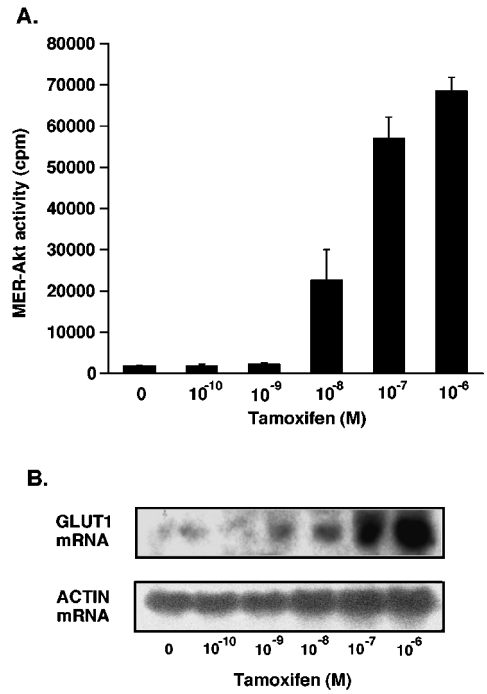

FIG. 4. **Concentration dependence of hydroxytamoxifen-stimulated MER-Akt1 activity and GLUT1 mRNA accumulation in Hepa1c1c7 cells.** Hepa1c1c7 cells stably expressing MER-Akt1 were serum-starved overnight and stimulated for 2 h (for Akt activity) or 18 h (for GLUT1 mRNA) with the indicated concentrations of hydroxytamoxifen. Cells were lysed and assayed for either MER-Akt1 activity (*A*) or GLUT1 mRNA (*B*) as described under "Experimental Procedures." *Data points* indicate the means (± S.E.) of three experiments.

increasing mRNA synthesis or by decreasing mRNA degradation. To distinguish between these possibilities, we used actinomycin D to inhibit mRNA synthesis, and we measured the half-life of GLUT1 mRNA in Hepa1c1c7 cells that stably express MER-Akt1. Our findings reveal no differences in the rates of GLUT1 mRNA decay in uninduced, insulin-induced, or hydroxytamoxifen-induced cells (Fig. 6). In all three cases, the half-life of GLUT1 mRNA is about 4 h. Therefore, these findings reveal no evidence for GLUT1 mRNA stabilization after hydroxytamoxifen or insulin treatment.

To test directly whether insulin and Akt activation stimulate *GLUT1* gene transcription, we performed nuclear run-on experiments using Hepa1c1c7 cells that stably express MER-Akt1. Our findings indicate that insulin and hydroxytamoxifen both increase *GLUT1* transcription about 3-fold (Fig. 7). These findings, together with the lack of change in mRNA degradation, imply that the induction of *GLUT1* gene expression arises primarily at the level of transcription.

DISCUSSION

In mammalian cells, a family of GLUT proteins mediates glucose uptake, thereby profoundly influencing cellular metabolism (1–3). The *GLUT1* gene, which encodes the most widely expressed glucose transporter, responds to several chemical and hormonal stimuli and makes an important contribution to the maintenance of intracellular homeostasis. Thus, an understanding of the events that control *GLUT1* gene expression may provide important insights into the molecular mechanisms by which cells adapt to changes in their environment.

Here, we show that an increase in *GLUT1* gene transcription substantially accounts for the accumulation of GLUT1 mRNA and protein that accompanies exposure of mouse hepatoma cells to insulin. Furthermore, we demonstrate that elevating Akt activity leads to increased *GLUT1* transcription and to mRNA and protein accumulation in the absence of insulin. Finally, we show that expression of a kinase inactive Akt1 inhibits the ability of insulin to induce GLUT1 mRNA. Therefore, we infer that an increase in Akt activity is an important event in the signaling pathway through which insulin regulates *GLUT1* gene expression.

Our findings are consistent with and extend prior observations that expression of a constitutively active Akt1 increases both the GLUT1 protein in 3T3-L1 adipocytes and the GLUT3 protein in L6 skeletal muscle cells (28, 29). Our observations are also consistent with a prior report implicating the $p21^{ras}$ protein in insulin-induced *GLUT1* gene expression (30) because $p21^{ras}$ can activate the PI 3-kinase/Akt pathway (31). In addition, our work is consistent with studies that implicate the mammalian target of rapamycin (mTOR) in the induction of the GLUT1 mRNA by insulin (7, 32) because Akt regulates mTOR (33), and we find that rapamycin inhibits the insulin-

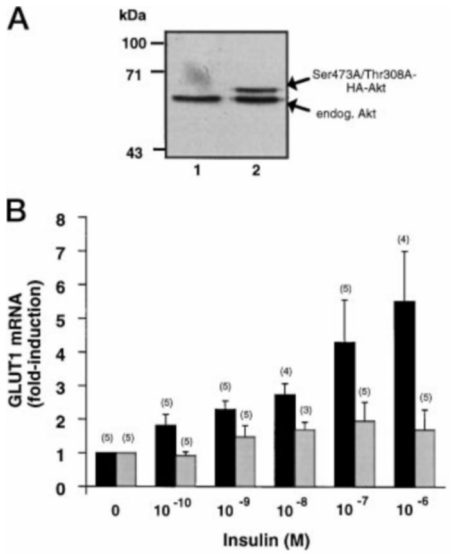

FIG. 5. **Effect of a kinase inactive Akt1 on the insulin-stimulated GLUT1 mRNA accumulation in Hepa1c1c7 cells.** *A,* expression of an inactive Akt1. Hepa1c1c7 cells were infected with either the empty retroviral vector or a retroviral vector encoding an inactive Akt1 mutant (Akt1-S473A/T308A-pWZL-neo). Amounts of the mutant Akt1 were determined by Western blotting of total cell lysates (10 μg/lane) from control cells (*lane 1*) and from cells infected with mutant Akt1 (*lane 2*). *B,* effect of inactive Akt1 on induction of GLUT1 mRNA by insulin. Hepa1c1c7 cells infected with either the empty retroviral vector (pWZL) or inactive Akt1 (Akt1-S473A/T308A-pWZL-neo) were serum-starved and treated for 18 h with the indicated concentrations of insulin. The cells were lysed, and total RNA was assayed for GLUT1 and actin mRNA by Northern analysis. The results were quantitated and are presented as fold inductions (± S.E.). *Black bars* represent data from the noninfected cells; *gray bars* represent the data from cells expressing inactive Akt1. Numbers in *parentheses* represent the number of experiments performed at the indicated insulin concentrations.

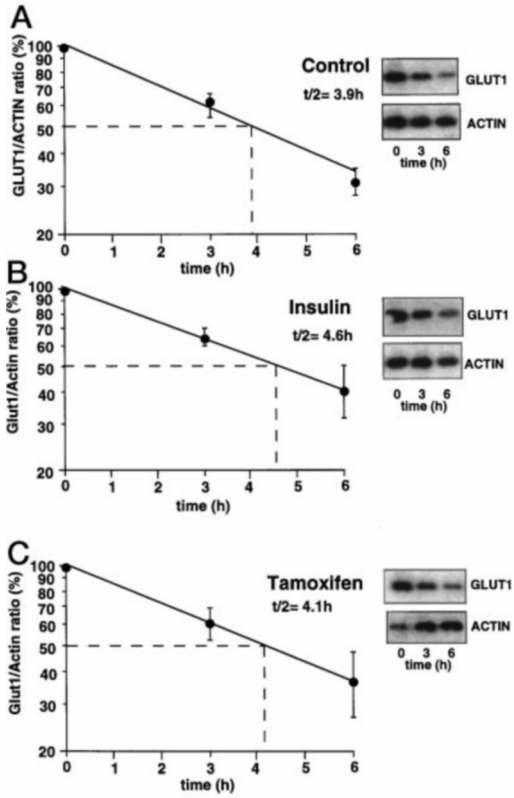

FIG. 6. **Effect of insulin and hydroxytamoxifen on the half-life of GLUT1 mRNA.** Hepa1c1c7 cells expressing MER-Akt1 were exposed to ethanol (as control) (*A*), insulin (1 μM) (*B*), or hydroxytamoxifen (1 μM) (*C*) in the presence of actinomycin D (5 μg/ml). Cells were harvested after 0, 3, or 6 h, and total RNA was assayed for GLUT1 and actin mRNA by blot hybridization. The autoradiograms shown are representative of three experiments. The amounts of GLUT1 and actin mRNA were quantified by phosphorimaging; the GLUT1 mRNA was normalized to actin and expressed as the percentage of GLUT1 mRNA at time 0. The values shown are means (± S.E.) of three experiments.

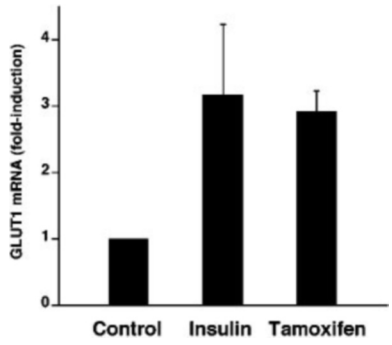

FIG. 7. **Nuclear run-on experiments.** Hepa1c1c7 cells expressing MER-Akt1 were exposed to ethanol (as a control), insulin (1 μM), or hydroxytamoxifen (1 μM). The cells were lysed, nuclei were isolated, and transcriptional run-on assays were performed as described under "Experimental Procedures." The radiolabeled mRNA was hybridized with immobilized DNA probes for GLUT1 and actin. The autoradiograms from three experiments were scanned, normalized for the amounts of actin present, and expressed as the fold induction over the nontreated controls.

induced increase in GLUT1 mRNA in the Hepa1c1c7 cells.[2] However, our findings are not consistent with a recent report that wortmannin, an inhibitor of the PI 3-kinase/Akt pathway, fails to inhibit the insulin-stimulated increase in *GLUT1* gene transcription in L6 skeletal muscle cells (32). Perhaps these findings reflect differences in cell type, because the $p21^{ras}$ protein also does not appear to play a role in insulin-induced *GLUT1* gene transcription in L6 cells (34). It is also possible that the lack of effect of wortmannin is due to the instability of this molecule. For example, we find that LY294002, another PI 3-kinase inhibitor (35), inhibits induction of GLUT1 mRNA by insulin in Hepa1c1c7 cells.[2]

From a mechanistic standpoint, we envision that Akt stimulates *GLUT1* transcription via phosphorylation of a particular protein(s). The target for phosphorylation might be a specific transcription factor(s) or a signaling component(s) that functions prior to the formation of a transcriptional complex at the GLUT1 promoter. The *GLUT1* gene contains multiple DNA elements that enhance transcription in concert with their cognate binding proteins. Akt might influence *GLUT1* gene expression by phosphorylating a protein that binds to the serum-responsive element, the cAMP-responsive element, and/or the 12-*O*-tetradecanoylphorbol-13-acetate-responsive element (26). For example, Akt might regulate *GLUT1* gene expression by its recently described ability to phosphorylate and regulate CREB (cAMP response element-binding protein) (36). Alternatively, insulin might regulate *GLUT1* gene expression via the hypoxia-responsive element and its cognate DNA-binding proteins hypoxia-inducible factor-1α and the aryl hydrocarbon receptor nuclear translocator (37). Akt could phosphorylate these transcription factors, thus influencing their interactions with each other or with DNA. A third possibility is that Akt could phosphorylate a cytoplasmic factor(s), thereby allowing the release of an associated transcription factor, as described for IκB kinase and activation of NF-κB (38). In addition, recent genetic studies have identified a forkhead transcription factor, DAF-16, that functions downstream of Akt in *Caenorhabditis*

[2] A. Barthel, S. T. Okino, J. Liao, K. Nakatani, J. Li, J. P. Whitlock, Jr., and R. A. Roth, unpublished observations.

elegans (39). Therefore, Akt might mediate *GLUT1* transcription via regulating a mammalian homolog of DAF-16. These appear to be interesting areas for future research.

Our studies demonstrate that activation of the Akt kinase cascade is sufficient to induce GLUT1 mRNA and protein accumulation via an increase in *GLUT1* gene transcription. The increase in GLUT1 activity may in part account for the ability of Akt to inhibit apoptosis because glucose uptake plays a critical role in regulating intracellular ATP levels and cell viability. Akt could also mediate the increase in *GLUT1* gene expression that follows hypoxia because such conditions can activate the PI 3-kinase/Akt pathway (40). However, we did not find that the PI 3-kinase inhibitor, LY294002, could block the hypoxia-induced GLUT1 mRNA accumulation. Thus, one of the roles of Akt in the maintenance of cellular homeostasis may be to integrate several types of environmental stimuli, leading to enhanced expression of a subset of genes, including *GLUT1*.

Acknowledgments—We thank Dr. Garry Nolan for the Phoenix retroviral packaging cell line and the retroviral vectors, Dr. Morris Birnbaum for the antibodies to Akt2, and Dr. Karlene Cimprich for a critical reading of the manuscript.

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