Dexamethasone-Induced Insulin Resistance in 3T3-L1 Adipocytes Is Due to Inhibition of Glucose Transport Rather Than Insulin Signal Transduction

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Glucocorticoids reportedly induce insulin resistance. In this study, we investigated the mechanism of glucocorticoid-induced insulin resistance using 3T3-L1 adipocytes in which treatment with dexamethasone has been shown to impair the insulin-induced increase in glucose uptake. In 3T3-L1 adipocytes treated with dexamethasone, the GLUT1 protein expression level was decreased by 30%, which possibly caused decreased basal glucose uptake. On the other hand, dexamethasone treatment did not alter the amount of GLUT4 protein in total cell lysates but decreased the insulin-stimulated GLUT4 translocation to the plasma membrane, which possibly caused decreased insulin-stimulated glucose uptake. Dexamethasone did not alter tyrosine phosphorylation of insulin receptors, and it significantly decreased protein expression and tyrosine phosphorylation of insulin receptor substrate (IRS)-1. Interestingly, however, protein expression and tyrosine phosphorylation of IRS-2 were increased. To investigate whether the reduced IRS-1 content is involved in insulin resistance, IRS-1 was overexpressed in dexamethasone-treated 3T3-L1 adipocytes using an adenovirus transfection system. Despite protein expression and phosphorylation levels of IRS-1 being normalized, insulin-induced 2-deoxy-D-[^{3}H]glucose uptake impaired by dexamethasone showed no significant improvement. Subsequently, we examined the effect of dexamethasone on the glucose uptake increase induced by overexpression of GLUT2-tagged p110α, constitutively active Akt (myristoylated Akt), oxidative stress (30 mU glucose oxidase for 2 h), 2 mmol/l 5-aminoimidazole-4-carboxamide ribonucleoside for 30 min, and osmotic shock (600 mmol/l sorbitol for 30 min). Dexamethasone treatment clearly inhibited the increases in glucose uptake produced by these agents. Thus, in conclusion, the GLUT1 decrease may be involved in the dexamethasone-induced decrease in basal glucose transport activity, and the mechanism of dexamethasone-induced insulin resistance in glucose transport activity (rather than the inhibition of phosphatidylinositol 3-kinase activation resulting from a decreased IRS-1 content) is likely to underlie impaired glucose transporter regulation. *Diabetes* 49:1700–1708, 2000

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2-DG, 2-deoxy-D-[^{3}H]glucose; AICAR, 5-aminoimidazole-4-carboxamide ribonucleoside; AMPK, 5′AMP-activated protein kinase; DMEM, Dulbecco's modified Eagle's medium; ECL, enhanced chemiluminescence; IRS, insulin receptor substrate; myrAkt, myristoylated Akt; PDK, phosphoinositide-dependent protein kinase; PI3-K, phosphatidylinositol 3-kinase; PI-3,4-P_2, phosphatidylinositol-3,4-diphosphate; PI-3,4,5-P_3, phosphatidylinositol-3,4,5-triphosphate; PMSF, phenylmethylsulfonyl fluoride.

Research has shown that glucocorticoids are hormones that induce insulin resistance and that their clinical use often exacerbates diabetes (1–3). This glucocorticoid-induced insulin resistance has been demonstrated not only in clinical cases but also in animal experiments (4,5) and in vitro experiments using isolated or cultured cells (6–8). For example, in skeletal muscle of glucocorticoid-treated rats, increases in glucose uptake induced by insulin, IGF-I, or hypoxia were found to be decreased (9,10). Similar observations were reported in primarily cultured adipocytes (11). Insulin-stimulated recruitment of GLUT4 to the cell surface is also reportedly inhibited by dexamethasone in muscle and adipose tissue (12).

To date, two possible mechanisms underlying dexamethasone-induced insulin resistance have been suggested. One possibility is the downregulation of insulin receptor substrate (IRS)-1 expression by dexamethasone (as has been observed in 3T3-L1 adipocytes [13,14]) because IRS-1 plays a major role in the activation of phosphatidylinositol 3-kinase (PI3-K), which is essential for GLUT4 translocation (15,16). On the other hand, in the liver, dexamethasone treatment reportedly decreased IRS-1 phosphorylation and IRS-1–associated PI3-K levels despite an increased IRS-1 protein content (17). When taking these reports into consideration, impaired PI3-K activation may be regarded as a cause of insulin resistance in both liver and muscle.

The other possibility is that dexamethasone impairs the GLUT4 translocation step independently of insulin signaling. This possibility may be supported by evidence that glucocorticoids inhibit not only insulin-induced but also hypoxia-induced GLUT4 translocation to the cell surface in skeletal muscle (10). Thus, whether the step in early insulin signaling in which IRS-1 is involved or whether the impairment of

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GLUT4 translocation machinery is the main cause of insulin resistance in muscle or adipose tissues remains unclear.

In this study, we attempted to determine which hypothesis is more likely to be the mechanism of dexamethasone-induced insulin resistance in adipocytes. For this purpose, we overexpressed IRS-1 in dexamethasone-treated 3T3-L1 cells using an adenovirus system to normalize the decreased IRS-1 content and examined the insulin responsiveness of glucose uptake. In addition, we investigated whether dexamethasone impairs the increased glucose uptake induced by overexpression of GLUT2-tagged p110α or constitutively active Akt (myristoylated Akt [myrAkt]), oxidative stress, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), or osmotic shock. Based on these results, we concluded that dexamethasone-induced insulin resistance affecting GLUT4 translocation in 3T3-L1 adipocytes is principally located beyond the early insulin-signaling step and is thus likely to involve the GLUT4 translocation machinery.

RESEARCH DESIGN AND METHODS

Antibodies. Affinity-purified antibodies against IRS-1, IRS-2, p85α, p110α, p110β, GLUT1, and GLUT4 were prepared as previously described (18,19). Antiphosphotyrosine monoclonal antibody (4G10) was purchased from Upstate Biotechnology (Lake Placid, NY). Anti-Akt antibody and anti-phospho-Akt (Ser[473]) antibody were purchased from New England Biolabs (Beverly, MA).
Cell culture. The 3T3-L1 fibroblasts were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% donor calf serum (Life Technologies) in an atmosphere of 10% CO_2 at 37°C. Two days after the fibroblasts had reached confluence, differentiation was induced by treating the cells with DMEM containing 0.5 mmol/l 3-isobutyl-1-methylxanthine, 4 μg/ml dexamethasone, and 10% fetal bovine serum for 48 h. Cells were fed DMEM supplemented with 10% fetal bovine serum every other day for the next 4–10 days. More than 90% of cells expressed the adipocyte phenotype.

The differentiated 3T3-L1 cells were treated with various concentrations of dexamethasone as indicated in the figure legends. H_2O_2 was generated by adding 30 mU/ml glucose oxidase (type II from *Aspergillus niger*; Sigma, St. Louis, MO) to serum-free DMEM supplemented with 0.5% bovine serum albumin for 2 h according to the method of Rudich and colleagues (20–22). AICAR stimulation was performed for 30 min by incubating the cells in Krebs-Ringer HEPES buffer containing 2 mmol AICAR after serum starvation for 3 h. Osmotic shock was performed for 30 min by incubating in base DMEM containing 600 mmol/l D-sorbitol after 2 h of serum starvation.
Gene transduction. Recombinant adenoviruses Adex1CA–IRS-1, Adex1CA-p110α, Adex1CA-myrAkt, and Adex1CA–Lac-Z were constructed by homologous recombination between the expression cosmid cassette containing corresponding cDNAs and the parental virus genome as described previously (23). The 3T3-L1 adipocytes were incubated with DMEM containing these adenoviruses for 6 h at 37°C, and growth medium was then added. Experiments were performed 2 days after infection with the adenoviruses. In the present study, recombinant adenoviruses were applied at a multiplicity of infection of ~200–300 plaque-forming units/cell, and 3T3-L1 adipocytes infected with Adex1CA–Lac-Z virus (23) were used as a control.
Immunoprecipitation and immunoblotting. The 3T3-L1 adipocytes in a 12-well culture dish were serum starved for 3 h in DMEM containing 0.2% bovine serum albumin. The cells were incubated with or without 10^{-6} mol/l insulin for 15 min, and then the cells were lysed at 4°C with ice-cold phosphate-buffered saline containing 1% Triton X-100, 1 μmol/l phenylmethylsulfonyl fluoride (PMSF), and 100 mmol/l sodium orthovanadate. Insoluble materials were removed by centrifugation at 15,000*g* for 10 min at 4°C. The cell lysates were incubated with the specific antibodies, and the immunocomplexes were precipitated with protein A or G sepharose (Pharmacia Biotech). These immunoprecipitates were subjected to SDS-PAGE, and immunoblotting using specific antibodies was then performed with enhanced chemiluminescence (ECL). Band intensities were quantified with a Molecular Imager GS-525 using Imaging Screen-CH.

To evaluate the translocation of GLUT4 to the plasma membrane, plasma membrane lawns were prepared by sonication as described previously (23). GLUT4 contents of the plasma membrane lawns were determined by immunoblotting using anti-GLUT4 antibody performed with an ECL kit.
PI3-K assay. After 3 h of serum starvation, 3T3-L1 adipocytes were incubated with or without 10^{-6} mol/l insulin for 15 min and were solubilized in ice-cold lysis buffer containing 20 mmol/l Tris, pH 7.5, 137 mmol/l NaCl, 1 mmol/l $CaCl_2$, 1 μmol/l PMSF, and 100 μmol/l sodium orthovanadate. Lysates were immunoprecipitated with anti–IRS-1 or anti-phosphotyrosine monoclonal antibody as described above. PI3-K activity in the immunoprecipitates was assayed as reported previously (24).
Glucose uptake. The cells were serum starved for 3 h. Next, glucose-free incubation was performed for 45 min in Krebs-Ringer phosphate buffer. Cells were then incubated with or without 10^{-6} mol/l insulin for 15 min, and 2-deoxy-D-[^{3}H]glucose (2-DG) uptake was measured as described previously (19).

RESULTS

Effect of dexamethasone on glucose transport activity and glucose transporter protein expression into 3T3-L1 adipocytes. The 24-h incubation with dexamethasone decreased 2-DG uptake into 3T3-L1 adipocytes under both basal and insulin-stimulated conditions. This effect of dexamethasone was concentration dependent, and 1 μmol/l dexamethasone decreased 2-DG uptake in the basal and 1-μmol/l insulin-stimulated conditions by 54 and 41%, respectively (Fig. 1*A*). Decreased 2-DG uptake resulting from dexamethasone was also observed when cells were stimulated with lower doses of insulin (Fig. 1*B*) or IGF-I (Fig. 1*C*). These dose-response curves suggest that dexamethasone impaired responsiveness to insulin or IGF-I but not sensitivity regarding glucose transport activity.

To determine the GLUT1 and GLUT4 glucose transporter expression levels in 3T3-L1 adipocytes, total cell lysates were immunoblotted with anti-GLUT1 antibody and anti-GLUT4 antibody, respectively. The amount of GLUT1 protein in the dexamethasone-treated cells was reduced to 70% of that in control cells (Fig. 2*A*). In contrast, expression of GLUT4 protein did not differ significantly between the dexamethasone-treated and control cells (Fig. 2*B*).

To assess GLUT4 translocation, we performed SDS-PAGE and Western blotting using anti-GLUT4 antibodies in plasma membrane lawns (Fig. 2*C*). We found that dexamethasone treatment decreased the insulin-stimulated GLUT4 translocation to the plasma membrane by 50%, which agrees well with the glucose uptake results.
Effect of dexamethasone on protein expression and tyrosine phosphorylation of IRS-1 and IRS-2. After 10^{-6} mol/l of insulin stimulation for 15 min, lysates from 3T3-L1 cells were immunoprecipitated and immunoblotted with anti-phosphotyrosine, anti–IRS-1, or anti–IRS-2 antibodies. Insulin-induced tyrosine phosphorylation of insulin receptors in 3T3-L1 adipocytes was not affected by dexamethasone treatment (Fig. 3*A*). Protein expression and insulin-induced tyrosine phosphorylation levels of IRS-1 in the dexamethasone-treated cells were decreased to 28 and 72% of those in control cells, respectively (Fig. 3*B* and *C*). Interestingly, protein expression and insulin-induced tyrosine phosphorylation levels of IRS-2 in the dexamethasone-treated cells were elevated to 154 and 145% of those in control cells, respectively (Fig. 3*D* and *E*).
Effects of dexamethasone on PI3-K subunit protein expression and insulin-induced PI3-K activation. Lysates from 3T3-L1 cells were immunoprecipitated and immunoblotted with anti-p85α, anti-p110α, or anti-p110β antibodies. The protein expression level of PI3-K subunits (i.e., p85α, p110α, and p110β) were not changed by dexamethasone treatment (data not shown).

PI3-K activity was assayed after insulin stimulation at with the indicated insulin or IGF-I concentrations for 15 min at

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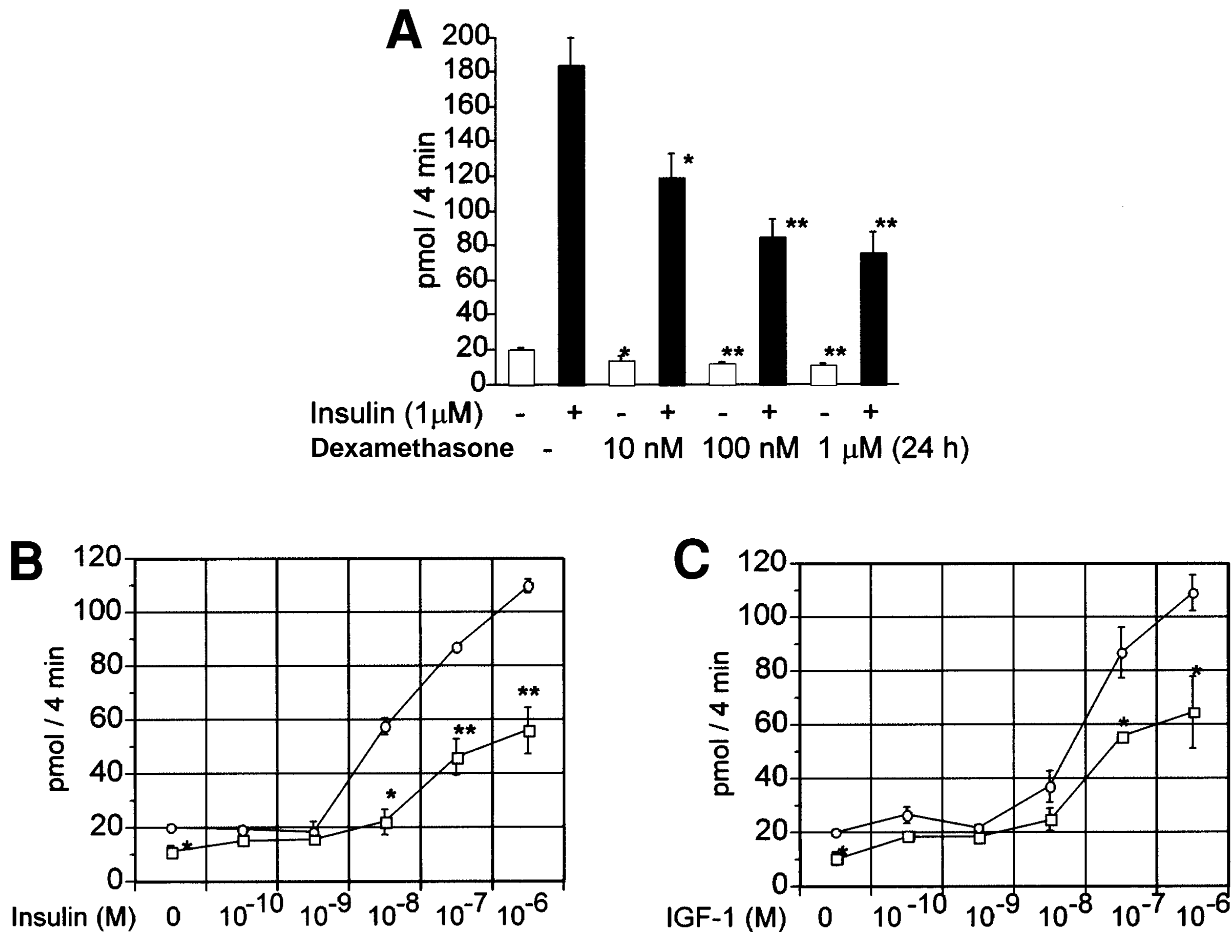

FIG. 1. Effect of dexamethasone on 2-DG uptake in 3T3-L1 adipocytes. *A*: The 3T3-L1 adipocytes were treated with the indicated dexamethasone concentrations for 24 h and then were incubated for an additional 15 min with or without insulin at 37°C. The assay was initiated by the addition of 2-DG. The assay was terminated after a 4-min incubation at 37°C by the addition of cold Krebs-Ringer phosphate buffer containing phloretin. *B* and *C*: The 3T3-L1 adipocytes were incubated with (□) or without (○) 1 μmol/l dexamethasone for 24 h and then were incubated for an additional 15 min with the indicated insulin (*B*) or IGF-I (*C*) concentrations at 37°C. The 2-DG uptake was assayed in these cells. Data are means ± SE of three independent experiments, each performed in triplicate. $^*P < 0.05$ and $^{**}P < 0.01$ vs. dexamethasone treatment (–).

37°C. PI3-K activity at 10^{-6} mol/l insulin stimulation in the anti-phosphotyrosine and anti–IRS-1 antibody immunoprecipitates prepared from dexamethasone-treated 3T3-L1 adipocytes were shown to be decreased to 61 and 65% of those from control cells, respectively (Fig. 4*A* and *C*). PI3-K activity at 10^{-6} mol/l IGF-I stimulation in the anti–IRS-1 antibody immunoprecipitates prepared from dexamethasone-treated 3T3-L1 adipocytes was shown to be decreased to 61% of those from control cells (Fig. 4*B*).

Effect of overexpressing IRS-1 on dexamethasone-induced insulin resistance. To reverse the reduction in IRS-1 expression caused by dexamethasone, an adenovirus containing IRS-1 cDNA was prepared. IRS-1 overexpression resulting from adenovirus transfection was shown to successfully normalize the level of IRS-1 expression in dexamethasone-treated cells (Fig. 5*A*). Similarly, the insulin-induced tyrosine phosphorylation level of IRS-1 was normalized and reached the control level (Fig. 5*B*). The association of PI3-K protein (p85 regulatory subunit) in anti–IRS-1 precipitates was also normalized (data not shown). However, the impaired insulin-induced 2-DG uptake showed minimal change, and glucose uptake into IRS-1 normalized dexamethasone-treated cells was only 51% of that from control cells (Fig. 5*C*).

Effect of dexamethasone on p110α-induced glucose transport activity. Next, we examined the effect of dexamethasone on glucose uptake stimulated by the overexpression of GLUT2-tagged p110α in 3T3-L1 adipocytes. The amount of p110α expressed was ~3 times that of endogenous p110α (Fig. 6*A*). In the absence of insulin, the overexpression of GLUT2-tagged p110α raised 2-DG uptake to 3.9-fold that of the control cells overexpressing Lac-Z, whereas that of control Lac-Z had no effect as reported previously (23). With dexamethasone treatment, the elevated glucose transport activity in p110α-overexpressing cells was significantly decreased by 56% (Fig. 6*B*).

Effects of dexamethasone on protein expression, Ser^{473} phosphorylation, and 2-DG uptake in myrAkt overexpressing 3T3-L1 adipocytes. First, we investigated whether dexamethasone treatment with dexamethasone affects the level of Akt expression or insulin-induced Ser phosphorylation of Akt. As shown in Fig. 7*A* and *B*, dex-

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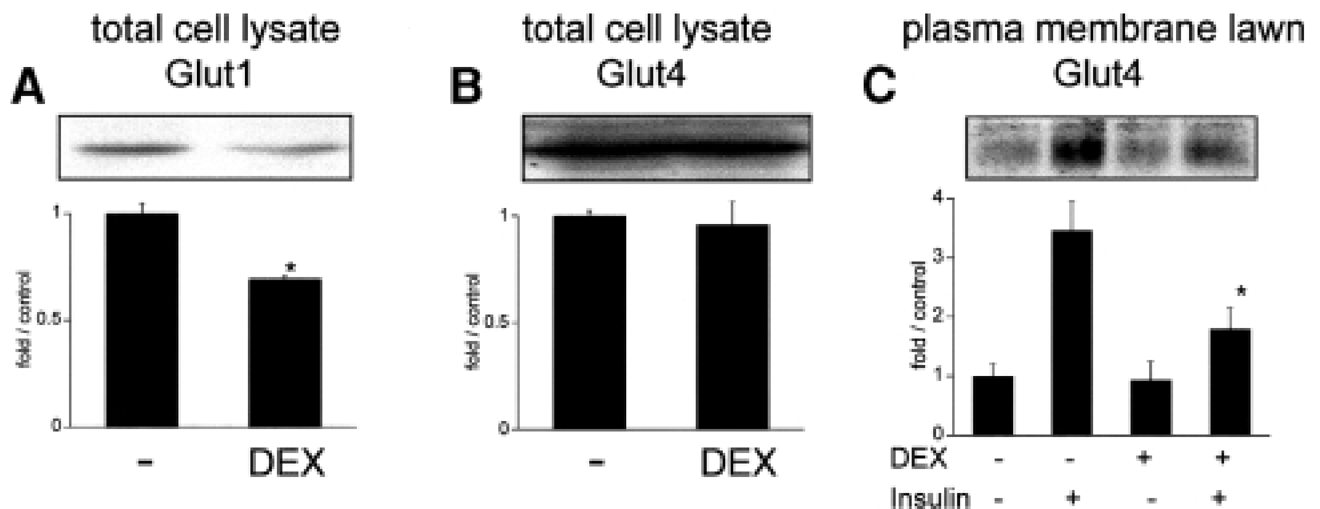

FIG. 2. Effect of dexamethasone (DEX) on glucose transporter protein expression and GLUT4 translocation in 3T3-L1 adipocytes. After incubation with or without 1 μmol/l dexamethasone for 24 h at 37°C, cellular contents of GLUT1 (*A*) and GLUT4 (*B*) were determined by immunoblotting using anti-GLUT1 antibody or anti-GLUT4 antibody performed with an ECL kit and then quantified and visualized with a Bio-Rad Molecular Imager (Richmond, CA). *C*: After treatment with or without 1 μmol/l dexamethasone for 24 h and an additional 15-min incubation with or without 100 nmol/l insulin at 37°C, plasma membrane lawns were prepared by sonication. GLUT4 contents of the plasma membrane lawns were determined by immunoblotting using anti-GLUT4 antibody performed with an ECL kit and were then quantified and visualized with a Bio-Rad Molecular Imager. Data are representative of three independent experiments. *$P < 0.05$ vs. dexamethasone treatment (–).

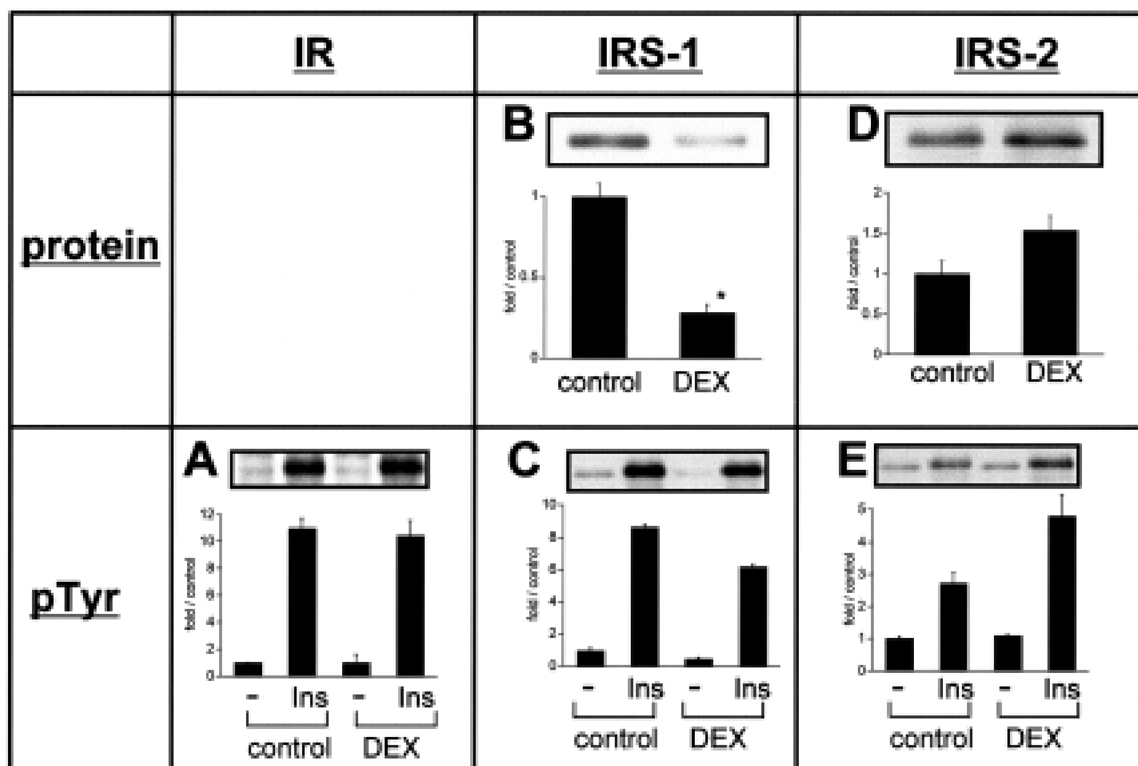

FIG. 3. Effect of dexamethasone (DEX) on protein expression and tyrosine phosphorylation of insulin receptor (IR), IRS-1, and IRS-2 3T3-L1 adipocytes treated with or without 1 μmol/l dexamethasone for 24 h and then incubated for an additional 15 min with or without insulin (Ins) at 37°C. The cells were solubilized, immunoprecipitated, and immunoblotted as described in RESEARCH DESIGN AND METHODS and then were quantified and visualized with a Bio-Rad Molecular Imager. *A*: Tyrosine phosphorylation of insulin receptors was detected using anti-phosphotyrosine antibody for immunoprecipitation and immunoblotting. *B*: Protein expression of IRS-1 was detected using anti–IRS-1 antibody for immunoprecipitation and immunoblotting. *C*: Tyrosine phosphorylation of IRS-1 was detected using anti–IRS-1 antibody for immunoprecipitation and anti-phosphotyrosine antibody for immunoblotting. *D*: Protein expression of IRS-2 was detected using anti–IRS-2 antibody for immunoprecipitation and immunoblotting. *E*: Tyrosine phosphorylation of IRS-2 was detected using anti–IRS-2 antibody for immunoprecipitation and anti-phosphotyrosine antibody for immunoblotting. Data are representative of three independent experiments. *$P < 0.05$ vs. control. pTyr, tyrosine phosphorylation.

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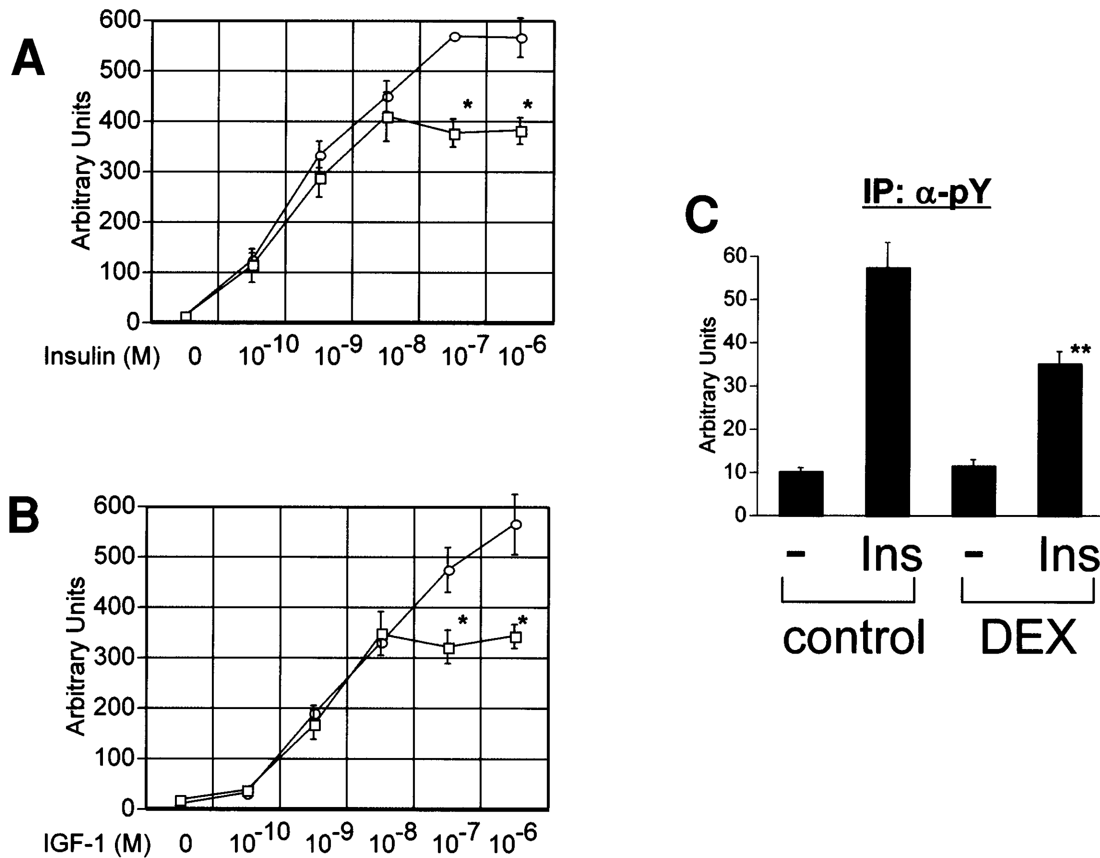

FIG. 4. Effect of dexamethasone (DEX) on PI3-K activities. ***A*** **and** ***B*****: After treatment with (□) or without (○) 1 μmol/l dexamethasone for 24 h and an additional 15-min incubation with the indicated insulin (Fig. 3*A*) or IGF-I (Fig. 3*B*) concentrations at 37°C, 3T3-L1 adipocytes were solubilized and immunoprecipitated using anti–IRS-1 antibody. PI3-K activities were assayed as described in RESEARCH DESIGN AND METHODS.** ***C*****: After treatment with or without 1 μmol/l dexamethasone for 24 h and an additional 15-min incubation with 10^{-6} mol/l insulin (Ins), PI3-K activities were assayed using anti-phosphotyrosine antibody (IP:α-pY). The resulting labeled lipids were extracted, separated by thin-layer chromatography, and then quantified with a Bio-Rad Molecular Imager. Data are means ± SE of three independent experiments. *$P < 0.05$ and **$P < 0.01$ vs. control.**

amethasone was demonstrated to have no effect on protein expression or the insulin-induced phosphorylation of Ser[473] of endogenous Akt.

Subsequently, we examined the effect of dexamethasone on glucose transport activity raised by the overexpression of constitutively active Akt. The amount of constitutively active Akt (myrAkt) expressed was approximately twice that of endogenous Akt (Fig. 7*A*). Although the Ser phosphorylation of endogenous Akt was observed almost exclusively in the presence of insulin, overexpressed myrAkt was highly Ser phosphorylated even in the absence of insulin, and insulin stimulation induced a slight additional increase in Ser phosphorylation (Fig. 7*B*). The overexpression of myrAkt in 3T3-L1 adipocytes increased glucose transport activity 8.2-fold compared with that of control Lac-Z adipocytes in the absence of insulin (Fig. 7*C*). These observations agree very well with those of previous reports (25). Although dexamethasone had no effect on either protein expression or Ser[473] phosphorylation of myrAkt in myrAkt-overexpressing 3T3-L1 adipocytes (Fig. 7*A* and *B*), 2-DG uptake into dexamethasone-treated cells was significantly lower (by 26%) than that into the non–dexamethasone-treated myrAkt-overexpressing cells (Fig. 7*C*).

Effects of dexamethasone on glucose uptake induced by osmotic shock, AICAR, and oxidative stress. Finally, we investigated the effect of dexamethasone on glucose transport activity increased via insulin-independent pathways. For this purpose, we used osmotic shock, AICAR, and oxidative stress, all of which have been reported to increase glucose transport activity without insulin receptor phosphorylation or PI3-K–Akt pathway activation (20–22,26–29). Osmotic shock (600 mmol/l sorbitol for 30 min) increased glucose transport activity 2.6-fold, whereas dexamethasone treatment suppressed glucose transport in the presence of sorbitol by ~58% (Fig. 8*A*). Similarly, 2 mmol/l AICAR for 30 min stimulated glucose transport 2.4-fold, whereas dexamethasone treatment suppressed glucose transport activity in the presence of AICAR by ~30% (Fig. 8*B*). Also similarly, oxidative stress (30 mU glucose oxidase for 2 h) increased glucose transport activity to 2.8-fold, whereas dexamethasone treatment suppressed glucose transport activity in the presence of glucose oxidase by ~43% (Fig. 8*C*).

DISCUSSION

Insulin resistance plays a major role in the onset and progression of diabetes. Although many factors cause insulin resistance (e.g., obesity, high-fat diets, insufficient exercise, stress), glucocorticoids are the most common hormones causing insulin resistance. Currently, although much remains unknown about how insulin exerts its metabolic function, researchers believe that the activation of PI3-K through IRS

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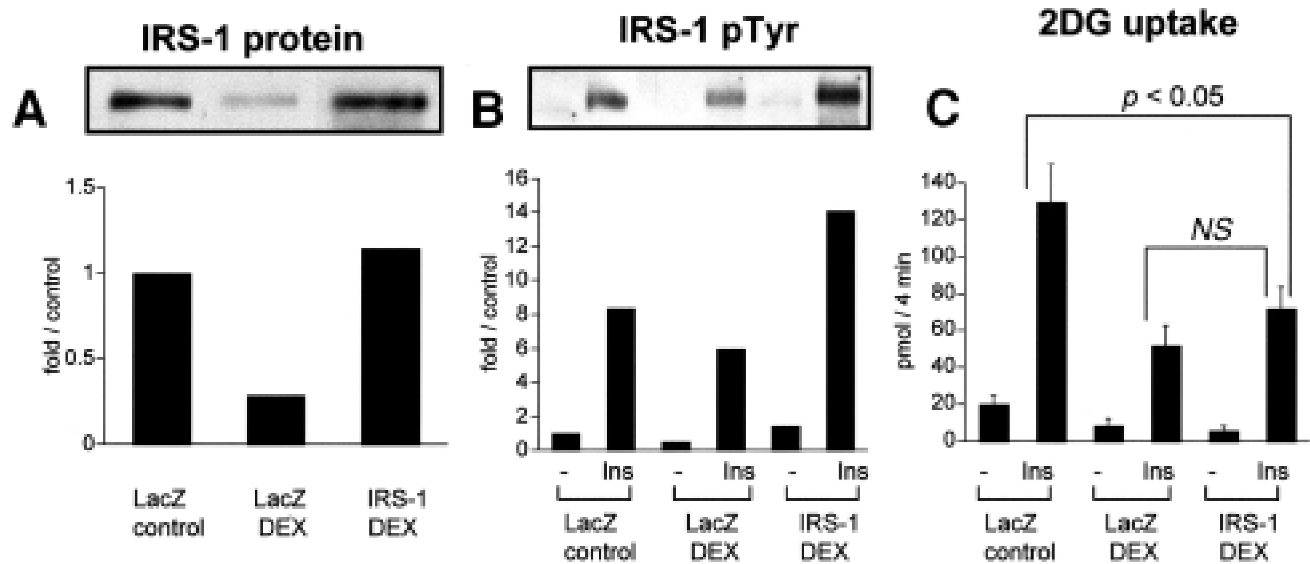

FIG. 5. Effect of overexpressing IRS-1 on dexamethasone (DEX)-induced insulin resistance. *A*, *B*, and *C*: The 3T3-L1 adipocytes overexpressing IRS-1 or Lac-Z were incubated with or without 1 μmol/l dexamethasone for 24 h and then were incubated for an additional 15 min with or without insulin (Ins) at 37°C. Cells were solubilized and immunoprecipitated using anti–IRS-1 antibody and were immunoblotted using anti–IRS-1 antibody (*A*) or anti-phosphotyrosine antibody (*B*). A representative blot and quantitation of three independent experiments are shown. *C*: The 2-DG uptake was assayed in these cells. Data are means ± SE of three independent experiments. pTyr, tyrosine phosphorylation.

proteins phosphorylated via the insulin receptor plays an important role in insulin's action in the liver, muscle, and adipose tissue (30). In addition, studies have demonstrated that most factors causing insulin resistance impair the early steps of insulin signaling leading to PI3-K activation (31–37).

Several studies have attempted to elucidate the molecular mechanism of dexamethasone-induced insulin resistance, and from the results obtained, three major hypothesizes have emerged: *1*) impaired PI3-K activation, *2*) downregulation of glucose transporter protein, and *3*) impairment of the translocation machinery of GLUT4. In dexamethasone-treated rats, PI3-K activation was shown to be impaired in both liver and muscle (17). In addition, the downregulation of IRS-1 by dexamethasone was reported in 3T3-L1 adipocytes (13).

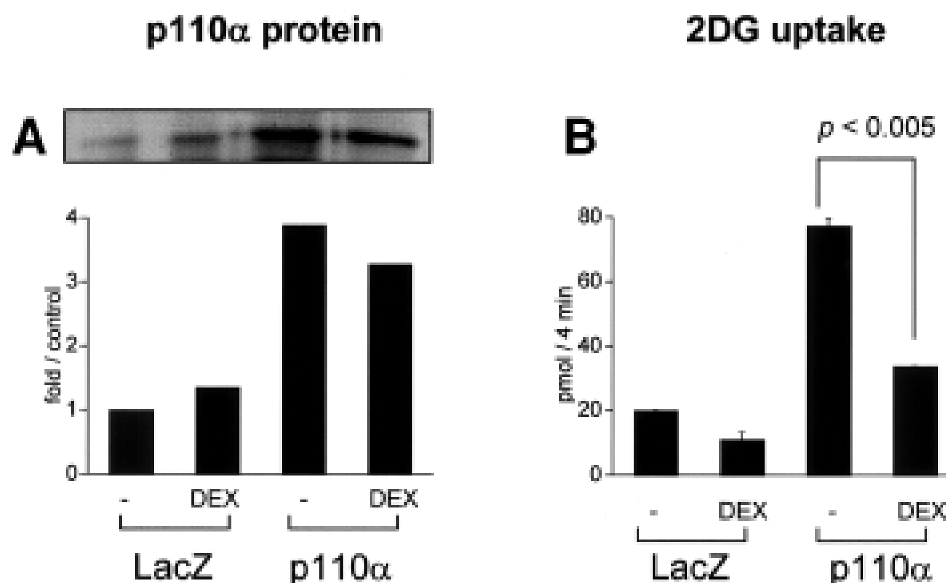

FIG. 6. Effect of dexamethasone (DEX) on p110α protein expression, PI3-K activities, and 2-DG uptake in p110α-overexpressing 3T3-L1 adipocytes. *A*: The protein expression level of p110α in p110α-overexpressing 3T3-L1 adipocytes. After treatment with or without 1 μmol/l dexamethasone for 24 h, cells were immunoprecipitated and immunoblotted with anti-p110α antibody. *B*: The 3T3-L1 adipocytes overexpressing p110α or Lac-Z were incubated with or without 1 μmol/l dexamethasone for 24 h and then for an additional 15 min with or without insulin at 37°C. The 2-DG uptake was assayed in these cells. Data are means ± SE of three independent experiments.

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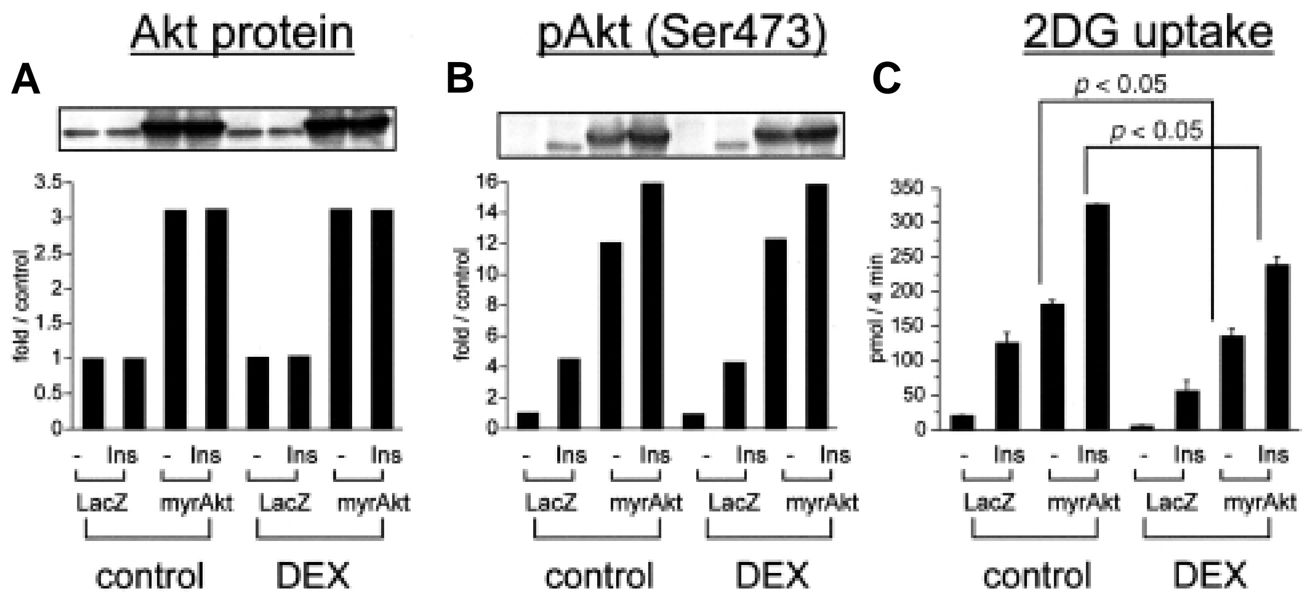

FIG. 7. Effects of dexamethasone (DEX) on protein expression, Ser phosphorylation, and 2-DG uptake in myrAkt-overexpressing 3T3-L1 adipocytes. The 3T3-L1 adipocytes overexpressing myrAkt or Lac-Z were incubated with or without 1 μmol/l dexamethasone for 24 h and then for an additional 15 min with or without insulin (Ins) at 37°C. Cells were solubilized and immunoblotted using anti-Akt antibody (*A*) and anti–phospho-Akt (Ser^{473}) antibody (*B*) and were then quantified and visualized with a Bio-Rad Molecular Imager. *C*: The 2-DG uptake was assayed in these cells. Data are representative of three independent experiments. pAkt, phosphorylated Akt.

In this study, we confirmed that dexamethasone decreases both basal and insulin-induced 2-DG uptake into 3T3-L1 cells and that decreased protein or tyrosine phosphorylation levels of IRS-1 in the dexamethasone-treated cells were comparable with those demonstrated in previous reports (13,14,38). These results are also consistent with primary cultured adipocyte studies conducted by Garvey et al. (11). Interestingly, protein expression and insulin-induced tyrosine phosphorylation levels of IRS-2 were increased in the dexamethasone-treated cells, but this may not have been enough to compensate for the decreased IRS-1 because PI3-K activities in the phosphotyrosine antibody immunoprecipitates were decreased in the dexamethasone-treated cells. Thus, we speculated that impairment of the early insulin signaling leading to PI3-K activation resulting from decreased IRS-1 expression may underlie the dexamethasone-induced insulin resistance. However, we found that normalization of the IRS-1 content reduced by dexamethasone did not ameliorate insulin resistance.

In addition, the observation that dexamethasone also decreased the glucose uptake induced by overexpression of p110α suggested that the major impaired step is located

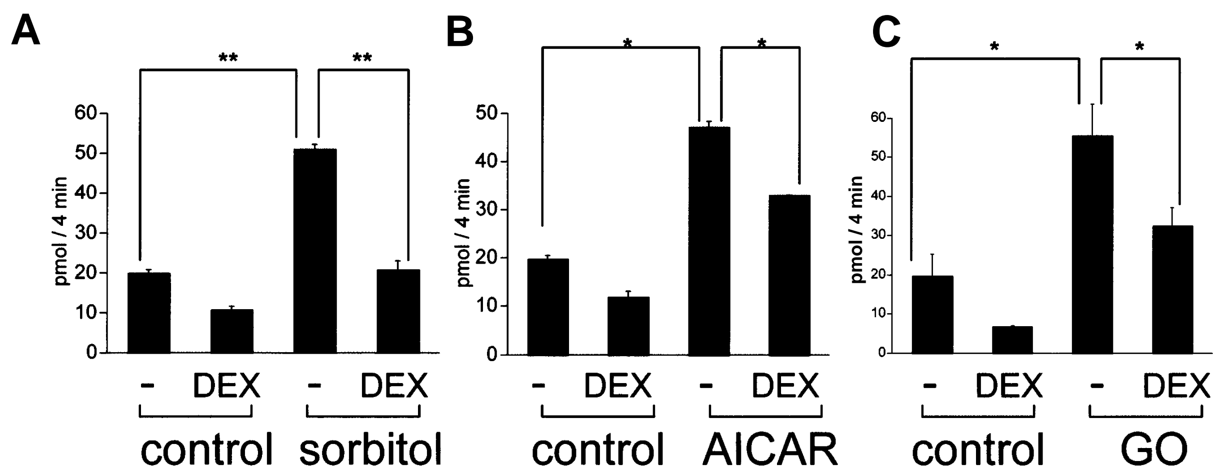

FIG. 8. Effect of dexamethasone (DEX) on insulin-independent enhancement of 2-DG uptake. After incubation with or without 1 μmol/l dexamethasone for 24 h, 3T3-L1 adipocytes were stimulated with 600 mmol/l sorbitol for 30 min (*A*), 2 mmol/l AICAR for 30 min (*B*), or 30 mmol/l glucose oxidase (GO) for 2 h (*C*). The 2-DG uptake was assayed as described in RESEARCH DESIGN AND METHODS. Data are means ± SE of three independent experiments. *$P < 0.05$; **$P < 0.01$.

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beyond early insulin signaling that precedes PI3-K activation. PI3-K produces phosphatidylinositol-3,4,5-triphosphate (PI-3,4,5-P_3) from phosphatidylinositol-4,5-diphosphate and phosphatidylinositol-3,4-diphosphate (PI-3,4-P_2) from phosphatidylinositol-4-phosphate. On binding with PI-3,4,5-P_3 or both PI-3,4,5-P_3 and PI-3,4-P_2, Akt is translocated to the plasma membrane, and the pleckstrin homology domain masking Thr^{308} in Akt is relieved. Akt is then sequentially phosphorylated at Thr^{308} by 3-phosphoinositide-dependent protein kinase (PDK) 1 and Ser^{473} by PDK2 (39). Membrane-targeted myristoylation of Akt reportedly becomes constitutively active and stimulates glucose uptake regardless of the presence of insulin (25). Although whether Akt is involved in insulin-induced glucose uptake remains controversial (25,40–45), one study recently reported that dominant-negative Akt (mutations K179A, T308A and S473A protein kinase B/Akt) markedly inhibits insulin-induced GLUT4 translocation (46). Thus, Akt/PKB may play a key role in the process of insulin-induced GLUT4 translocation. We found that dexamethasone treatment had no effect on protein expression or Ser^{473} phosphorylation of endogenous Akt, although dexamethasone treatment attenuated PI3-K activation. One possibility is that the PI3-K activity decreased by dexamethasone is still sufficient for full activation of Akt. Dexamethasone also had no effect on either protein expression or Ser^{473} phosphorylation of myristoylated Akt overexpressed in 3T3-L1 adipocytes. However, dexamethasone apparently inhibited myristoylated Akt-induced 2-DG uptake. Thus, the inhibitory effect of dexamethasone on glucose transport activity very likely lies downstream from PI3-K or Akt activation.

Glucose transport activity is reportedly enhanced by oxidative stress (20–22), osmotic shock (26,27), and AICAR (28,29) without insulin stimulation, as reported previously. Oxidative stress and osmotic shock stimulate GLUT4 translocation in the absence of insulin. AICAR has been reported to increase glucose uptake in isolated rat skeletal muscle via stimulation of 5′AMP-activated protein kinase (AMPK) activity, which has been shown to be increased during skeletal muscle contraction. The activation of AMPK activity by AICAR was also demonstrated in adipocytes (47). In 3T3-L1 adipocytes, we observed increased glucose uptake with AICAR stimulation. Studies have demonstrated that these stimulants do not affect insulin-induced phosphorylation of IRS-1 or PI3-K activation (21,27,48). Indeed, although the mechanisms of glucose transport activity enhancement by these agents remain unknown, osmotic shock or AICAR-induced glucose transport cannot be inhibited by wortmannin, which is a specific inhibitor of PI3-K. Dexamethasone treatment attenuated the increases in glucose transport activity induced by oxidative stress, osmotic shock, and AICAR. Thus, the inhibition of glucose transport by dexamethasone may be located at the final step of GLUT4 translocation.

Finally, we discuss the effect of dexamethasone on the expression of glucose transporter isoforms that, in turn, have effects on basal and insulin-stimulated glucose uptake. For example, in rat skeletal muscle and L6 muscle cells, both GLUT1 and GLUT4 protein expressions were reported to increase with dexamethasone treatment (9,49–52). However, in adipocytes, dexamethasone reportedly decreases GLUT1 protein expression but does not change GLUT4 protein expression (53,54). Our 3T3-L1 data were consistent with those reported for adipocytes (i.e., GLUT1 protein expression decreased to 70% of the normal level, whereas GLUT4 was unchanged with dexamethasone treatment). Thus, the decrease in GLUT1 protein observed herein may be attributable to decreased basal glucose uptake. The reduction in GLUT1 protein may also contribute to reduce insulin-induced glucose uptake. However, we do not consider this alteration to be a potential cause of the decrease in insulin-induced 2-DG uptake because GLUT4, which is mainly responsible for insulin-induced glucose uptake, was not changed by dexamethasone.

Glucocorticosteroids serve as nuclear receptor ligands and regulate DNA transcription. Thus, glucocorticosteroid stimulation affects the expression of various proteins. Glucose transport inhibition by dexamethasone may also be caused by altered expression of some unknown protein(s) (e.g., a decrease in motor protein in GLUT4-containing vesicles or an increase in the inhibitor of GLUT4 translocation). However, further research is required to assess these possibilities.

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