Glucocorticoid-Induced Insulin Resistance: Dexamethasone Inhibits the Activation of Glucose Transport in Rat Skeletal Muscle by Both Insulin- and Non-Insulin-Related Stimuli

Steven P. Weinstein, Tania Paquin, Alla Pritsker, and Richard S. Haber

To test the hypothesis that glucocorticoids inhibit muscle glucose transport apart from changes in early insulin-signaling events, we determined the effect of glucocorticoid treatment on the activation of glucose transport by both insulin and non-insulin-related stimuli (insulin-like growth factor [IGF] I and hypoxia) in rat skeletal muscle. Male Sprague-Dawley rats were treated with dexamethasone (Dex) (0.8 mg/kg for 2 days) and compared with pair-fed controls. 2-[^{3}H]deoxyglucose (2-[^{3}H]DG) uptake in isolated soleus muscles was measured under conditions in which uptake reflects glucose transport activity. In control muscles, 2-[^{3}H]DG uptake was stimulated 10-fold by insulin (10 nmol/l) or IGF-I (50 nmol/l) and sixfold by hypoxia. Dex treatment decreased 2-[^{3}H]DG uptake at all concentrations of insulin tested, reducing maximal insulin-stimulated 2-[^{3}H]DG uptake by 41 ± 11% (mean ± SE, $P < 0.05$) and basal 2-[^{3}H]DG uptake by 38 ± 6% ($P < 0.01$). Dex treatment also inhibited 2-[^{3}H]DG uptake at all concentrations of IGF-I tested, reducing maximal IGF-I–stimulated 2-[^{3}H]DG uptake by 29 ± 2% ($P < 0.01$), and decreased hypoxia-stimulated 2-[^{3}H]DG uptake by 61% ($P < 0.01$). Dex treatment increased soleus GLUT4 protein content by 11%. Thus, Dex treatment reduces basal glucose transport and decreases the maximal response of skeletal muscle glucose transport to insulin, the related hormone IGF-I, and the non-insulin-related stimulus hypoxia. These findings support the hypothesis that, in addition to altering early insulin-signaling events, glucocorticoids may also act by inhibiting the glucose transport system, per se, perhaps by affecting GLUT4 subcellular trafficking. *Diabetes* 44:441–445, 1995

The diabetogenic effect of glucocorticoid hormones results from both hepatic and peripheral resistance to the action of insulin (1). In the setting of glucocorticoid excess, insulin fails to normally suppress hepatic glucose production and to normally stimulate peripheral glucose utilization (2). The decrease in insulin-stimulated peripheral glucose utilization reflects reduced insulin-stimulated glucose uptake into skeletal muscle (1), the main site of insulin-mediated glucose disposal (3).

The mechanism by which glucocorticoids inhibit insulin-stimulated glucose uptake in skeletal muscle is unknown. Glucocorticoids could potentially inhibit glucose uptake at one or more points along the pathway through which insulin stimulates glucose transport. Insulin acts by binding to insulin receptors at the cell surface, resulting in receptor autophosphorylation and activation of receptor tyrosyl kinase activity (4). Although the intermediate insulin-signaling events involved in the activation of glucose transport are uncertain, the final event in skeletal muscle, as well as in other insulin-sensitive peripheral tissues (adipose tissue and heart), appears to be the translocation of GLUT4 from intracellular sites to the plasma membrane (5). The finding that glucocorticoids reduce maximal insulin-stimulated glucose transport in rat adipocytes (6–8) suggests that glucocorticoids inhibit glucose transport, at least in part, by a postreceptor mechanism (9). Indeed, studies in rat skeletal muscle have shown that insulin receptor levels and insulin binding are not reduced by glucocorticoid treatment (10–12). Several laboratories have investigated effects of glucocorticoids on postbinding events thought to be important in insulin signaling. Insulin-stimulated receptor autophosphorylation in muscle has been reported to be either unchanged (10,12) or decreased (11) by glucocorticoid treatment. Insulin-stimulated tyrosyl phosphorylation of insulin receptor substrate-1, a major substrate for the receptor tyrosyl kinase, was found to be unchanged in muscle (11,12), but glucocorticoids decreased the association/activation of phosphatidylinositol 3-kinase with insulin receptor substrate-1 in response to insulin (11,12).

In addition, glucocorticoids have been shown to reduce cellular glucose uptake even in the absence of insulin (13–17), suggesting that glucocorticoids might reduce insulin-stimulated glucose transport in skeletal muscle, in part, by inhibiting the glucose transport system per se. To test this hypothesis, we examined the effect of in vivo dexamethasone (Dex) treatment on the activation of glucose transport by insulin and by two other potent stimuli, insulin-like growth factor (IGF) I and hypoxia, in isolated rat soleus muscles. IGF-I stimulates glucose transport in skeletal muscle by binding to IGF-I receptors, activating a signaling pathway that appears to converge with that of insulin (18–20). Hypoxia stimulates glucose transport via a signaling pathway that appears to be distinct from that of insulin but similar to

From the Department of Medicine, Mount Sinai School of Medicine, New York, New York.

Address correspondence and reprint requests to Dr. Steven P. Weinstein, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1055, New York, NY 10029.

Received for publication 11 August 1994 and accepted in revised form 19 December 1994.

Dex, dexamethasone; 2-[^{3}H]DG, 2-[^{3}H]deoxyglucose; IGF, insulin-like growth factor.

that of contractile activity, a potent physiological stimulus of glucose transport in skeletal muscle (21–24). We report herein that Dex inhibits the activation of glucose transport in skeletal muscle by insulin, by the related hormone IGF-I, and by the non-insulin-related stimulus hypoxia. Our findings support the hypothesis that glucocorticoids reduce glucose transport in skeletal muscle, at least in part, by inhibiting the muscle glucose transport system per se.

RESEARCH DESIGN AND METHODS

Bovine insulin (24.4 IU/mg), radioimmunoassay-grade bovine serum albumin, and glucose assay kits (catalog no. 16–10) were obtained from Sigma (St. Louis, MO). Human recombinant IGF-I was purchased from Boehringer Mannheim (Indianapolis, IN). Dex sodium phosphate was from American Reagent Laboratories (Shirley, NY). 2-[1,2-^{3}H(*N*)]deoxy-D-glucose (26 Ci/mmol) and D-[1-^{14}C]mannitol (49 mCi/mmol) were purchased from Du Pont-NEN (Boston, MA).

Treatment of animals. Male Sprague-Dawley rats (100–110 g) were obtained from Charles River (Wilmington, MA) and were fed standard rat food. A short (2-day) period of Dex treatment was chosen to avoid substantial muscle atrophy, an effect of glucocorticoids that could potentially render difficult the interpretation of changes in muscle hexose uptake (25). Treated rats received Dex in drinking water at a concentration of 2.5 mg/l; control rats received tap water alone. In 11 experiments, the observed average daily water consumption in the Dex-treated groups was 32 ml (SD = 4 ml) and 29 ml (SD = 6 ml) on day 1 and day 2, respectively; based on these measurements, treated rats received a Dex dose of 0.8 $mg \cdot kg^{-1} \cdot day^{-1}$ (SD = 0.1 $mg \cdot kg^{-1} \cdot day^{-1}$). Because food intake was reduced in Dex-treated animals (11,17,26), control rats were pair-fed by giving all rats a daily ration of food equal to that eaten by treated rats in preliminary experiments. In 11 experiments, the daily food consumption in control and Dex-treated rats was 13 ± 1 (mean ± SE) and 13 ± 1 g, respectively, on day 1, and 12 ± 1 and 10 ± 1 g, respectively, on day 2. After 2 days of treatment, fasted (4-h) rats were killed by carbon dioxide inhalation, and soleus muscles were rapidly excised for hexose uptake measurements or preparation of detergent extracts for immunoblotting.

Determination of serum glucose concentration. Blood was obtained either by cardiac puncture immediately postmortem or from the femoral vein in anesthetized (sodium pentobarbital, 50 mg/kg i.p.) animals. Serum glucose was measured by the hexokinase method with a kit (see above).

Hexose uptake measurements in isolated soleus muscles. A modification of the method of Hansen et al. (27) was used to measure 2-[^{3}H]deoxyglucose (2-[^{3}H]DG) uptake under conditions in which uptake reflects glucose transport activity. Excised unsplit muscles were placed directly into small flasks and preincubated for 60 min at 29°C in gassed (95% O_2/5% CO_2) uptake buffer (Krebs-Henseleit bicarbonate buffer containing 6.5 mmol/l glucose, 2 mmol/l sodium pyruvate, and 0.1% bovine serum albumin). In experiments in which only insulin was used to stimulate glucose transport, muscles were incubated in the presence or absence of insulin (0–74 nmol/l) for 30 min, an interval sufficient for maximal insulin effect. In experiments in which IGF-I was used to stimulate glucose transport, muscles were incubated in the presence or absence of IGF-I (0–100 nmol/l) for 60 min, since the stimulation of glucose transport by IGF-I is maximal by this time (18). In experiments in which the effects of hypoxia and insulin were compared, muscles were incubated in the presence or absence of insulin or in uptake buffer gassed with 95% N_2/5% CO_2 (hypoxic uptake buffer) for 60 min, an interval sufficient for maximal stimulation of glucose transport by hypoxia (22). After washing and further incubation for 10 min in fresh glucose-free uptake buffer (with or without hormone additions, and either oxygenated or hypoxic, as appropriate), uptakes were started by adding tracer concentrations of 2-[1,2-^{3}H(*N*)]deoxy-D-glucose (1.5 μCi/ml, 57 nmol/l) and D-[1-^{14}C]mannitol (0.3 μCi/ml). Uptakes were routinely terminated after 30 min by blotting the muscles, quickly excising connective tissue, and freezing the muscles in liquid nitrogen. The weighed muscles were then extracted in 10% trichloroacetic acid, and ^{3}H and ^{14}C radioactivity in the extracts and in the uptake medium was determined by dual-channel liquid scintillation counting. The extracellular space was calculated from the uptake of [^{14}C]mannitol, and intracellular 2-[^{3}H]DG uptake was calculated by subtracting the amount of 2-[^{3}H]DG in the extracellular space from the total 2-[^{3}H]DG uptake. Uptakes under all conditions were linear for at least 30 min.

GLUT4 protein quantitation. Paired soleus muscles were excised from each animal, frozen in liquid N_2, weighed, and extracted in 30 vol of detergent (0.5% Triton X-100/0.5% sodium deoxycholate) buffer, as previously described (17). Protein concentration in the detergent extracts was determined in 1:10 dilutions in water by the method of Lowry et al. (28), with bovine serum albumin as a standard. GLUT4 protein in extracts was determined by quantitative immunoblotting with antiserum against the COOH-terminus of GLUT4, as previously described (17).

Statistical analysis. Unless noted otherwise, data are expressed as means ± SE. For single comparisons, *P* values were calculated by unpaired (two-tailed) Student's *t* test. Data involving multiple comparisons were analyzed by analysis of variance, and *P* values for differences between groups were calculated by a post hoc Tukey's protected *t* test, using GB-STAT software from Dynamic Microsystems (Silver Spring, MD).

RESULTS

Effect of 2-day Dex treatment regimen on body weight, muscle weight, and serum glucose. Animals in both control (n = 70) and Dex-treated (n = 70) groups initially weighed 104 ± 1 g; by the end of the treatment period (2 days), the weights of pair-fed control animals had increased to 116 ± 1 g, whereas the weights of Dex-treated animals had fallen to 98 ± 1 g. Soleus muscles from control and Dex-treated animals weighed 43 ± 1 mg (n = 132) and 38 ± 1 mg (n = 132), respectively. In three experiments, the serum glucose concentration was 10.8 ± 0.6 mmol/l (n = 22) and 19.7 ± 1.2 mmol/l (n = 22) in blood obtained immediately postmortem from control and Dex-treated animals, respectively ($P < 0.001$). In an additional experiment in which blood was obtained from anesthetized animals, the serum glucose concentration was 7.2 ± 1.3 and 13.4 ± 1.6 mmol/l in control and Dex-treated animals (n = 4 per group), respectively ($P < 0.1$).

Effect of Dex treatment on basal and insulin-stimulated 2-[^{3}H]DG uptake in skeletal muscle. Figure 1 shows the concentration-response relationship for the action of insulin on 2-[^{3}H]DG uptake in soleus muscles from control and Dex-treated rats. In control muscles, maximally effective concentrations of insulin increased 2-[^{3}H]DG uptake ~10-fold. (The fold increase at 10 nmol/l insulin was 9.5 ± 1.1 in four independent experiments.) 2-[^{3}H]DG uptake was maximal or near maximal at 10 nmol/l insulin in muscles from both control and Dex-treated animals. 2-[^{3}H]DG uptake in muscles from Dex-treated animals was markedly reduced at all concentrations of insulin. In four experiments, Dex treatment reduced maximal insulin-stimulated (10 nmol/l) 2-[^{3}H]DG uptake by 41 ± 11% (mean ± SE of the inhibition in each experiment) ($P < 0.05$). Insulin-stimulated (10 nmol/l) 2-[^{3}H]DG uptake was 76 ± 2 $pmol \cdot g^{-1} \cdot 30\ min^{-1}$ in control muscles (n = 18) and 46 ± 4 $pmol \cdot g^{-1} \cdot 30\ min^{-1}$ in Dex-treated muscles (n = 18, $P < 0.001$). In addition, Dex reduced basal 2-[^{3}H]DG uptake by 38 ± 6% ($P < 0.01$). Basal 2-[^{3}H]DG uptake was 8.2 ± 0.6 $pmol \cdot g^{-1} \cdot 30\ min^{-1}$ in control muscles (n = 16) and 5.1 ± 0.5 $pmol \cdot g^{-1} \cdot 30\ min^{-1}$ in Dex-treated muscles (n = 16) ($P < 0.002$).

Effect of Dex treatment on IGF-I–stimulated 2-[^{3}H]DG uptake in skeletal muscle. To determine whether glucocorticoids inhibit the activation of muscle glucose uptake by the related hormone IGF-I, the effect of IGF-I on 2-[^{3}H]DG uptake was examined in soleus muscles from control and Dex-treated rats (Fig. 2). 2-[^{3}H]DG uptake was maximal or near maximal at 50 nmol/l IGF-I in muscles from both control and Dex-treated animals, and 2-[^{3}H]DG uptake in muscles

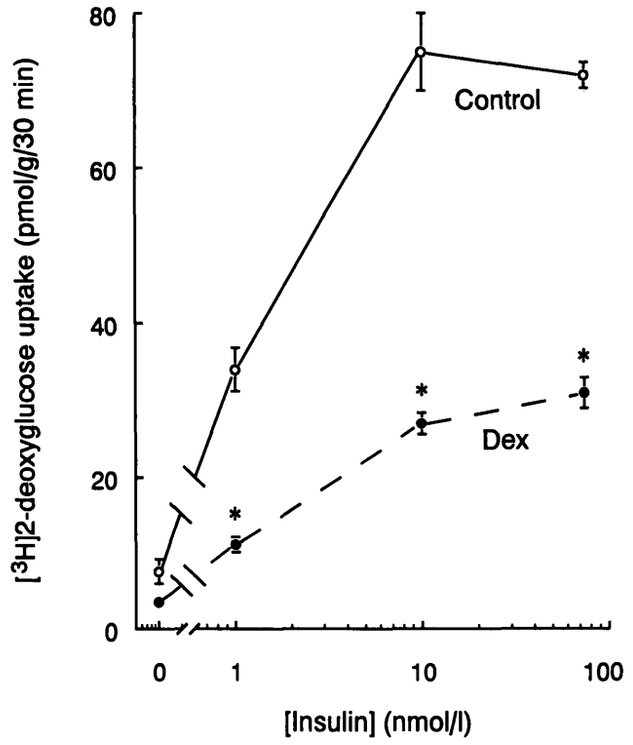

FIG. 1. Concentration-response curve for the effect of insulin on 2-[^{3}H]DG uptake in isolated soleus muscles from control and Dex-treated rats. Rats were treated with or without Dex for 2 days, after which 2-[^{3}H]DG uptake was measured in isolated soleus muscles at 0, 1, 10, and 74 nmol/l insulin, as described in METHODS. Each point represents the mean value of four muscles from separate rats. Error bars indicate 1 SE; the SE for the Dex-treated zero-insulin group is too small to illustrate. *P < 0.01 for Dex-treated vs. control rats at the same insulin concentration.

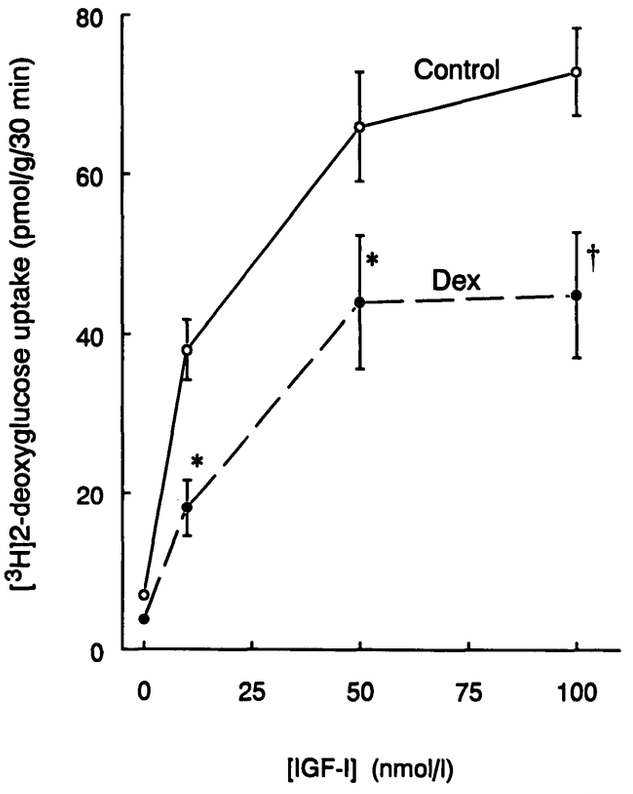

FIG. 2. Concentration-response curve for the effect of IGF-I on 2-[^{3}H]DG uptake in isolated soleus muscles from control and Dex-treated rats. Uptakes were measured at 0, 10, 50, and 100 nmol/l IGF-I. Each point represents the mean value of four muscles from separate rats. Error bars represent 1 SE; the SE for the Dex-treated zero–IGF-I group is too small to illustrate. *P < 0.05 for Dex-treated vs. control rats at the same IGF-I concentration; †P < 0.01 for Dex-treated vs. control rats at the same IGF-I concentration.

from Dex-treated animals was reduced at all concentrations of IGF-I. IGF-I (50 nmol/l) stimulated 2-[^{3}H]DG uptake 9.6 ± 1.6-fold (n = three experiments) in control muscles, an effect comparable to that of a maximally effective concentration of insulin (see above) (18,29,30). Dex treatment reduced IGF-I–stimulated (50 nmol/l) 2-[^{3}H]DG uptake by 29 ± 2% (percent inhibition in three experiments; P < 0.01). IGF-I–stimulated (50 nmol/l) 2-[^{3}H]DG uptake was 69 ± 2 pmol · g^{-1} · 30 min^{-1} in control muscles (n = 14) and 47 ± 3 pmol · g^{-1} · 30 min^{-1} in Dex-treated muscles (n = 14) (P < 0.001).

Effect of Dex treatment on hypoxia-stimulated 2-[^{3}H]DG uptake in skeletal muscle. To determine whether glucocorticoids inhibit the activation of muscle glucose transport by a non-insulin-related stimulus, the effect of hypoxia on 2-[^{3}H]DG uptake was examined in soleus muscles from control and Dex-treated rats (Fig. 3). In experiments in which the effect of hypoxia was compared with that of insulin, hypoxia stimulated 2-[^{3}H]DG uptake 6.4-fold, and insulin at 1 and 10 nmol/l stimulated 2-[^{3}H]DG uptake 7.3- and 10-fold, respectively, in control muscles (Fig. 3). Dex treatment inhibited hypoxia-stimulated 2-[^{3}H]DG uptake by 61% (P < 0.01) and reduced insulin-stimulated 2-[^{3}H]DG uptake by 63% (P < 0.01) and 42% (P < 0.01) at 1 and 10 nmol/l insulin, respectively (Fig. 3).

Effect of Dex treatment on GLUT4 protein content in skeletal muscle. We have previously reported that treatment of older rats (250–275 g) with Dex for 7 days decreases insulin-stimulated glucose uptake without reducing GLUT4

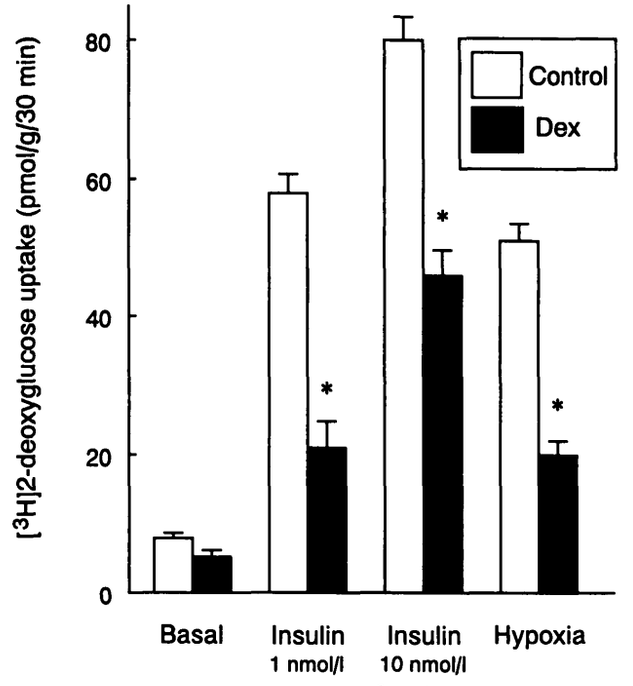

FIG. 3. Effect of insulin and hypoxia on 2-[^{3}H]DG uptake in isolated soleus muscles from control and Dex-treated rats. Each bar represents the mean value of eight muscles from separate rats (data are pooled from two experiments). Error bars indicate 1 SE. *P < 0.01 for Dex-treated vs. control rats.

protein content in skeletal muscle (17). To confirm that the effects of the current 2-day Dex treatment regimen on muscle glucose transport were not due to reductions in GLUT4 protein content, detergent extracts of soleus muscles were analyzed by quantitative immunoblotting. Detergent-extractable protein yields in soleus muscles from control and Dex-treated animals were similar (76 ± 3 and 80 ± 2 mg/g [n = 8 animals per group], respectively; $P > 0.2$). GLUT4 content per gram of extract protein in soleus muscles, expressed as a percentage of the mean control value in each of two experiments, was 100 ± 3% in control subjects and 111 ± 3% in Dex-treated animals (n = 8 animals per group) ($P < 0.05$), indicating that GLUT4 protein content in soleus muscles actually increased slightly with Dex treatment, as previously reported (17,26).

DISCUSSION

Activation of glucose transport in skeletal muscle occurs in response to a number of stimuli. Insulin and related hormones (e.g., IGF-I) stimulate glucose transport via a signaling pathway that appears to be distinct from that of non-insulin-related stimuli (e.g., hypoxia and contractile activity) (18–24). Indeed, mounting evidence suggests that activation of glucose transport by these two pathways may be mediated by translocation of GLUT4 to the cell surface from different intracellular pools (22,31,32). Therefore, to test the hypothesis that, in addition to altering early insulin-signaling events, glucocorticoids reduce muscle glucose transport by inhibiting the glucose transport system per se, we examined the effect of in vivo Dex treatment on the activation of 2-[^{3}H]DG uptake by insulin, the related hormone IGF-I, and the non-insulin-related stimulus hypoxia in isolated rat soleus muscles.

Treatment of animals with Dex for 2 days caused weight loss and hyperglycemia, which are expected effects of glucocorticoid excess. Dex treatment reduced basal and insulin-stimulated hexose uptake in skeletal muscle, an effect previously shown for Dex and other glucocorticoids (15–17,26,33,34). The decrease in basal hexose uptake suggests that glucocorticoids exert an action on the glucose transport system in muscle that is independent of any effects on insulin signal transduction. Moreover, the concentration-response relationship shown herein for the action of insulin on 2-[^{3}H]DG uptake (Fig. 1) clearly demonstrates that glucocorticoids reduce maximal insulin-stimulated hexose uptake in skeletal muscle, consistent with an effect of glucocorticoids distal to the insulin receptor (9).

We next examined the effect of Dex treatment on the response of soleus muscles to two other activators of glucose transport, IGF-I and hypoxia. Dex treatment reduced 2-[^{3}H]DG uptake at all concentrations of IGF-I tested (Fig. 2), decreasing maximal IGF-I–stimulated 2-[^{3}H]DG uptake by 29 ± 2%. This observation indicates that the inhibitory effect of glucocorticoids on insulin-stimulated glucose transport in skeletal muscle does not depend solely on alterations at the level of the insulin receptor per se. In other hyperinsulinemic models of insulin resistance (e.g., gold thioglucose-obese mice, obese [*fa/fa*] Zucker rats, old rats, and spontaneously diabetic BB/W rats), IGF-I–stimulated skeletal muscle hexose uptake or IGF-I–stimulated whole body hexose disposal is also reduced (18,30,35,36).

The response of 2-[^{3}H]DG uptake to the non-insulin-related stimulus hypoxia was also inhibited (by 61%) in skeletal muscle from Dex-treated animals (Fig. 3). To the extent that insulin and hypoxia stimulate glucose transport via separate pathways, the finding that glucocorticoids inhibit the stimulation of muscle glucose transport by both pathways suggests that in addition to altering early insulin-signaling events, glucocorticoids may act, in part, by inhibiting the glucose transport system per se. Such an effect of glucocorticoids on the glucose transport system in muscle may be viewed in the context of current understanding of GLUT4 subcellular trafficking. In the basal state, most GLUT4 resides intracellularly, but insulin rapidly induces a reversible increase in the fractional partitioning of GLUT4 to the surface pool, resulting in increased glucose transport (5,37,38). In skeletal muscle, contractile activity and hypoxia have also been shown to acutely increase plasma membrane GLUT4 content (22,31). Studies examining the mechanism of GLUT4 translocation in adipocytes have demonstrated that insulin increases the rate constant for incorporation of intracellular GLUT4 into the plasma membrane, with either no change (39) or a decrease (40) in the rate constant for internalization of plasma membrane GLUT4. In rat adipocytes, the glucocorticoid-induced inhibition of basal and insulin-stimulated glucose transport is associated with a redistribution of glucose transporters from the plasma membrane to intracellular sites (7,8). Based on these observations and the results presented herein, we postulate that glucocorticoids reduce skeletal muscle glucose transport, at least in part, by altering the kinetics of GLUT4 subcellular trafficking in favor of increased GLUT4 partitioning to intracellular sites. In this context, glucocorticoids might act by inhibiting the incorporation of intracellular GLUT4 into the plasma membrane and/or by promoting the internalization of plasma membrane GLUT4. Such an action could explain the observation that glucocorticoids inhibit basal, as well as stimulated, glucose transport in skeletal muscle and adipocytes. Alternatively, glucocorticoids might inhibit a distal signaling event common to the pathway activated by insulin and by IGF-I and the pathway activated by non-insulin-related stimuli or might reduce the intrinsic transport activity of GLUT4. Investigations aimed at elucidating the effect of glucocorticoids on subcellular GLUT4 trafficking in skeletal muscle are likely to provide additional insights into the mechanisms involved in glucocorticoid-induced insulin resistance.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Research Grants DK-02057 and DK-41674 and by an Irma T. Hirschl Career Scientist Award (R.S.H.).

Parts of this study were published previously in abstract form in *Diabetes* 43 (Suppl. 1):229A, 1994.

REFERENCES

1. Amatruda JM, Livingston JN, Lockwood DH: Cellular mechanisms in selected states of insulin resistance: human obesity, glucocorticoid excess, and chronic renal failure. *Diabetes Metab Rev* 1:293–317, 1985
2. Pagano G, Cavallo-Perin P, Cassader M, Bruno A, Ozzello A, Masciola P, Dall'omo AM, Imbimbo B: An in vivo and in vitro study of the mechanism of prednisone-induced insulin resistance in healthy subjects. *J Clin Invest* 72:1814–1820, 1983
3. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP: The effect of insulin on the disposal of intravenous glucose: results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes* 30:1000–1007, 1981
4. Goldfine ID: The insulin receptor: molecular biology and transmembrane signaling. *Endocr Rev* 8:235–255, 1987

5. Pessin JE, Bell GI: Mammalian facilitative glucose transporter family: structure and molecular regulation. *Annu Rev Physiol* 54:911–930, 1992
6. Olefsky JM: Effect of dexamethasone on insulin binding, glucose transport, and glucose oxidation of isolated rat adipocytes. *J Clin Invest* 56:1499–1508, 1975
7. Carter-Su C, Okamoto K: Effect of insulin and glucocorticoids on glucose transporters in rat adipocytes. *Am J Physiol* 252:E441–E453, 1987
8. Garvey WT, Huecksteadt TP, Monzon R, Marshall S: Dexamethasone regulates the glucose transport system in primary cultured adipocytes: different mechanisms of insulin resistance after acute and chronic exposure. *Endocrinology* 124:2063–2073, 1989
9. Kahn C: Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. *Metab Clin Exp* 27:1893–1902, 1978
10. Block NE, Buse MG: Effects of hypercortisolemia and diabetes on skeletal muscle insulin receptor function in vitro and in vivo. *Am J Physiol* 256:E39–E48, 1989
11. Giorgino F, Almahfouz A, Goodyear LJ, Smith RJ: Glucocorticoid regulation of insulin receptor and substrate IRS-1 tyrosine phosphorylation in rat skeletal muscle in vivo. *J Clin Invest* 91:2020–2030, 1993
12. Saad MJA, Folli F, Kahn JA, Kahn CR: Modulation of insulin receptor, insulin receptor substrate-1, and phosphatidylinositol 3-kinase in liver and muscle of dexamethasone-treated rats. *J Clin Invest* 92:2065–2072, 1993
13. Horner HC, Munck A, Lienhard GE: Dexamethasone causes translocation of glucose transporters from the plasma membrane to an intracellular site in human fibroblasts. *J Biol Chem* 262:17696-17702, 1987
14. Grunfeld C, Baird K, Van Obberghen E, Kahn CR: Glucocorticoid-induced insulin resistance in vitro: evidence for both receptor and postreceptor defects. *Endocrinology* 109:1723–1730, 1981
15. Herman MS, Ramey ER: Effect of hydrocortisone and epinephrine on glucose uptake by the rat diaphragm. *Endocrinology* 67:650–656, 1960
16. Skillman CA, Fletcher HP: The effect of insulin and hydrocortisone on the in vivo tissue uptake of 2-deoxyglucose in mice. *J Pharmacol Methods* 12:125–140, 1984
17. Haber RS, Weinstein SP: Role of glucose transporters in glucocorticoid-induced insulin resistance: GLUT4 isoform in rat skeletal muscle is not decreased by dexamethasone. *Diabetes* 41:728–735, 1992
18. Poggi C, Le Marchand-Brustel Y, Zapf J, Froesch ER, Freychet P: Effects and binding of insulin-like growth factor I in the isolated soleus muscle of lean and obese mice: comparison with insulin. *Endocrinology* 105: 723–730, 1979
19. Yu K-T, Czech MP: The type I insulin-like growth factor receptor mediates the rapid effects of multiplication-stimulating activity on membrane transport systems in rat soleus. *J Biol Chem* 259:3090–3095, 1984
20. Keller SR, Lamphere L, Lavan BE, Kuhne MR, Lienhard GE: Insulin and IGF-I signaling through the insulin receptor substrate 1. *Mol Reprod Dev* 35:346–352, 1993
21. Randle PJ, Smith GH: Regulation of glucose uptake by muscle. 2. The effects of insulin, anaerobiosis and cell poisons on the penetration of isolated rat diaphragm by sugars. *Biochem J* 70:501–508, 1958
22. Cartee GD, Douen AG, Ramlal T, Klip A, Holloszy JO: Stimulation of glucose transport in skeletal muscle by hypoxia. *J Appl Physiol* 70:1593–1600, 1991
23. Holloszy JO, Narahara HT: Studies on tissue permeability. X. Changes in permeability to 3-*O*-methylglucose associated with contraction of isolated frog muscle. *J Biol Chem* 240:3493–3500, 1965
24. Nesher R, Karl IE, Kipnis DM: Dissociation of effects of insulin and contraction on glucose transport in rat epitrochlearis muscle. *Am J Physiol* 249:C226–C232, 1985
25. Henriksen EJ, Holloszy JO: Effect of diffusion distance on measurement of rat skeletal muscle glucose transport in vitro. *Acta Physiol Scand* 143:381–386, 1991
26. Guillaume-Gentil C, Assimacopoulos-Jeannet F, Jeanrenaud B: Involvement of non-esterified fatty acid oxidation in glucocorticoid-induced peripheral insulin resistance in vivo in rats. *Diabetologia* 36:899–906, 1993
27. Hansen PA, Gulve EA, Holloszy JO: Suitability of 2-deoxyglucose for in vitro measurement of glucose transport activity in skeletal muscle. *J Appl Physiol* 76:979–985, 1994
28. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275, 1951
29. Dimitriadis G, Parry-Billings M, Bevan S, Dunger D, Piva T, Krause U, Wegener G, Newsholme EA: Effects of insulin-like growth factor I on the rates of glucose transport and utilization in rat skeletal muscle in vitro. *Biochem J* 285:269–274, 1992
30. Dardevet D, Sornet C, Attaix D, Baracos VE, Grizard J: Insulin-like growth factor 1 and insulin resistance in skeletal muscles of adult and old rats. *Endocrinology* 134:1475–1484, 1994
31. Douen AG, Ramlal T, Rastogi S, Bilan PJ, Cartee GD, Vranic M, Holloszy JO, Klip A: Exercise induces recruitment of the "insulin-responsive glucose transporter." *J Biol Chem* 265:13427-13430, 1990
32. Coderre L, Vallega G, Pilch PF: Association of GLUT4 vesicles with glycogen particles in skeletal muscle: identification of a contraction-sensitive pool (Abstract). *Diabetes* 43 (Suppl. 1):159A, 1994
33. Riddick FA, Reisler DM, Kipnis DM: The sugar transport system in striated muscle: effect of growth hormone, hydrocortisone and alloxan diabetes. *Diabetes* 11:171–178, 1962
34. Marfaing P, Ktorza A, Berthault MF, Predine J, Picon L, Penicaud L: Effects of counterregulatory hormones on insulin-induced glucose utilization by individual tissues in rats. *Diabete Metab* 17:55–60, 1991
35. Jacob RJ, Sherwin RS, Greenawalt K, Shulman GI: Simultaneous insulin-like growth factor I and insulin resistance in obese Zucker rats. *Diabetes* 41:691–697, 1992
36. Jacob RJ, Sherwin RS, Bowen L, Fryburg D, Fagin KD, Tamborlane WV, Shulman GI: Metabolic effects of IGF-I and insulin in spontaneously diabetic BB/w rats. *Am J Physiol* 260:E262–E268, 1991
37. Wilson CM, Cushman SW: Insulin stimulation of glucose transport activity in rat skeletal muscle: increase in cell surface GLUT4 as assessed by photolabelling. *Biochem J* 299:755–759, 1994
38. Lund S, Holman GD, Schmitz O, Pedersen O: GLUT4 content in the plasma membrane of rat skeletal muscle: comparative studies of the subcellular fractionation method and the exofacial photolabelling technique using ATB-BMPA. *FEBS Lett* 330:312–318, 1993
39. Satoh S, Nishimura H, Clark AE, Kozka IJ, Vannucci SJ, Simpson IA, Quon MJ, Cushman SW, Holman GD: Use of bismannose photolabel to elucidate insulin-regulated GLUT4 subcellular trafficking kinetics in rat adipose cells. *J Biol Chem* 268:17820-17829, 1993
40. Jhun BH, Rampal AL, Liu H, Lachaal M, Jung CY: Effects of insulin on steady state kinetics of GLUT4 subcellular distribution in rat adipocytes: evidence of constitutive GLUT4 recycling. *J Biol Chem* 267:17710-17715, 1992