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The Insulin-dependent Biosynthesis of GLUT1 and GLUT3 Glucose Transporters in L6 Muscle Cells Is Mediated by Distinct Pathways

ROLES OF p21^{ras} AND pp70 S6 KINASE*

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Insulin binding results in rapid phosphorylation of insulin receptor substrate-1 to activate p21^{ras} and mitogen-activated protein kinase. Insulin also activates the ribosomal protein S6 kinase (pp70 S6 kinase) independently of the Ras pathway. Chronic (18 h) treatment of L6 muscle cells with insulin increases glucose transport activity severalfold due to biosynthetic elevation of the GLUT1 and GLUT3 but not the GLUT4 glucose transporters. Here we investigate the roles of p21^{ras} and pp70 S6 kinase in the insulin-mediated increases in GLUT1 and GLUT3 expression. L6 cells were transfected with the dominant negative Ras(S17N) under the control of a dexamethasone-inducible promoter. Induction of Ras(S17N) failed to block the insulin-mediated increase in GLUT1 glucose transporter protein and mRNA; however, it abrogated the insulin-mediated increase in GLUT3 glucose transporter protein and mRNA. Inhibition of pp70 S6 kinase by rapamycin, on the other hand, eliminated the insulin-mediated increase in GLUT1 but had no effect on that of GLUT3 in both parental and Ras(S17N) transfected L6 cells. These results suggest that the biosynthetic regulation of glucose transporters is differentially determined, with pp70 S6 kinase and p21^{ras} playing active roles in the insulin-stimulated increases in GLUT1 and GLUT3, respectively.

Insulin mediates a wide spectrum of biological responses including stimulation of glucose influx and metabolism in muscle and adipocytes, transport of amino acids, transcription of specific genes and mitogenesis (1, 2). These are determined by signals initiated by insulin binding, leading to rapid autophosphorylation of receptor tyrosine residues (3) and tyrosine phosphorylation of Shc (4) and IRS-1.[1] IRS-1 serves as a docking protein for Src homology 2 (SH2) domain proteins including phosphatidylinositol 3-kinase, and GRB2, an adaptor protein linked to Sos, a guanine nucleotide exchange factor (3, 5). Association of IRS-1 or Shc with GRB2-Sos results in the release of GDP from p21^{ras} and a consequent increase in the amount of GTP-p21^{ras} (5). Like all GTP-binding proteins, p21^{ras} cycles between inactive GDP-bound and active GTP-bound conformations (6). Ras binds directly to the serine/threonine kinase Raf-1, which in turn phosphorylates and activates MAPK/ERK kinase to phosphorylate and activate MAPK (ERK) (7–10). Insulin also stimulates the ribosomal protein S6 kinases pp70 S6 kinase and pp90 S6 kinase (3, 11). The two S6 kinases, originally identified by their ability to phosphorylate the 40 S ribosomal protein S6, are regulated by distinct mechanisms, as MAPK directly phosphorylates and activates pp90 S6 kinase but has no effect on pp70 S6 kinase (3, 5), whereas phosphatidylinositol 3-kinase is required for activation of pp70 S6 kinase but not pp90 S6 kinase or MAPK (12).

The L6 cell line is derived from neonatal rat thigh skeletal muscle and retains several properties of skeletal muscle (13, 14). During all stages of their development, L6 cells express the GLUT1 glucose transporter, a ubiquitous isoform that is widely distributed and is believed to provide cells with basal glucose requirements (15). They also express the GLUT3 glucose transporter, which is expressed in fetal (16) and regenerating muscle (17) and in neuronal cells of the brain (18). In contrast, the fat/muscle-specific GLUT4 glucose transporter is not expressed until alignment and onset of cell fusion into myotubes (15, 19, 20). Our laboratory has previously demonstrated that in response to chronic treatment with insulin (for several hours), glucose transport activity increases severalfold due to an increase in GLUT1 mRNA and protein levels (2, 21). We also observed that sustained insulin-like growth factor-1 treatment leads to an increase in GLUT3 mRNA and protein levels (22). By contrast, prolonged insulin treatment was associated with a small decrease in the levels of GLUT4 protein and mRNA (21). Similar observations on GLUT1 and GLUT4 have been made in 3T3-F442A adipocytes (23).

Despite long standing recognition of the signaling cascades that link the insulin receptor to the nucleus, it remains unknown whether they participate in the insulin-induced regulation of expression of specific genes with the exception of those encoding for transcription factors (24). Therefore, the objective of this study was to investigate the role of the signaling cascade, in particular the role of p21^{ras} and pp70 S6 kinase, in the insulin-induced increases in GLUT1 and GLUT3 expression after prolonged (18 h) treatment with the hormone. Using a constructed L6 cell line transfected with a dominant negative Ras, Ras(S17N) under the control of a dexamethasone-inducible promoter (25), and using rapamycin, a specific inhibitor of pp70 S6 kinase (11, 26), we investigated the roles of p21^{ras} and pp70 S6 kinase in the insulin-mediated regulation of expression of GLUT1 and GLUT3 protein and mRNA.

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[1] The abbreviations used are: IRS-1, insulin receptor substrate-1; GRB2, growth receptor-bound protein 2; MAPK, mitogen-activated protein kinase; ERK, extracellular signal regulated kinase; pp70 S6 kinase, 70-kDa ribosomal protein S6 kinase; pp90 S6 kinase, 90-kDa ribosomal protein S6 kinase; GLUT, glucose transporter; CHO, Chinese hamster ovary.

EXPERIMENTAL PROCEDURES

Materials—Porcine insulin was obtained from Sigma. Rapamycin was purchased from Calbiochem. Polyclonal anti-GLUT1 (Ra-GLUTRANS) antibody was purchased from East Acres Biologicals (Southbridge, MA). Polyclonal anti-mouse GLUT3 antibody was a kind gift from Dr. I. Simpson (National Institutes of Health). The monoclonal antibody 6H to the α1 subunit of the Na^+/K^+-ATPase was a kind gift from Dr. M. Caplan (Department of Cellular and Molecular Physiology, Yale University). Monoclonal anti-phosphotyrosine antibody was purchased from Upstate Biotechnology Inc. (Lake Placid, NY). Plasmids containing full-length cDNAs for GLUT1 (prGT4–12) and GLUT3 (pmGLUT3–6) were kindly provided, respectively, by Dr. M. Birnbaum (Department of Cell Biology, Harvard Medical School) and Dr. C. F. Burant (Department of Medicine, University of Chicago).

Cell Culture and Incubations—Construction of the rat L6 skeletal muscle cell line transfected with a plasmid containing Ras(S17N) under a mouse mammary tumor virus promoter inducible by dexamethasone was described previously (25). Parental cells and cells transfected with Ras(S17N) were maintained in myoblast monolayer culture in α-minimal essential medium containing 2% (v/v) fetal bovine serum and 1% (v/v) antibiotic/antimycotic solution (10,000 units/ml penicillin G, 10 mg/ml streptomycin, 25 mg/ml amphotericin B) in 80-cm^2 flasks in an atmosphere of 5% CO_2 at 37 °C. Cells were maintained in continuous passages (<8) by trypsinization of subconfluent cultures using 0.25% trypsin. For total membrane preparation and RNA isolation, myoblasts were seeded in 10-cm diameter dishes at approximately 4×10^4 cells/cm^2. The cells were fed fresh medium every 48 h. Ras(S17N) was induced with 1.5 μM dexamethasone for 24 h before stimulation with 100 nM insulin for 18 h.

Total Membrane Preparation and Immunoblotting—Total membranes of myoblasts were isolated as described previously (20). Protein content was determined by the Bio-Rad Bradford procedure (27). Membrane samples (50 μg) were subjected to SDS-polyacrylamide gel electrophoresis on 10% polyacrylamide gels essentially according to Laemmli (28). Glucose transporter isoforms and the α1 subunit of the Na^+/K^+-ATPase were detected by immunoblotting analysis as described previously (20) using specific antibodies to each protein. Antibodies were diluted in 150 mM NaCl, 50 mM Tris-HCl, 1% (w/v) bovine serum albumin, 0.04% Nonidet P-40, 0.2% NaN_3 (pH 7.5) (anti-GLUT1 polyclonal antibody, 1:2000; anti-GLUT3 polyclonal antibody, 1:500; anti-α1 Na^+/K^+-ATPase monoclonal antibody, 1:1000) and visualized by ^{125}I-protein A (for polyclonal antibodies) or ^{125}I-sheep anti-mouse IgG (for monoclonal antibody).

RNA Isolation and Northern Blot Hybridization—Total RNA was isolated and Northern blots were performed as described previously (21, 22).

Detection of MAPK Phosphorylation—Parental and Ras(S17N) transfected L6 cells were treated with or without 1.5 μM dexamethasone for 24 h, then stimulated with or without 100 nM insulin for 5 min, and lysed essentially according to Lamphere and Lienhard (29). Briefly, cells were lysed in a solution containing 4% SDS, 10 mM dithiothreitol, 115 mM Tris/HCl (pH 6.8), 10% glycerol, 0.25 mg/ml bromphenol blue, protease inhibitors (100 μM phenylmethanesulfonyl fluoride, 10 μM E-64, 1 μM pepstatin, and 1 μM leupeptin), and phosphatase inhibitors (40 mM sodium fluoride, 7.5 mM sodium pyrophosphate, and 1.5 mM sodium orthovanadate). Lysates were passed five times through a 27-gauge needle to sheer the DNA and boiled for 3 min. A 30-μg sample of total protein from each condition was subjected to SDS-7.5% polyacrylamide gels. MAPK (ERK) phosphorylation was detected by immunoblotting using anti-phosphotyrosine monoclonal antibody (1:5000 dilution) followed by sheep anti-mouse immunoglobulin conjugated to horseradish peroxidase (1:5000 dilution) and visualized by the enhanced chemiluminescence method.

Statistical Analysis—Autoradiograms were quantified by laser scanning densitometry using a PDI model DNA 35 scanner with version 1.3 of the discovery series one-dimensional gel analysis software. Statistical analysis was performed using the analysis of variance test (Fisher, multiple comparisons).

RESULTS

Insulin Stimulation of GLUT1 Expression Is Mediated by pp70 S6 Kinase but Not by p21^{ras}—L6 cells transfected with dominant negative Ras(S17N) under the control of a dexamethasone-inducible promoter were treated with/without dexamethasone for 24 h prior to chronic (18 h) exposure to insulin. As shown in Fig. 1*A* and quantified in Fig. 1*B*, in the absence of dexamethasone insulin caused a 103% increase in total content of GLUT1 protein above basal levels. Although dexamethasone increased the basal level of GLUT1 protein, insulin still caused a 66% increase in GLUT1 protein above the value in the presence of the glucocorticoid. There was no difference between the net magnitudes of the insulin-induced gains in GLUT1 protein in the presence or absence of dexamethasone; hence the insulin-stimulated increase in GLUT1 glucose transporter is in large part a p21^{ras}-independent event.

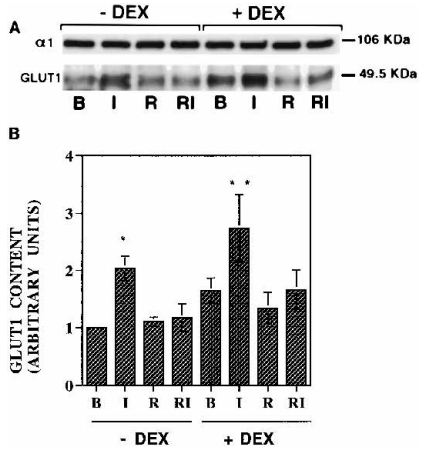

FIG. 1. **Inhibition of insulin-induced elevation in GLUT1 protein by rapamycin but not by expression of Ras dominant negative mutant.** *A*, L6 cells overexpressing Ras(S17N) were treated with or without 1.5 μM dexamethasone (*DEX*) for 24 h prior to treatment without (basal (*B*)) or with 100 nM insulin (*I*) in the presence or absence of 30 ng/ml rapamycin (*R*) for 18 h. Total membranes were prepared and immunoblotted as described under "Experimental Procedures." The content of the immunoreactive α1 Na^+/K^+-ATPase subunit is illustrated for assessment of equality of protein loading. This is a representative blot of four independent experiments. *B*, the results of four independent experiments were densitometrically scanned. The content of GLUT1 protein in basal cells in the absence of dexamethasone was assigned a value of 1.0, and other values were expressed in relative units. Values represent means ± S.E. of four independent experiments. *, significance at 95% compared with basal cells in the absence of dexamethasone. **, significance at 95% compared with basal cells in the presence of dexamethasone.

On the other hand, rapamycin, a specific inhibitor of pp70 S6 kinase, almost completely eliminated the increase in GLUT1 glucose transporter protein in response to insulin (shown in Fig. 1*A* and quantified in Fig. 1*B*). This was observed under control conditions (in the absence of dexamethasone) as well as when the dominant negative p21^{ras} was induced (in the presence of dexamethasone). Therefore, the insulin-stimulated increase in GLUT1 protein is a pp70 S6 kinase-dependent event.

Parallel results were obtained with GLUT1 mRNA; chronic treatment with insulin resulted in a 101% increase in GLUT1 mRNA above the basal value (mean of four independent experiments). The induction of dominant negative Ras by dexamethasone failed to block the insulin-mediated increase in GLUT1 mRNA. Insulin still caused a 128% increase in GLUT1 mRNA above the value in the presence of dexamethasone (mean of four independent experiments). These results indicate that insulin action on GLUT1 gene expression occurs independently of p21^{ras}.

Insulin Stimulation of GLUT3 Expression Is Mediated by p21^{ras} but Not by pp70 S6 Kinase—As demonstrated in Fig. 2*A* and quantified in Fig. 2*B*, insulin caused a 204% elevation in the total content of GLUT3 protein above basal levels. By contrast, when Ras(S17N) was induced by dexamethasone, the ability of insulin to increase GLUT3 protein was almost completely abrogated, suggesting that the insulin-stimulated increase in GLUT3 protein is p21^{ras}-dependent. Moreover, unlike

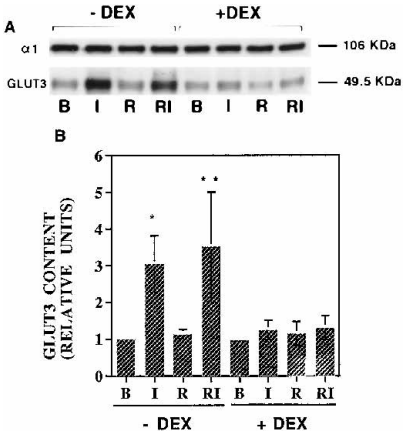

FIG. 2. **Inhibition of insulin-induced elevation in GLUT3 protein by expression of Ras dominant negative but not by rapamycin.** *A*, L6 cells overexpressing Ras(S17N) were treated with or without 1.5 μM dexamethasone (*DEX*) for 24 h prior to treatment without (basal (*B*)) or with 100 nM insulin (*I*) in the presence or absence of 30 ng/ml rapamycin (*R*) for 18 h. Total membranes were prepared and subjected to immunoblot analysis as described under "Experimental Procedures." The content of immunoreactive α1 Na^+/K^+-ATPase subunit is illustrated for assessment of equality of protein loading. This is a representative blot of four independent experiments. *B*, the results of four independent experiments were densitometrically scanned. The content of GLUT3 protein in basal cells in the absence of dexamethasone was assigned a value of 1.0, and other values were expressed in relative units. Values represent means ± S.E. of four independent experiments. *, significance at 95% compared with the basal cells in the absence of dexamethasone. **, significance at 95% compared with rapamycin alone.

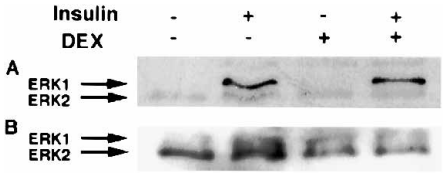

FIG. 3. **Inhibition of insulin-induced phosphorylation of MAPK (ERK) in cells overexpressing Ras(S17N) but not in parental L6 cells.** Parental (*A*) or Ras(S17N)-transfected (*B*) cells were treated with or without 1.5 μM dexamethasone (*DEX*) for 24 h, then stimulated with or without 100 nM insulin for 5 min, and lysed, and MAPK phosphorylation was detected in the whole lysates as described under "Experimental Procedures." This is a representative blot of two independent experiments.

the GLUT1 protein, the level of GLUT3 was not affected by dexamethasone itself as shown in Fig. 2. This further highlights the difference in the regulation of the expression of both proteins.

Rapamycin, on the other hand, was without effect on the insulin-stimulated increase in GLUT3 protein. In the absence of dexamethasone, rapamycin did not block the ability of insulin to increase GLUT3 (Fig. 2). Furthermore, rapamycin did not interfere with the ability of Ras(S17N), when induced by dexamethasone, to eliminate the insulin-mediated increase in GLUT3 glucose transporter as shown in Fig. 2. Thus, the insulin-mediated increase in GLUT3 is a pp70 S6 kinase-independent event.

Parallel results were obtained for GLUT3 mRNA. GLUT3 mRNA rose by 104% above the basal levels in response to chronic treatment with insulin (mean of four independent experiments). Unlike the effects on GLUT1 mRNA, however, the induction of dominant negative Ras was associated with an insulin-mediated increase in GLUT3 mRNA of only 9% above basal levels (mean of four independent experiments). These results indicate that insulin action on GLUT3 gene expression is a $p21^{ras}$-dependent event.

Effect of Dexamethasone on MAPK Phosphorylation in Parental and Ras(S17N)-transfected L6 Cells—To verify that indeed the presence of dexamethasone induced the expression of Ras(S17N) without having an inhibitory effect of its own, the insulin-induced MAPK phosphorylation was examined in parental and Ras(S17N)-transfected L6 cells in the presence and absence of dexamethasone. As shown in Fig. 3*A*, dexamethasone did not affect the insulin-induced phosphorylation of MAPK in parental L6 cells. However, the presence of dexamethasone diminished MAPK phosphorylation in Ras(S17N)-overexpressing cells (Fig. 3*B*).

Effects of Rapamycin and Dexamethasone on the Insulin-mediated Increases in GLUT1 and GLUT3 in Parental L6 Cells—In order to verify that the inhibition of the insulin-induced increase in GLUT1 by rapamycin seen in cells transfected with Ras(S17N) (Fig. 1) was not an artifactual effect due to the process of transfection, a similar set of experiments was performed in parental L6 cells. As shown in Fig. 4*A*, rapamycin abolished the insulin-induced increase in GLUT1 protein (compare *lanes 2* and *4* of *panel A*). Interestingly, the elevation in GLUT1 by dexamethasone alone was also abolished by rapamycin (compare *lanes 5* and *7* of *panel A*), an observation also seen in Ras(S17N)-transfected cells. Hence, both insulin- and dexamethasone-induced elevations in GLUT1 protein are pp70 S6 kinase-dependent events.

To confirm that the inhibition of the insulin-mediated increase in GLUT3 seen earlier after induction of Ras(S17N) by dexamethasone (Fig. 2) is not due to an inhibitory effect of dexamethasone itself, the elevation in GLUT3 in response to insulin was examined in the presence and absence of dexamethasone in parental L6 cells. As shown in Fig. 4*B*, dexamethasone had no effect on the insulin-mediated increase in GLUT3 (compare *lanes 2* and *6* of *panel B*). Furthermore, rapamycin did not affect the increase in GLUT3 in response to insulin (*lane 4* of *panel B*). Hence, insulin action on GLUT3 is indeed a $p21^{ras}$-dependent and pp70 S6 kinase-independent phenomenon.

DISCUSSION

Exposure of L6 muscle cells to insulin for several hours increases total levels of both GLUT1 and GLUT3 glucose transporters but has no effect on GLUT4 expression (2, 21). Here we examined the roles of $p21^{ras}$ and pp70 S6 kinase as mediators in the pathway(s) by which insulin elevates GLUT1 and GLUT3 expression, since both $p21^{ras}$ and pp70 S6 kinase appear to mediate communication between the insulin receptor and nuclear events.

Ras(S17N) expressed in L6 muscle cells has reduced affinity for GTP and inhibits the activity of endogenous $p21^{ras}$ by interfering with Sos needed for Ras activation (25). Induction of Ras(S17N) by dexamethasone was also shown to diminish the ability of insulin to stimulate the tyrosine phosphorylation of MAPK (Ref. 25 and Fig. 3*B*). Moreover, dexamethasone itself had no inhibitory effect on the insulin-induced MAPK phosphorylation in parental L6 (Fig. 3*A*). Hence, L6 cells expressing Ras(S17N) provide a useful system for studying the role of $p21^{ras}$ in insulin signaling.

In agreement with the work of others (30), the use of the glucocorticoid dexamethasone was associated with an increase in total GLUT1 protein in parental (Fig. 4*A*) and in Ras(S17N)-transfected L6 cells (Fig. 1). However, induction of Ras(S17N) by dexamethasone failed to eliminate the insulin-mediated increase in total GLUT1 protein or mRNA, suggesting that insulin action on GLUT1 gene expression occurs independently of $p21^{ras}$. The failure of Ras(S17N) to block the insulin-mediated increase in GLUT1 is not a result of a dysfunctional mutation since in the same cells GLUT3 expression was prevented (Fig.

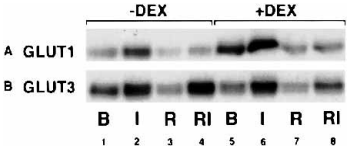

FIG. 4. **Effects of rapamycin and dexamethasone on the insulin-mediated increases in GLUT1 and GLUT3 in parental L6 cells.** Parental L6 cells were treated with or without 1.5 μM dexamethasone (*DEX*) for 24 h prior to treatment without (basal (*B*)) or with 100 nM insulin (*I*) in the presence or absence of 30 ng/ml rapamycin (*R*) for 18 h. Total membranes were prepared and subjected to immunoblot analysis using specific anti-GLUT1 (*A*) or anti-GLUT3 (*B*) antibodies as described under "Experimental Procedures."

2) and MAPK phosphorylation was diminished (Fig. 3*B*). In another study, microinjection of dominant inhibitory forms of p21^{ras} or neutralizing antibodies directed against p21^{ras} in 3T3-L1 adipocytes blocked the insulin-induced increase in GLUT1 at the cell surface (31). However, that study did not examine if GLUT1 expression was prevented. Therefore, it is possible that the dominant inhibitory forms of p21^{ras} or the neutralizing antibodies directed against p21^{ras} interfered with the machinery sorting GLUT1, thus preventing it from reaching the cell surface. The disparity between the results of Hausdorff *et al.* (31) and those reported here could also be due to the differences between a stable expression and microinjection or to cell type-specific regulation of GLUT1. Indeed, dominant inhibitory mutants of p21^{ras} interfere with the insulin-induced activation of the collagenase promoter in CHO and HeLa cells but not in A14 cells (24).

Supporting the idea of Ras-independent signaling pathways for insulin-induced nuclear events, insulin provoked normal DNA synthesis in CHO cells transfected with mutant mSos protein devoid of the guanine nucleotide exchange activity (CHO-IR/ΔSos cells), yet the insulin-stimulated formation of GTP-bound p21^{ras} and the phosphorylation of MAPK were markedly inhibited (32). Moreover, consistent with our results, Kozma *et al.* (33) observed that the total amount of GLUT1 protein was similar in parental 3T3-L1 cells and in those expressing an activated mutant N-Ras^{61k} protein. The possible involvement of p21^{ras} in the *acute* regulation of glucose transport mediated by rapid translocation of glucose transporters to the cell surface remains controversial. Microinjection of activating or inhibitory forms of p21^{ras} had no effect on the rapid action of insulin in 3T3-L1 adipocytes (31), but introducing p21^{ras} neutralizing antibodies attenuated the rapid effects of the hormone in cardiac myocytes (34).

Considering the importance of pp70 S6 kinase in the regulation of protein synthesis (26), we asked whether insulin increases GLUT1 protein level in L6 muscle cells by activation of this kinase. The drug rapamycin blocks the activation of pp70 S6 kinase (11, 26), and its effect is selective insofar as it does not block the activation of other kinases such as Raf-1, MAPK, or pp90 S6 kinase (11, 26). Indeed, inhibition of pp70 S6 kinase by rapamycin blocked the ability of insulin to increase GLUT1 protein in parental cells (Fig. 4*A*) and in cells transfected with Ras(S17N) (Fig. 1). Moreover, rapamycin also blocked the dexamethasone-induced elevation in GLUT1 protein in both parental and transfected L6 cells. pp70 S6 kinase has been dissociated from playing a role in the *acute* translocation of GLUT4 to the cell surface by insulin (11). The data presented herein demonstrate that pp70 S6 kinase might be a mediator of some of the *chronic* effects of insulin, such as the stimulation of GLUT1 expression but not of GLUT3. It is also likely that the dexamethasone-induced increase in GLUT1 is a pp70 S6 kinase-dependent event.

Information on the regulation of the GLUT3 gene and its protein product remains scarce possibly due to the absence of this glucose transporter from adult human or rodent muscle. In this study we demonstrated for the first time that chronic treatment with insulin, like insulin-like growth factor-1, leads to an increase in GLUT3 protein and mRNA in L6 muscle cells. We also addressed for the first time the mechanism by which the GLUT3 glucose transporter protein increases in response to prolonged exposure to insulin. In contrast to GLUT1, the insulin-induced increases in GLUT3 protein and mRNA were found to be p21^{ras}-dependent. The inhibition of insulin action on GLUT3 after induction of Ras(S17N) by dexamethasone was not due to any inhibitory effects of dexamethasone itself. Dexamethasone did not affect the insulin-induced increase in GLUT3 in parental L6 (Fig. 4*B*). In addition, rapamycin did not interfere with the ability of insulin to stimulate GLUT3 protein expression in parental (Fig. 4*B*) or transfected cells (Fig. 2), thus ruling out a role for pp70 S6 kinase in the mechanism by which GLUT3 glucose transporter protein is induced by insulin.

In conclusion, signaling for the insulin-dependent synthesis of GLUT1 and GLUT3 glucose transporters occurs through different pathways; expression of the former is governed by pp70 S6 kinase whereas expression of the latter is determined via p21^{ras}.

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REFERENCES

1. Khan, C. R., and White, M. F. (1988) *J. Clin. Invest.* **82,** 1151–1156
2. Walker, P. S., Ramlal, T., Sarabia, V., Koivisto, U.-M., Bilan, P. J., Pessin, J. E., and Klip, A. (1990) *J. Biol. Chem.* **265,** 1516–1523
3. White, M. F., and Khan, C. R. (1994) *J. Biol. Chem.* **269,** 1–4
4. Sasaoka, T., Draznin, B., Leitner, J. W., Langlois, W. J., and Olefsky, J. M. (1994) *J. Biol. Chem.* **269,** 10734–10738
5. Keller, S. R., and Lienhard, G. E. (1994) *Trends Cell Biol.* **4,** 115–118
6. Buday, L., and Downward, J. (1993) *Mol. Cell. Biol.* **13,** 1903–1910
7. Pumiglia, K., Chow, Y. H., Fabian, J., Morrison, D., Decker, S., and Jove, R. (1995) *Mol. Cell. Biol.* **15,** 398–406
8. Schlessinger, J. (1993) *Trends Biochem. Sci.* **18,** 273–275
9. Crews, C. M., and Erikson, R. L. (1993) *Cell* **74,** 215–217
10. Blenis, J. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 5889–5892
11. Fingar, D. C., Hausdorff, S. F., Blenis, J., and Birnbaum, M. J. (1993) *J. Biol. Chem.* **268,** 3005–3008
12. Cheatham, B., Vlahos, C. J., Cheatham, L., Wang, L., Blenis, J., and Khan, C. R. (1994) *Mol. Cell. Biol.* **14,** 4902–4911
13. Yaffe, D. (1968) *Proc. Natl. Acad. Sci. U. S. A.* **61,** 477–483
14. Sargeant, R., Mitsumoto, Y., Sarabia, V., Shillabeer, G., and Klip, A. (1993) *J. Endocrinol. Invest.* **16,** 147–162
15. Mitsumoto, Y., Liu, Z., and Klip, A. (1993) *Endocr. J.* **1,** 307–315
16. Kayano, T., Kuumoto, H., Eddy, T., Fan, Y.-S., Byers, M. G., Shows, T. B., and Bell, G. I. (1988) *J. Biol. Chem.* **263,** 12245–12248
17. Guillet-Deniau, I., Leturque, A., and Girard, J. (1994) *J. Cell Sci.* **107,** 487–496
18. Maher, F., Vannucci, S., Takeda, J., and Simpson, I. A. (1992) *Biochem. Biophys. Res. Commun.* **182,** 703–711
19. Mitsumoto, Y., and Klip, A. (1992) *J. Biol. Chem.* **267,** 4957–4962
20. Mitsumoto, Y., Burdett, E., Grant, A., and Klip, A. (1991) *Biochem. Biophys. Res. Commun.* **175,** 652–659
21. Koivisto, U. M., Martinez-Valdez, H., Bilan, P. J., Burdett, E., Ramlal, T., and Klip, A. (1991) *J. Biol. Chem.* **266,** 2615–2621
22. Klip, A., Ramlal, T., Bilan, P. J., Marette, A., Liu, Z., and Mitsumoto, Y. (1993) *Cell. Signalling* **5,** 519–529
23. Hainque, B., Guerre-Millo, M., Hainault, I., Moustaid, N., Wardzala, L. J., and Lavau, M. (1990) *J. Biol. Chem.* **265,** 7982–7986
24. Medema, R. H., Wubbots, R., and Bos, J. L. (1991) *Mol. Cell. Biol.* **11,** 5963–5967
25. Skolnik, E. Y., Batzer, A., Li, N., Lee, C. H., Lowenstein, E., Mohammadi, M., Margolis, B., and Schlessinger, J. (1993) *Science* **260,** 1953–1955
26. Jefferies, H. B. J., Reinhard, C., Kozma, S. C., and Thomas, G. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 4441–4445
27. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254
28. Laemmli, K. (1970) *Nature* **227,** 680–685
29. Lamphere, L., and Lienhard, G. E. (1992) *Endocrinology* **131,** 2196–2202
30. Sivitz, W. I., and Pasley, A. M. (1995) *Biochem. Biophys. Res. Commun.* **206,** 51–56
31. Hausdorff, S. F., Frangioni, J. V., and Birnbaum, M. J. (1994) *J. Biol. Chem.* **269,** 21391–21394
32. Sakaue, M., Bowtel, D., and Kasuga, M. (1995) *Mol. Cell. Biol.* **15,** 379–388
33. Kozma, L., Baltensperger, K., Klarlund, J., Porras, A., Santos, E., and Czech, M. P. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 4460–4464
34. Manchester, J., Kong, X., Lowry, O. H., and Lawrence, J. C., Jr. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 4644–4648