Transcriptional Activation of the Glut1 Gene in Response to Oxidative Stress in L6 Myotubes*

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Exposure of L6 myotubes to prolonged low grade oxidative stress results in increased Glut1 expression at both the protein and mRNA levels, leading to elevated glucose transport activity. To further understand the cellular mechanisms responsible for this adaptive response, the Glut1 transcription rate and mRNA stability were assessed. Nuclear run-on assays revealed 2.0- and 2.4-fold increases in Glut1 transcription rates in glucose oxidase- and xanthine/xanthine oxidase-pretreated cells, respectively. Glut1 mRNA stability was increased with both treatments compared with the control (7.8 ± 1.3, 6.0 ± 2.0, and 2.4 ± 0.5 h, respectively). The serum-responsive element and AP-1 (but not the cAMP-responsive element) showed increased binding capacity following oxidative stress. Both activation of AP-1 binding and elevation of Glut1 mRNA were prevented by cycloheximide. The involvement of enhancer 1 of the Glut1 gene was demonstrated using transfected 293 cells. Induction of Glut1 mRNA in response to oxidative stress differed from its activation by chronic insulin exposure as demonstrated by the ability of rapamycin to inhibit the latter without an effect on the former. In conclusion, oxidative stress increases the Glut1 transcription rate by mechanisms that may involve activation of AP-1 binding to enhancer 1 of the Glut1 gene.

Glucose transport is a rate-limiting step in the metabolism of many cell types and therefore in energy production (1). This process is mediated by a family of transmembrane glycoproteins differing in their kinetics and tissue distribution (2). Among the six known glucose transporters, Glut1 is the ubiquitous glucose transporter. Its gene expression is regulated in different cell types by various stimuli. These include hypoglycemia (3), hypoxia (4), mitochondria inhibitors (5), prolonged insulin exposure (6), tumor necrosis factor α (7), and iron chelators (8). It appears that under such stress conditions, energy requirements are increased, and induction of the Glut1 isomorph is therefore instrumental in cellular adaptation. However, the cellular mechanisms involved in this process are still unclear.

Increased oxidative stress has been suggested to play a role in many pathophysiological conditions. Altered transcriptional regulation of various genes, postulated to be mediated by transcriptional activation factors such as AP-1 and NF-κB, is a well described cellular reaction to oxidative stress (9–11). Striated muscle appears to be specifically exposed to oxidative stress in ischemia-reperfusion injury (12, 13) as well as in conditions resulting in systemic increase in oxidative stress parameters, including diabetes mellitus (14, 15). In this disease, oxidative stress has been suggested to be related to impaired insulin action, supporting a physiologically relevant consequence of skeletal muscle exposure to increased oxidative stress (16–18).

We have recently assessed the effect of oxidative stress on glucose transport and metabolism in L6 myotubes and 3T3-L1 adipocytes (19, 20). We observed that exposure of both cell types to prolonged low grade oxidative stress caused a time- and dose-dependent increase in Glut1 expression at both the protein and mRNA levels, resulting in increased basal glucose transport activity.

Recently, the transcriptional regulation of the Glut1 gene has been shown to involve regulatory elements that include a promotor and two enhancers (21, 22). These elements include potential binding sites for various transcriptional activation factors, including the serum-responsive element (SRE), the cAMP-responsive element (CRE), and AP-1-binding sites (TRE). In this study, we aimed to elucidate the cellular mechanisms responsible for Glut1 activation in response to oxidative stress. We demonstrate that following exposure to oxidative stress, the Glut1 gene transcription rate is enhanced, concomitant with increased stability of mRNA transcripts. This transcriptional activation is at least partly mediated by enhancer 1 of the Glut1 gene and requires de novo protein synthesis. Increased binding activity of AP-1 and SRE to DNA is observed in response to oxidative stress and may mediate Glut1 transcriptional activation in response to oxidative stress.

EXPERIMENTAL PROCEDURES

Materials—Tissue culture medium, serum, and reagents were obtained from Biological Industries (Beit-Haemek, Israel). Cytochalasin B, 2-deoxyglucose, cycloheximide, xanthine, xanthine oxidase, and glucose oxidase were obtained from Sigma. Recombinant human insulin was from Novo Nordic (Bagsvaerda, Denmark). Rapamycin was from Calbiochem. 2-Deoxy[3H]glucose and 5-[α-32P]dCTP were obtained from Rotem Industries (Dimona, Israel). [γ-32P]ATP and [3H]-acetyl coenzyme A were obtained from NEN Life Science Products. EcoRI and BglII were from Pharmacia LKB (Uppsala, Sweden). The polyclonal antibodies raised against the C-terminal sequences of Glut1 were obtained from East Acres Biologicals. The plasmids pGT1-1.3CAT, pGT1-1.3CAT/SBb, pGT1-1.3CAT/Bxb, and pGT1-1.3CAT/Bxb/SBb were constructed as described previously (22).

Cell Culture—L6 muscle cells were grown in monolayers to the stage

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¶¶ The abbreviations used are: SRE, serum-responsive element; CRE, cAMP-responsive element; TRE, 12-O-tetradecanoylphorbol-13-acetate-responsive element; Xan/XO, xanthine/xanthine oxidase; Glic/GO, glucose/glucose oxidase; CAT, chloramphenicol acetyltransferase; kb, kilobase pairs.
Transcriptional Activation of Glut1 by Oxidative Stress

of myotubes as described previously (4, 20). The cells were grown in 24-well plates for transport determinations or in 10-cm diameter dishes for RNA, transcriptionally active nucleus, and nuclear protein preparations.

Oxidative Stress—Reactive oxygen species were generated continuously by two different sources of oxidant-generating systems as described previously (19, 20) using the enzyme/substrate mixture of xanthine/xanthine oxidase (Xan/XO) or glucose/glucose oxidase (Glc/GO). The first catalyzes the conversion of xanthine to uric acid with reduction of O2 to O2−, H2O2, and OH−, whereas the second catalyzes the reaction of glucose to glucuronic acid and H2O.

Hexose Transport Determinations—Hexose uptake was measured for 10 min using 10 μM 2-deoxy[3H]glucose (1 μCi/ml) as described previously (4, 20).

RNA Preparation and Northern Blot Analysis—Total cellular RNA was extracted from L6 myotubes after incubation in the indicated conditions using a single-step extraction procedure with acid guanidium thiocyanate/phenol/chloroform as described by Chomczynski and Sacchi (23). Northern blot analysis was performed according to Sambrook et al. (24) as described previously (19, 20).

Nuclear Run-on Transcription Analysis—Cells were disrupted using a Dounce homogenizer and ice-cold buffer containing 10 mM Tris-HCl (pH 7.4), 5 mM MgCl2, 0.1 mM EDTA, 50 mM NaPO4 (pH 7.7), and 1 mM DTT. Nuclei were isolated using the procedure described by Mahajan and Thompson (25). The final pellet was resuspended in 0.05 ml of 75 mM NaCl, 20 mM Tris-HCl (pH 8.0), 1 mM dithiothreitol, and 0.5 mM EDTA in 50% glycerol and frozen at −70 °C.

Transcription assays were carried out in a 0.2-ml reaction volume in buffer containing 52% glycerol, 100 mM KCl, 50 mM HEFES (pH 7.4), 5 mM magnesium acetate, 4 mM dithiothreitol, 0.1 mM EDTA, 4 units of RNasin, 1 mM CTP, 1 mM GTP, and 1 mM ATP plus 0.5 μCi of [α-32P]UTP. The reaction was allowed to proceed for 30 min at 30 °C and terminated by the addition of 1 mM MgCl2 and 7000 units/ml RNase-free DNase I for 15 min at 37 °C. The mixture was then digested with 300 μg/ml proteinase K in the presence of 0.1% SDS. The labeled RNA transcripts were isolated using the acid phenol method (25) and resuspended in 100 μl of water.

Nylon filters (Zeta Probe, Bio-Rad) containing fixed and denatured plasmids with either Glut1 or β-actin (10 μg) cDNA probes were prehybridized for 1 h at 50 °C in 6 × SSPE (1 × SSPE = 0.18 M NaCl, 10 mM NaPO4 (pH 7.7), and 1 mM EDTA), 10 × Denhardt’s reagent, 1% SDS, and 100 μg/ml denatured salmon sperm DNA. Hybridization was carried out at 65 °C for 36 h in 50% formamide, 6 × SSPE, 1% SDS, and 100 μg/ml denatured salmon sperm DNA plus 7 × 10−6 cm2/ml radiolabeled RNA transcripts. Subsequent to hybridization, the filters were washed three times under low stringency conditions (2 × SSPE and 1.0% SDS) for 60 min at 37 °C. The washed filters were subjected to autoradiography and quantitated by laser scanning densitometry.

Transfection and Chloramphenicol Acetyltransferase (CAT) Measurements—L6 myotubes were incubated in a final reaction volume of 20 μl containing 0.5 ng of 32P-labeled cDNA in a medium containing 10 mM glucose (Glc/GO) or glucose oxidase (Glc) or to 50 mM xanthine and 20 mM xanthine oxidase (Xan/XO), after which the 2-deoxyglucose uptake and Glut1 protein and mRNA levels were determined as described under “Experimental Procedures.” Values are means ± S.E. The number of independent experiments is indicated in parentheses.

RESULTS

A 24-h exposure of L6 myotubes to 50 milliunits/ml glucose oxidase in medium containing 5 mM glucose (Glc/GO) or to 50 milliunits/ml xanthine oxidase in the presence of 50 μM xanthine (Xan/XO) caused an ~2-fold increase in glucose transport activity attributed to elevated levels of Glut1 protein (Table I). Cycloheximide, a protein synthesis inhibitor, prevented elevation of both glucose transport activity and Glut1 protein expression (20), indicating that reactive oxygen species stimulated the de novo synthesis of this glucose transporter. The activation of glucose transport activity was reversible when, following treatment, cells were further exposed to fresh medium. Full recovery to pretreatment 2-deoxyglucose uptake activity was observed after 40 h, with a t1/2 of 17.5 h. Steady-state mRNA levels were increased to a similar extent by oxidation (Table I), suggesting an increased transcription rate of the Glut1 gene and/or increased mRNA stability. To address this question, nuclear run-on and mRNA stability assays were performed, respectively. Following treatment with oxidant-generating systems for 24 h, transcriptionally active nuclei were isolated from cells, and in vitro transcription was performed for 30 min as described under “Experimental Procedures.” This was followed by Northern dot-blot analysis using an empty plasmid or a plasmid containing the full-length cDNA probes of either Glut1 or β-actin. The empty plasmid did not react with nascent mRNA (data not shown). However, nuclei isolated from cells exposed to either Glc/GO or Xan/XO had a higher Glut1 transcription rate compared with the control, with no similar effect observed on the transcription rate of β-actin (Fig. 1). Densitometric analysis of three independent experiments indicated 2.0- and 2.4-fold increases in Glut1 transcription rates in Glc/GO- and Xan/XO-pretreated cells, respectively, compared with control cells. This suggests a specific effect of oxidative stress on the transcriptional regulation of the Glut1 gene. Under the same conditions, Glut1 mRNA stability was evaluated. Following a 24-h exposure to Glc/GO or Xan/ XO, cells were further incubated with the mRNA synthesis

labeled with [γ-32P]ATP using polynucleotide kinase, annealed, and subsequently purified on a Sephadex G-50 column. Equivalent protein amounts (10 μg) were incubated in a final reaction volume of 20 μl containing 0.5 ng of 32P-labeled double-stranded probe, 2 μg of DNA, 3 μl of poly(d-Ⅹ), 20 μg of bovine serum albumin, 20 μM HEFES (pH 8.0), 4 μl Tris (pH 7.9), 50 mM KCl, 2 mM EDTA, and 500 μg dithiothreitol. After incubation at room temperature for 20 min, samples were electrophotographically separated on a nondenaturing 5% polyacrylamide gel. The gel was dried and autoradiographed. For the competition experiments, a 100-fold molar excess of unlabeled oligonucleotide or nonrelated oligonucleotide was added to the binding reaction.

Statistics—The significance of each result was compared only with the control using Student’s t test.

| Table I | Effect of a 24-h exposure to Glc/GO or Xan/XO on 2-deoxyglucose uptake and Glut1 protein and mRNA content in L6 myotubes |
|---------|---------------------------------------------------------------|
| Glc/GO  | Xan/XO                                                        |
| 2-DG− uptake (fold of control) | 2.65 ± 0.43 (10) | 2.2 ± 0.7 (8) |
| Glut1 protein (arbitrary units) | 2.1 ± 0.4 (5) | 1.95 ± 0.2 (5) |
| Glut1 mRNA (arbitrary units) | 2.3 ± 0.7 (4) | 2.0 ± 0.3 (4) |

*2DG, 2-deoxyglucose.
inhibitor actinomycin D. The half-life of pre-existing Glut1 mRNA was increased in both Glc/GO- and Xan/XO-treated cells compared with control cells ($t_{1/2} = 7.8 \pm 1.3, 6.0 \pm 2.0$, and $2.4 \pm 0.5$ h, respectively; $p < 0.05$ for each treatment versus the control) (Fig. 2). These results suggest that both increased transcription rate and mRNA stability may account for the increased steady-state Glut1 mRNA content following oxidation.

The regulatory elements of the Glut1 gene include a promoter ($-1.3$kb to $+137$ base pairs) and two enhancers (enhancer 1, $-3.3$kb to $-2.7$kb; and enhancer 2, $+16.7$kb to $+18$kb), which have been shown to include consensus sequences for the binding of various transcription factors, including SRE, CRE, and the AP-1-binding site (TRE) (21, 22). To assess the involvement of these regulatory elements in oxidative stress-induced Glut1 gene activation, plasmids containing the Glut1 promoter and the CAT reporter gene either alone or with enhancer 1, enhancer 2, or both (Fig. 3) were transfected into 293 cells. These cells exhibited an $\sim50\%$ increase in 2-deoxyglucose uptake activity following exposure to Xan/XO (data not shown).

To control for CAT activity, cells were simultaneously transfected with plasmids containing $\beta$-galactosidase as described above. Cells were then exposed for 3 h to Xan/XO, after which both CAT and $\beta$-galactosidase activities were measured. Cells containing a plasmid with the promoter alone (pGT1-1.3CAT) did not exhibit an increased CAT/$\beta$-galactosidase activity ratio following oxidation compared with control cells. A maximal $4.2 \pm 0.25$-fold increase in the CAT/$\beta$-galactosidase activity ratio was observed following oxidation of cells transfected with a plasmid containing both enhancers (pGT1-1.3CAT/Bxb/SBb). This effect could be attributed mainly to enhancer 1, as a $2.4 \pm 0.21$-fold increase in the CAT/$\beta$-galactosidase activity ratio was observed in cells containing the pGT1-1.3CAT/SBb plasmid, without a similar effect in cells transfected with a plasmid containing enhancer 2.

Enhancer 1 of the Glut1 gene has been shown to contain consensus sequences for the binding of various transcriptional activation factors. These include two AP-1-binding sites (TRE), one SRE, and one CRE (21). To evaluate the potential role of the respective transcription factors in inducing Glut1 gene transcription, we assessed whether oxidation caused increased binding activity of nuclear protein extracts to prelabeled oligonucleotides utilizing the gel electromobility shift assay. L6 myotubes were incubated for 8 h in the absence or presence of Glc/GO or Xan/XO, after which nuclear protein extracts were prepared as described. Fig. 4 demonstrates increased AP-1 and SRE (but not CRE) binding activity (data not shown) following exposure of the cells to oxidative stress.

AP-1 activation may involve activation of its two precursors, c-fos and c-jun. To further evaluate the respective role of the AP-1 transcription factor in Glut1 gene transcriptional activation, steady-state mRNA levels of c-fos and c-jun were measured in L6 myotubes exposed to Glc/GO or Xan/XO. As shown in Fig. 5A, c-fos and c-jun mRNA levels were increased following oxidation, without a similar effect on $\beta$-actin mRNA levels (data not shown). The ability of cycloheximide to inhibit both AP-1 activation and Glut1 mRNA elevation following oxidation was assessed. Fig. 5B demonstrates that activation of AP-1 DNA-binding activity was inhibited when L6 myotubes were exposed to oxidative stress in the presence of $5 \mu$g/ml cycloheximide. Under similar conditions, the elevation of Glut1 mRNA levels was prevented by cycloheximide (Fig. 5C). Taken together, these results confirm the role of de novo protein syn-

**Fig. 1.** Nuclear run-on assay of nuclei isolated from L6 myotubes exposed to Xan/XO and Glc/GO. L6 myotubes were treated for 24 h with 20 milliunits/ml xanthine oxidase in the presence of $50 \mu$M xanthine (Xa/XO) or with Glc/GO, after which transcriptionally active nuclei were isolated and subsequently used for transcriptional run-on assays using Glut1 and $\beta$-actin probes as described under “Experimental Procedures.” Shown is a representative experiment performed three times for each treatment.

**Fig. 2.** Glut1 mRNA stability in control and Xan/XO- and Glc/GO-treated L6 myotubes. L6 myotubes were incubated for 24 h with Xan/XO (Xa/XO) or Glc/GO (G/GO), after which cells were rinsed three times with phosphate-buffered saline, and $10 \mu$g/ml actinomycin D was added at time 0. Cells were further incubated in the absence (●) and presence (○) of either Xan/XO or Glc/GO. At the indicated time intervals, cellular RNA was extracted, after which Northern blot analysis was performed using Glut1 and $\beta$-actin probes as described under “Experimental Procedures” (A). Blots were analyzed by video densitometry, and the Glut1 mRNA/$\beta$-actin mRNA ratio was calculated (B). Shown is a representative experiment performed three times for each treatment. cont, control.
thesis in both activation of AP-1 DNA-binding activity and induction of the Glut1 gene in response to oxidative stress.

Fig. 4 demonstrates the increased activation of SRE binding to DNA following oxidative stress. This transcription factor has been shown to mediate Glut1 transcriptional activation in response to insulin exposure (22). In L6 myotubes, chronic insulin-induced elevation of Glut1 protein is inhibited by rapamycin, a pp70S6K inhibitor, but not by the expression of a Ras dominant-negative mutant (28). In accordance, the Ras/mitogen-activated protein kinase cascade was not involved in increasing the expression of Glut1 in L6 myotubes in response to oxidative stress, as no increase in immunoreactive active mitogen-activated protein kinase was observed (data not shown).

To compare the insulin-induced activation of the Glut1 gene with the effect of oxidative stress, Glut1 mRNA levels and glucose transport activity were evaluated following exposure to Glc/GO or insulin for 24 h in the presence of rapamycin. L6 myotubes were incubated with either 100 nm insulin or Glc/GO for 24 h in the absence and presence of rapamycin. Under these conditions, cell viability as detected by protein recovery and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test was not affected. Fig. 6B demonstrates that rapamycin did not exert an effect on 2-deoxyglucose uptake activity of control cells. However, 100 ng/ml rapamycin totally prevented insulin-induced elevation of Glut1 mRNA levels (Fig. 6A) and glucose transport activity (Fig. 6B). In contrast, no similar effect of rapamycin was ob-

Fig. 3. Effect of Xan/XO on the CATβ-galactosidase activity ratio in 293 cells transfected with plasmids containing various regulatory regions of the Glut1 gene. 293 cells were transfected with plasmids containing β-galactosidase and the Glut1 promotor region either alone or with enhancer 1, enhancer 2, or both as described under “Experimental Procedures.” Following a 3-h exposure to Xan/XO, CAT and β-galactosidase activities were measured as described under “Experimental Procedures.” Results are means ± S.E. of three independent experiments.

Fig. 4. Gel electromobility shift assays of nuclear protein extracts from L6 myotubes exposed to Xan/XO or Glc/GO. Nuclear extracts were obtained from L6 myotubes exposed to either Xan/XO or Glc/GO for 8 h, and gel mobility shift assays were performed using 32P-labeled oligonucleotides as described under “Experimental Procedures.” Specificity of the DNA binding was assessed using a 100-fold molar excess of unlabeled oligonucleotide (S) or a nonrelated 32P-labeled oligonucleotide (N). Shown are representative experiments performed for four times for AP-1 and three times for SRE. C, control; X, Xan/XO; G, Glc/GO.

Fig. 5. Effect of Xan/XO, Glc/GO, and cycloheximide on c-fos, c-jun, and Glut1 mRNA levels and AP-1 binding activity. A, L6 myotubes were treated for 24 h with Xan/XO or Glc/GO, after which total RNA was extracted and analyzed for the amount of c-fos and c-jun mRNAs as described under “Experimental Procedures.” B, cells were incubated as described for A in the absence or presence of 5 μg/ml cycloheximide (CHX), followed by a gel electromobility shift assay as described in the legend to Fig. 4. C, cells were treated with cycloheximide as described for B and assayed for Glut1 and β-actin mRNAs (data not shown) as described under “Experimental Procedures.” All blots were representative of two to four independent experiments. C, control; X, Xan/XO; G, Glc/GO.

Fig. 6. Rapamycin prevents insulin-induced (but not Glc/GO-induced) Glut1 mRNA and glucose transport activation. L6 myotubes were treated with or without 100 nm insulin or 25 milliunits/ml glucose oxidase for 24 h in the absence (□) or presence of 30 ng/ml (○) or 100 ng/ml (■) rapamycin (rapa), a pp70S6K inhibitor. Glut1 mRNA levels (A) and glucose transport activity (B) were assessed as described under “Experimental Procedures.” Shown are a representative Northern blot performed three times and the means ± S.E. of 2-deoxyglucose (2DG) uptake values obtained from four independent experiments, each performed at least in duplicate. *, p < 0.01 compared with the control in the absence of rapamycin; †, p < 0.01 compared with chronic (chr.) insulin in the absence of rapamycin; ‡, p < 0.001 compared with chronic insulin in the absence of rapamycin. G/GO, Glc/GO; prot., protein.
erved on oxidative stress-induced elevation of Glut1 mRNA levels and glucose transport activity. Taken together, these results provide evidence that oxidative stress-induced Glut1 transcriptional activation is distinct from Glut1 activation by insulin.

**DISCUSSION**

With accumulating evidence suggesting a significant role for oxidative stress in diverse pathophysiological conditions, there is increasing interest in understanding cellular response to oxidative stress. The ability of oxidative stress to alter gene expression has been shown in many cell types and in various experimental models (10, 11). We have previously demonstrated that the expression of the glucose transporter isomor Glut1 is increased in both L6 myotubes and 3T3-L1 adipocytes in response to oxidative stress, resulting in increased glucose transport and metabolism (19, 20). These are believed to provide the elevated energy required for the recruitment of repair and antioxidant defense mechanisms. In this study, we investigated further the cellular mechanisms by which Glut1 expression is increased following exposure to oxidative stress.

The results of this study indicate that in L6 myotubes, the increase in steady-state Glut1 mRNA levels following oxidative stress can be attributed to both an enhanced Glut1 transcription rate, as determined by nuclear run-on assay (Fig. 1), and increased mRNA stability (Fig. 2). The fact that steady-state Glut1 mRNA levels are increased by not more than 2–2.5-fold (Table I) suggests that additional mechanisms may be involved in regulating Glut1 mRNA levels following oxidative stress. For example, increased mRNA stability may develop as a consequence of an earlier occurring enhancement of the transcription rate if mRNA degradation mechanisms become rate-limiting.

A combination of increased mRNA stability and elevated transcription rate in the regulation of this glucose transporter gene expression has been observed in clone 9 cells exposed to the oxidative phosphorylation inhibitor sodium azide (29). Increased stability of Glut1 mRNA was reported in cells exposed to tumor necrosis factor α (7) and phorbol ester and glucose deprivation (30), whereas an increased transcription rate without an alteration in mRNA stability was reported in ARL 15 cells treated with thyroid hormone (31, 32). The direct cellular mechanism responsible for elevated steady-state Glut1 mRNA levels in response to anoxia, iron chelators, malignant transformation, heat shock, and viral infection has yet to be determined. However, it appears that increasing Glut1 gene expression represents a common cellular response to various stress conditions, including oxidative stress, by mechanisms that result in transcriptional activation and/or stabilization of pre-existing mRNA transcripts. Alternative mechanisms to increase Glut1 protein content to enhance glucose flux may occur at the protein stability level.

The transcriptional regulation of Glut1 in response to oxidative stress is mediated at least partly by enhancer 1 of the regulatory region of this gene (Fig. 3). Among the three transcription factors known to have a potential binding site on enhancer 1, AP-1 and SRE are shown to be activated in response to oxidative stress (Fig. 4), whereas CRE is not. While this excludes the direct or indirect (through the activation of another gene) involvement of CRE in Glut1 transcriptional activation, the contribution of AP-1 or SRE activation to this process cannot be conclusively sorted out by this study. The dependence of this process on de novo protein synthesis is demonstrated by the ability of cycloheximide to inhibit elevation of Glut1 mRNA levels following oxidation. Under the same conditions, oxidative stress-induced AP-1 activation is also inhibited, which may provide circumstantial evidence for a direct or indirect role for AP-1 in Glut1 transcriptional activation following oxidative stress.

H2O2 has been shown to increase cellular protein tyrosine phosphorylation by altering the tyrosine kinase-to-phosphatase balance and was further demonstrated to exhibit insulinomimetic effects (33, 34). The effect of chronic exposure to insulin on Glut1 induction was studied using various experimental models. In L6 myotubes, the activation of Glut1 following an 18-h exposure to insulin was inhibited by rapamycin, a pp70S6K inhibitor (28). In NIH/3T3HIR3.5 cells transfected with hybrid genes containing the enhancer and promoter of the Glut1 gene, a 1-h exposure to insulin resulted in activation of SRE and enhancer 1 (22). To compare the effect of chronic insulin with the effect of prolonged oxidative stress on Glut1 gene expression, we assessed the ability of rapamycin to prevent Glut1 activation. While rapamycin completely prevented the activation of glucose transport and Glut1 following chronic exposure to insulin, no similar effect on the induction of Glut1 in response to oxidative stress was observed (Fig. 6). Thus, the activation of Glut1 by prolonged low grade oxidative stress may not be identical to that elicited by chronic exposure to insulin.

In conclusion, the cellular response of L6 myotubes to oxidative stress involves the transcriptional activation of the Glut1 gene, mediated at least partly by enhancer 1 of the Glut1 gene, and requires de novo protein synthesis. This effect may not be attributed to an insulinomimetic effect of H2O2. The respective role of various transcription factors including AP-1 and SRE in mediating this response requires further study.

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Transcriptional Activation of Glut1 by Oxidative Stress

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