Stimulation of GLUT-1 glucose transporter expression in response to hyperosmolarity

DAW-YANG HWANG AND FARAMARZ ISMAIL-BEI G I

Departments of Physiology and Biophysics and Medicine, Case Western Reserve University, Cleveland, Ohio 44106

Received 26 September 2000; accepted in final form 8 June 2001

Hwang, Daw-Yang, and Faramarz Ismail-Beigi. Stimulation of GLUT-1 glucose transporter expression in response to hyperosmolarity. Am J Physiol Cell Physiol 281: C1365–C1372, 2001.—Glucose transporter isoform-1 (GLUT-1) expression is stimulated in response to stressful conditions. Here we examined the mechanisms mediating the enhanced expression of GLUT-1 by hyperosmolarity. GLUT-1 mRNA, GLUT-1 protein, and glucose transport increased after exposure of Clone 9 cells to 600 mosmol/l (produced by addition of mannitol). The stimulation of glucose transport was biphasic: in the early phase (0–6 h) a ~2.5-fold stimulation of glucose uptake was associated with no change in the content of GLUT-1 mRNA, GLUT-1 protein, or GLUT-1 in the plasma membrane, whereas the ~17-fold stimulation of glucose transport during the late phase (12–24 h) was associated with increases in both GLUT-1 mRNA (~7.5-fold) and GLUT-1 protein content. Cell sorbitol increased after 3 h of exposure to hyperosmolarity. The increase in GLUT-1 mRNA content was associated with an increase in the half-life of the mRNA from 2 to 8 h. A 44-bp region in the proximal GLUT-1 promoter was necessary for basal activity and for the two- to threefold increments in expression by hyperosmolarity. It is concluded that the increase in GLUT-1 mRNA content is mediated by both enhanced transcription and stabilization of GLUT-1 mRNA and is associated with increases in GLUT-1 content and glucose transport activity.

glucose transporter isoform-1; sorbitol; aldose reductase; GLUT-1 messenger ribonucleic acid; GLUT-1 promoter; GLUT-1 messenger ribonucleic acid half-life

THE ADAPTIVE RESPONSE OF CELLS to stressful conditions is mediated by a variety of specific transcriptional and posttranscriptional mechanisms. GLUT-1, a member of the facilitative glucose transporter family of proteins, is among a subset of genes and gene products that respond to stress (21, 23). GLUT-1-mediated glucose transport is augmented by a variety of stressful stimuli and conditions, including hypoxia, a rise in intracellular calcium concentration, inhibition of oxidative phosphorylation, transformation, and incubation in medium low in glucose or high in pH (15, 34). Detailed study of the glucose transport response to some of the above stimuli demonstrates that the enhancement of glucose transport is biphasic with the early phase being mediated by posttranslational mechanisms and the late phase involving enhancement of GLUT-1 gene expression (2, 27).

The generalized cellular response to hyperosmolar stress has been examined in considerable detail, and the specific pathways and mechanisms underlying this response continue to be under active study. After the acute phase of the response, which is mediated predominantly by changes in the ionic fluxes across the plasma membrane (and hence in the ionic composition of cells), there is a chronic adaptive phase that involves net cellular accumulation of osmolytes and a further adjustment of the internal ionic composition (10, 18). These osmolytes are either accumulated from the external medium after the induction of their specific transporters (e.g., betaine, myo-inositol) or are synthesized within the cells (e.g., sorbitol).

In the present study, we describe and examine the mechanisms underlying the stimulation of GLUT-1-mediated glucose transport after exposure to hyperosmolar stress. Our interest to study this regulation stems from suggestions that GLUT-1 is considered to be a stress-response protein (32, 34). An increase in the content of GLUT-1 in response to prolonged (24 h) exposure to hyperosmolarity has been described in L6 cells (30), but mechanisms underlying the response are not known. We chose Clone 9 cells (a nontransformed rat liver cell line) for investigation because this cell line has been used in other studies focused on hyperosmolarity (16) and because previous results have shown that glucose transport is rate limiting for glucose metabolism in these cells (7, 20). In addition, GLUT-1 appears to be the only isoform of the GLUT family that is expressed in these cells, which serves to simplify the analysis (26). A preliminary report of some of these findings has been presented (14).

MATERIALS AND METHODS

Materials. Clone 9 cells were obtained from American Type Culture Collection (Rockville, MD). [α-32P]dCTP (3,000 Ci/mmol) and 3-O-methyl-d-[3H]glucose (3-[3H]OMG; 3.4 mCi/mmol) were purchased from NEN Life Science Products (Boston, MA) and Amersham Pharmacia Biotech (Piscataway, NJ), respectively. Random primed DNA labeling kit and...
FuGENE 6 were from Roche Molecular Biochemicals (Indianapolis, IN). Nitrocellulose paper (BA-S 50) was obtained from Schleicher and Schuell (Keene, NH). Quickhyb was obtained from Stratagene (La Jolla, CA). Dulbecco’s modified Eagle’s medium (DMEM), Hanks’ balanced salt solution, calf serum, horseradish peroxidase-conjugated anti-rabbit antibody, and streptavidin-agarose bead were obtained from Life Technologies (Grand Island, NY). Culture dishes were obtained from Corning (Acton, MA). Qiagen Plasmid Maxi Kit was obtained from Qiagen (Valencia, CA). Dual luciferase reporter assay system, pGL2-Basic plasmid, pRL-TK plasmid, Wizard Plus Miniprep system, and restriction endonucleases were purchased from Promega (Madison, WI). Sulfo-NHS-SS-biotin was from Pierce (Rockford, IL). Standard chemicals were obtained from Sigma (St. Louis, MO).

**Reporter constructs.** The 6.3 kbp rat GLUT-1 promoter region was deleted serially from its 5’-end to prepare constructs containing different segments of the GLUT-1 promoter, as previously described (3). Some reporter constructs were prepared by PCR amplification of specific regions of the promoter that were then subcloned into pGL2-Basic luciferase reporter vector. In all instances the products were verified by sequencing of both DNA strands.

**Cell culture, transfection, and luciferase reporter assays.** Clone 9 cells were passed and maintained in DMEM containing 10% calf serum at 37°C with 8% CO₂ (20). Cells were employed between passages 29 and 45. The medium was changed to serum-free DMEM for all experiments. Culture media were made hyperosmolar by the addition of various osmolytes, usually mannitol at 300 mosmol/l. Cells (in duplicate or triplicate culture dishes) were cotransfected (at 60% confluence) with 2 μg of pGL2-Basic plasmid constructs containing various segments of the GLUT-1 promoter and expressing the firefly luciferase and 0.2 μg of pRL-TK plasmid (expressing *Renilla* luciferase) employing FuGENE 6. After 48 h, the media were changed to serum-free isotonic or hypertonic medium, and the incubation continued for an additional 24 h before measurement of luciferase activity. In experiments designed to measure basal GLUT-1 promoter activity, the activity of firefly luciferase was corrected against *Renilla* luciferase activity. In experiments testing the effect of hyperosmolarity, firefly luciferase activity was normalized against the protein content of control and experimental culture plates, because *Renilla* luciferase activity increased dramatically in response to hyperosmolarity.

**Measurement of 3-[3H]OMG uptake.** Culture plates in triplicate were incubated for 60 s in glucose uptake medium, as described (20). The uptake medium consisted of 1.0 ml DMEM containing 5 μCi 3-[3H]OMG and 1 μl of either DMSO alone or DMSO containing cytochalasin B (CB) such that the final concentration of the latter was 50 μM. In cells incubated in hyperosmolar medium, the osmolarity of the uptake medium was adjusted to hyperosmolar levels before use. Cells were harvested in 1 ml of H₂O, and the radioactivity was determined by scintillation spectrometry. CB-inhibitable 3-OMG transport was calculated as the difference between the uptake in the absence and presence of CB. Uptakes in control and treated cells were performed in parallel.

**Measurement of sorbitol.** Sorbitol was measured by enzymatic assay using sorbitol dehydrogenase (19). In short, cells were washed with ice-cold phosphate-buffered saline (PBS) twice and collected in 1 ml PBS, and an aliquot was used for measurement of protein. Two milliliters of ice-cold 6% perchloric acid was added, and tubes were incubated on ice for 10 min before centrifugation for 10 min at 14,000 g. The supernatant was neutralized with a solution of 3 M potassium carbonate and 0.5 M triethanolamine to pH 7.9 followed by centrifugation at 14,000 g for 10 min at 4°C. Five-hundred-microliter samples were mixed with 1 ml of a solution containing 50 mM glycine, 1.2 mM β-nicotinamide adenine dinucleotide, and 2.5 μU of sorbitol dehydrogenase for 30 min at 25°C followed by measurement of the optical density at 340 nm.

**Cell surface biotinylation and isolation of plasma membrane.** Previous described methods were employed without modification (28) except sulfo-NHS-SS-biotin was used. Briefly, cells on two or three 100-mm culture dishes were rinsed with ice-cold PBS followed by incubation on a shaking platform for 30 min with 1.5 ml biotinylation buffer containing sulfo-NHS-SS-biotin. After washing and lysis of cells, streptavidin-agarose beads were added, and the tubes were rotated for 30 min at 4°C. Pellets were washed, and proteins were eluted from the beads with Laemmli loading buffer devoid of 2-mercaptoethanol and bromphenol blue (for measurement of protein using a Bio-Rad kit); this was followed by SDS-PAGE and Western blot analysis.

**SDS-PAGE and Western blotting.** Whole cell lysates were prepared by scraping the cells into 100 μl of lysis buffer (150 mM NaCl, 5 mM EDTA, 1% Nonidet P-40, 0.1 mM phenylmethylsulfonyl fluoride; pH 7.4). Lysates were cleared of nuclei by centrifugation at 14,000 g for 1 min. Protein samples were separated by 10% SDS-PAGE and transferred to nitrocellulose membrane. The membrane was blocked by 5% nonfat milk and incubated with rabbit anti-GLUT-1 IgG (Chemicon International) at 1:3,000 dilution in Tris-buffered saline-Tween 20 (TBST: 50 mM Tris, 150 mM NaCl, and 0.05% Tween 20, vol/vol). The secondary antibody was 1:10,000 dilution of horseradish peroxidase-conjugated anti-rabbit antibody diluted in TBST. Immunoreactive bands were visualized on the Kodak X-Omat film with Western blot luminol reagent (Santa Cruz) and quantified by densitometry.

**Northern blot analysis.** Clone 9 cells were exposed to isotonic or hypertonic medium for various times as indicated in different experiments. Cytoplasmic RNA was isolated and fractionated as described previously (26). Blots were probed using rat GLUT-1 (26) or aldose reductase cDNA (11) labeled with [α-32P]dCTP. A 915-bp aldose reductase cDNA fragment was prepared by RT-PCR of Clone 9 cell cytoplasmic RNA employing upstream and downstream oligonucleotides with the following sequences: 5’-aggtgatgccatggcagt-3’ and 5’-tgcccatgctgcaagaggggtcttgctggaag-3’ (11). Membranes were probed at 68°C overnight using Quick-Hyb and washed four times for 15 min each with a solution containing 0.1% SDS and 1× standard saline citrate (SSC) at 58°C. The blots were autoradiographed using X-ray film or by employing a Phosphorimager (Molecular Dynamics). Relative intensities of the specific mRNA bands were normalized against the 28S rRNA band measured by ethidium bromide staining of the membrane.

**Statistical analysis.** Results are expressed as means ± SE. Unpaired Student’s *t*-test was used, and *P* < 0.05 was considered significant (29).

**RESULTS**

**Effect of hyperosmolarity on GLUT-1-mediated glucose transport, GLUT-1 protein and mRNA content, and cell surface GLUT-1 content.** Figure 1 shows the time course of the effect of hyperosmolarity (produced by the addition of 300 mosmol/l mannitol to the medium) on the rate of cytochalasin B (CB)-inhibitable glucose transport in Clone 9 cells. In repeated experi-
ments it was found that the rate of CB-inhibitable 3-OMG uptake increased ~2.5 fold ($P < 0.05$) at 3 and 6 h after exposure to hyperosmolarity, and thereafter the rate increased markedly, reaching 15- and 25-fold of control levels by 24 and 48 h, respectively.

We next determined whether the stimulation of glucose transport in response to hyperosmolarity is associated with an increase in the content of GLUT-1 mRNA and GLUT-1 transporter. In initial experiments the effect of different levels of hyperosmolarity on the expression of GLUT-1 mRNA was examined (Fig. 2). After 24 h of incubation, GLUT-1 mRNA content increased by the addition of mannitol at either 200 or 300 mosmol/l (but not 50 or 100 mosmol/l) to the culture medium. In all subsequent experiments mannitol added at 300 mosmol/l was employed. After the addition of mannitol, the content of GLUT-1 mRNA remained unchanged up to 9 h, increased significantly by 12 h, and reached near-plateau levels at 24–48 h (Fig. 3A). The time course of changes in GLUT-1 protein after exposure of cells to hyperosmolarity is shown in Fig. 3B. The content of GLUT-1 was unchanged at 3 h, increased significantly at 6 h (2.4 ± 0.7-fold), and increased to 9- and 11-fold of the control level at 12 and 24 h, respectively. The time point of 6 h is shown in Fig. 3B because the increase in cell GLUT-1 content occurred well before any increase in cell content of GLUT-1 mRNA.

Results of several experiments that were performed to quantitatively measure the effect of hyperosmolarity on the rate of glucose transport, the content of GLUT-1 mRNA, and GLUT-1 protein after 3 and 24 h of exposure are summarized in Table 1. As is apparent from data in Table 1 (and Figs. 1 and 3), the glucose transport response to hyperosmolarity is biphasic with the early phase occurring with no change in the content of GLUT-1 mRNA or GLUT-1 protein; in contrast, the stimulation of glucose transport during the late phase is associated with significant increases in the contents of both GLUT-1 mRNA and GLUT-1.

To test whether the observed increase in GLUT-1 mRNA expression in response to hyperosmolarity is specific to mannitol, or occurs with the use of other osmolytes, Clone 9 cells were exposed to dextrose, mannitol, NaCl, and urea (all added to a final osmolality of 600 mosmol/l) (Fig. 4). GLUT-1 mRNA content was significantly increased after exposure to dextrose, mannitol, and NaCl but was only slightly increased after exposure to urea; the smaller response to urea presumably reflects the fact that this reagent is not a perfect osmolyte. The interpretation of results employing NaCl may be difficult because of potential secondary changes in other intracellular ions.

Table 1. Effect of hyperosmolarity on glucose transport, GLUT-1 protein, and GLUT-1 mRNA content at 3 and 24 h

<table>
<thead>
<tr>
<th></th>
<th>3 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose transport</td>
<td>$2.7 ± 0.1^a$</td>
<td>$16.3 ± 0.7^a$</td>
</tr>
<tr>
<td>GLUT-1 protein</td>
<td>$1.1 ± 0.1$</td>
<td>$8.0 ± 1.0^a$</td>
</tr>
<tr>
<td>GLUT-1 mRNA</td>
<td>$1.2 ± 0.4$</td>
<td>$7.5 ± 1.4^a$</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 6$ for each value. Results are expressed as fold increase normalized to respective values in cells incubated in isotonic medium for the same period. $^aP < 0.05$ compared with controls.
We next determined whether the stimulation of glucose transport during the early phase of the response to hyperosmolarity is associated with an increase in the content of GLUT-1 in the plasma membrane. A previously described procedure for cell surface biotinylation followed by isolation of plasma membrane was employed (1, 17, 28). Cells treated with 250 μM CoCl₂ for 24 h were used in these studies as a control, since this treatment is associated with an increase in cell and plasma membrane GLUT-1 content (1, 3, 17). The results showed no significant changes in plasma membrane GLUT-1 protein content after 3 h of incubation in hyperosmotic vs. control medium (1.1 ± 0.3-fold, n = 4; Fig. 5). This suggests the increased glucose uptake at the 3-h time point is not due to increased membrane GLUT-1 protein and is consistent with an activation mechanism.

**Effect of hyperosmolarity on aldose reductase mRNA expression and cell sorbitol content.** Aldose reductase, an enzyme that is induced in response to hyperosmolarity, catalyzes the production of sorbitol from glucose. The effect of hyperosmolarity on aldose reductase mRNA expression was hence determined (Fig. 6). In accordance with previous reports that the content of aldose reductase mRNA is enhanced in these and other cells (10, 16), we found a significant increase in aldose reductase mRNA (~2.5-fold) as early as 3 h after exposure to hyperosmolarity. Higher degrees of stimulation were evident at later time points.

We next determined whether the stimulatory effect of hyperosmolarity on glucose transport is associated with an increase in cellular content of sorbitol. Table 2 summarizes the cellular levels of sorbitol after 3 and 24 h of incubation in hyperosmolar medium. The increased expression of aldose reductase mRNA noted above (and presumably aldose reductase protein) is associated with significant increases in cellular content of sorbitol.

**Mechanism of induction of GLUT-1 mRNA in response to hyperosmolarity.** The increase in the content of GLUT-1 mRNA can be mediated by increased GLUT-1 gene transcription, decreased GLUT-1 mRNA degradation, or a combination of both mechanisms. To dissect the effects of hyperosmolarity on GLUT-1 mRNA expression, the RNA synthesis inhibitor, actinomycin D, was employed to determine whether changes in the rate of GLUT-1 mRNA degradation play a role in the observed induction of the mRNA. Cells were pretreated with isotonic or hypertonic media for 24 h before addition of actinomycin D, and cytoplasmic RNA was collected after 0, 2, 4, and 6 h of exposure to the inhibitor. (The time of addition of actinomycin D was varied to enable harvesting of the cells at the same time.) Preexposure of cells to hyperosmolarity increased the relative content of GLUT-1 mRNA by ~9-fold compared with cells incubated under isotonic conditions (Fig. 7, A and B). The half-life of GLUT-1 mRNA incubated in isotonic medium was 2.2 ± 0.1 h (n = 3), which is comparable to previous observations in these cells (26). In these studies, the half-life of GLUT-1 mRNA from each of three experiments was plotted, and the resulting half-lives were averaged. After 24 h of incubation in hypertonic medium, the half-life of GLUT-1 mRNA increased to 7.9 ± 1.8 h (n = 3; P < 0.05). These results suggest that the increase in the content of GLUT-1 mRNA in response to hyperosmolarity is mediated in part by an increase in the stability of the mRNA.

To elucidate the potential role of enhanced transcription of the GLUT-1 gene in the response to hyperosmolarity, we employed reporter constructs containing different regions of the ~6 kbp rat GLUT-1 promoter (3); cells were cotransfected with pRL-TK plasmid expressing the *Renilla* luciferase to control for transfection efficiency. Clone 9 cells were transiently transfected

---

**Table 2. Effect of hyperosmolarity on sorbitol content of Clone 9 cells**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Sorbitol, nmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotonic, 3 h</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>Hypertonic, 3 h</td>
<td>25 ± 2.9*</td>
</tr>
<tr>
<td>Hypertonic, 24 h</td>
<td>48 ± 5.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE and are the results of 5 separate experiments. Cells were incubated in control or hyperosmolar media for the periods indicated. *P < 0.05.
and assayed for luciferase activity after 24-h exposure to either isotonic or hypertonic medium. Luciferase expression measured under basal (isotonic) condition showed some change with 5’-truncations of the promoter from ~6 kbp to ~104 bp upstream of the major transcription start-site of the gene (33), but the promoter remained active (Fig. 8, A and B). Since these constructs included 133 bp of the 5’-untranslated region of GLUT-1 mRNA (which contains an AP-1 site and a glucocorticoid response element), additional constructs devoid of this region were also prepared and tested (constructs E and F). These latter constructs were nearly as active as their respective controls under basal conditions. However, further 5’-truncation of the promoter to −85, −66, and −60 or to −11 (constructs G–J) resulted in a marked decrease in the basal rate of transcription.

The effect of hypertonic media on luciferase expression in transfected cells was next examined. In these experiments, firefly luciferase activity was normalized against cell protein since the expression of Renilla was stimulated by hyperosmolality. All constructs demonstrating significant expression of luciferase under basal conditions showed a significant (and nearly equivalent) response to exposure to the hypertonic medium. This included a modification of construct C in which we mutated the consensus CAT box located in the proximal region of the GLUT-1 promoter (data not shown). Inclusion of proximal promoter up to ~104 bp was associated with significant increases in luciferase activities after exposure to hyperosmolality. Constructs G and H exhibited some basal activity and responded significantly to hyperosmolality. In contrast, GLUT-1 promoter constructs I and J (containing less of the promoter) showed a small amount of basal expression but failed to show any increase in luciferase expression in response to hypertonic medium. Cells transfected with pGL2-Basic (devoid of GLUT-1 promoter) showed a small amount of luciferase expression, which was not stimulated in response to exposure to hyperosmolality (data not shown).

### DISCUSSION

The present study focused on the stimulation of glucose transport in response to exposure to hyperosmolar conditions and yielded new insights on the mechanisms underlying this response. Results showed that the stimulation of GLUT-1-mediated glucose transport is biphasic with the early phase (0–3 h) being mediated entirely by posttranslational mechanisms, while the late phase (12–48 h) is associated with a dramatic enhancement of GLUT-1 gene expression. We demonstrated that sorbitol levels increased in cells as early as 3 h and that the stimulation of glucose transport during the early phase is not associated with an increase in the content of GLUT-1 in the plasma membrane. We made the further novel observation that the increase in cellular content of GLUT-1 mRNA in response to hyperosmolality is mediated by both increased transcription and decreased degradation of GLUT-1 mRNA. Finally, we localized the region of the GLUT-1 promoter necessary for its basal expression and for the positive transcriptional response to hyperosmolality to a 44-bp segment in the proximal 5’-flanking region of the gene.

The chronic phase of the adaptive response to hyperosmolality is characterized by enhanced expression of several genes encoding proteins that mediate the accumulation of organic osmolytes; the specific genes are those encoding betaine, myo-inositol and taurine transporters, and the enzyme aldose reductase (10, 12). The transporters serve to increase the intracellular concentration of organic osmolytes by facilitating their transport from the external medium, while, in contrast, aldose reductase functions to convert intracellular glucose to sorbitol. The expression of these genes has been shown to be stimulated at the transcriptional level, and osmotic response elements have been identified in their promoter regions (8, 9); posttranscriptional mechanisms have also been demonstrated in some instances (31). In addition to the above, tissues differ in their usage of organic osmolytes during the adaptive response. For example, the concentration of all of the above osmolytes is upregulated in kidney medullary cells in response to hyperosmolality, while all except sorbitol are accumulated in the rat brain during adaptation to acute and chronic hypernatremia (5, 18).
The increase in the content of GLUT-1 mRNA in response to hyperosmolarity occurred after a significant delay period (>9 h). This finding suggests that the measured changes in rates of synthesis and degradation of the mRNA also occurred after a significant delay period. The reason for this delay is not known. It is possible, for example, that changes occurring during the acute phase of the response, such as alterations in cell volume, ionic composition, or actin skeleton (24), help initiate the subsequent responses. In this context, the induction of aldose reductase mRNA and increased cellular content of sorbitol (a product of glucose metabolism), which was observed as early as 3 h, occurred significantly before the increase in the abundance of GLUT-1 mRNA. It is hence possible that the enhanced synthesis of sorbitol plays an important role in the induction of GLUT-1 mRNA. This issue requires further study.

The region of GLUT-1 promoter necessary for the response to hyperosmolarity was demarcated to a 44-bp region of the proximal promoter (from −104 to −60). Moreover, this 44-bp region does not contain a classical osmotic response element nor is there such an element present in the published 600-bp segment of the proximal GLUT-1 promoter (33). The lack of a previously described consensus osmotic response element raises the possibility that the induction of the GLUT-1 gene may occur secondary to the induction of one or more genes. It is alternatively possible that exposure to hyperosmolarity results in a stimulation of the transcriptional protein complex of the GLUT-1 gene. Such a mechanism would help explain why the promoter region mediating the hyperosmolar response appears to correspond to the region necessary for basal transcription. A similar finding concerning basal and stimulated expression was recently reported in the transcriptional response of the aldose reductase gene to hyperosmolarity in Clone 9 cells (16). Finally, the 44-bp segment located within the proximal promoter region of the GLUT-1 gene mentioned above is highly GC rich (~75%) and contains a typical Sp1 binding site (and other Sp1-like binding sites) that has been reported to be of importance in the developmental regulation of the GLUT-1 gene in neonatal rat heart (25). In addition, an Sp1 binding site has also been implicated as being responsible for the induction of serum- and
glucocorticoid-inducible protein kinase under hyperosmotic stress (4). Whether this transcription factor plays an important role in the response to hyperosmolarity is not known. Further studies are necessary to determine the molecular basis of the stimulation of GLUT-1 gene transcription as well as the posttranscriptional stabilization of GLUT-1 mRNA in response to hyperosmolarity.

The small but significant increase in glucose uptake in the early phase (0 to 3 h) cannot be attributed to altered GLUT-1 gene expression or to increased translation of GLUT-1 and hence represents posttranslational regulation of GLUT-1-mediated glucose transport. Moreover, we found no evidence of an increase in the content of GLUT-1 in the plasma membrane at this early time point, suggesting an activation rather than a translocation mechanism. Acute stimulation of glucose uptake in the presence of a constant amount of cell GLUT-1 has also been observed in response to other stimuli (such as inhibition of oxidative phosphorylation) that can be classified as cellular stress (34). Stimulation of p38 mitogen-activated protein kinase (MAPK) has been implicated in the acute glucose transport response to stress (2), and p38 activation has been implicated in the increase in GLUT-1 content in response to hyperosmolarity after 24 h of exposure (30). Whether the early phase of the response to hyperosmolarity involves the p38 MAPK pathway needs to be verified. In addition, hyperosmolarity has been recently reported to acutely stimulate AMP-activated protein kinase (AMPK) (13), and we have observed that stimulation of the kinase is associated with an increase in GLUT-1-mediated glucose transport through an activation mechanism (1). It remains to be determined, however, whether the stimulation of AMPK mediates the acute glucose transport response to hyperosmolarity. In addition, we did observe a small but significant increase in cell GLUT-1 protein content 6 h after exposure to hyperosmolarity, a time at which no increase in the content of GLUT-1 mRNA had yet occurred. This finding suggests that the observed induction of GLUT-1 at this time point could be in part mediated at the translational level, in support of the possibility that the regulation of GLUT-1 expression and function can be mediated at multiple levels.

Previous results have shown that the transcription of GLUT-1 gene is enhanced in response to hypoxia (or by exposure to cobalt chloride as a surrogate of hypoxia), to inhibition of oxidative phosphorylation by azide, to an ionophore-induced increase in the concentration of cytosolic calcium, and after transformation (15, 34). The response to these stimuli, in addition to the response to hyperosmolarity shown here, may represent a generalized response of the GLUT-1 gene to cellular stress. Comparison of the findings of the present study with previous results identifying regions of the GLUT-1 promoter necessary for stimulation by hypoxia and azide demonstrates that different regions of the promoter mediate each of these responses. Specifically, a 666-bp region some ~6 kbp upstream to the transcriptional start site mediates the transcriptional response of the promoter to azide, while a ~480-bp region located ~2,500 bp upstream of the start site (and which contains a hypoxia-response element) is necessary for the stimulation by hypoxia or cobalt chloride (3, 6). Still another element in the mentioned ~480-bp region partly mediates the response to transformation (22). Finally, results of the present study showed that the region mediating the response to hyperosmolarity is in close proximity to the transcriptional start site. These findings suggest that the regions of the GLUT-1 promoter that mediate the transcriptional response of the gene to each of the above stressful stimuli (azide, hypoxia, transformation, and hyperosmolarity) are markedly different. The results hence imply that the above stimuli do not share a common transcriptional stress response pathway, and that different trans-acting factors are likely involved in the induction of the GLUT-1 gene in response to each of these stimuli.

This study was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-45945.

REFERENCES


