Cell volume regulates liver phosphoenolpyruvate carboxykinase and fructose-1,6-bisphosphatase genes

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Kaiser, Stephan. Cell volume regulates liver phosphoenolpyruvate carboxykinase and fructose-1,6-bisphosphatase genes. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G509–G517, 1998.—Hypertonic-induced cell shrinkage increases glucose release in H-4-II-E rat hepatoma cells. This is paralleled by a concomitant increase in the mRNA levels of the rate-limiting enzymes of the pathway of gluconeogenesis, phosphoenolpyruvate carboxykinase (PCK) and fructose-1,6-bisphosphatase (FBP), of seven- and fivefold, respectively. In contrast, hypertonic-induced swelling of the cells results in a transient decrease in PCK and FBP mRNAs to 15% and 39% of control levels. The antagonistic effects of hyper-osmotic and hypotonicity mimic the counteracting effects of adenosine 3',5'-cyclic monophosphate (cAMP) and insulin on PCK and FBP mRNA levels. The hypertonic-induced increase in mRNA levels is due to an enhanced transcriptional rate, whereas the decrease in mRNAs caused by hypertonicity results from a decrease in transcription as well as mRNA stability. The inductive effect of hypertonicity does not require ongoing protein synthesis and acts independently of the cAMP-dependent protein kinase and protein kinase C pathways. These results suggest that cell volume changes in liver cells may play an important role in regulating hepatic glucose metabolism by altered gene expression.

CELL VOLUME HAS LONG BEEN recognized as an important determinant for cellular function and metabolism in a variety of prokaryotic as well as eukaryotic cells (8). More recently, it has become clear that especially in the kidney and the brain certain genes are controlled by hyperosmotic challenge (7). This is not surprising in view of the importance of a naturally occurring osmotic gradient in the medulla of the kidney and of the sensitivity of brain function toward alterations in osmotic pressure. Regarding the liver, a control of cellular function by cell volume appears less obvious; however, changes in cell volume have been shown to affect metabolic liver function (15). When hepatocytes are exposed to anisotonic medium, the cells are able to counterregulate their volume within minutes. This volume-regulatory response, however, restores cell volume only partially, thus leaving the cells in a slightly swollen or shrunken state throughout the anisotonic exposure. Recent studies have shown that these cell volume changes occur not only under anisotonic conditions but also under the influence of hormones such as insulin and adenosine 3',5'-cyclic monophosphate (cAMP) (1) or during cumulative substrate uptake of certain amino acids such as glutamine (15). Among other effects, the changes in cell volume lead to subsequent alterations in hepatic carbohydrate metabolism. Thus cell swelling inhibits and cell shrinkage stimulates lactate, pyruvate, and glucose release in the isolated perfused rat liver, pointing to an inhibition or stimulation of glycogenolysis during cell swelling or shrinkage, respectively (15). Furthermore, glycogen synthesis has been shown to be stimulated during hyposmotic cell swelling (24). Although the effects of cell volume on metabolic flux rates have been studied extensively, little information is available regarding the effects of cell volume on the regulation of gene expression in the liver. Recent observation of a transcriptional regulation of albumin gene expression by osmotic pressure (26) has indicated a regulatory mechanism also on the molecular level.

Because previous reports showed that cell volume changes affect glucose metabolism in the liver and in isolated hepatocytes, the possibility of an effect of cell volume changes on hepatic gluconeogenesis and the expression of the genes coding for the two key enzymes phosphoenolpyruvate carboxykinase (PCK) and fructose-1,6-bisphosphatase (FBP) (27) was investigated. PCK and FBP activities are not subject to allosteric regulation but rather are determined solely by the abundance of enzyme. Therefore, the chief mechanism by which PCK and FBP activities are controlled is by changes in gene expression. The expression of the PCK gene is increased by cAMP, dexamethasone, thyroid hormone, and retinoic acid (27) and inhibited by insulin (25), the phorbol ester phorbol 12-myristate 13-acetate (PMA) (9), vanadate (4), okadaic acid (27), and lithium (5). Expression of the FBP gene is also increased in the presence of cAMP, whereas it is decreased by insulin (11, 12). Most importantly, the dominant antagonistic regulation of both genes is exerted by insulin and cAMP, and these two hormones also exert counteracting effects on liver cell volume.

The present study shows that cell volume changes modulate both PCK and FBP gene expression in H-4-II-E rat hepatoma cells. This response is also found in primary rat and human hepatocytes. Anisotonic-induced cell volume changes stimulate alterations in the rate of transcription as well as in mRNA stability and involve a signal transduction pathway apparently distinct from cAMP-dependent protein kinase and protein kinase C.

EXPERIMENTAL PROCEDURES

Materials. H-4-II-E cells were from American Type Culture Collection (ATCC; CRL-1548 and CRL-1560). Tissue culture media, fetal calf serum, and ribonuclease (RNase) T1 were from GIBCO-BRL. [α-32P]dCTP (sp act 3,000 Ci/mmol), [α-32P]UTP (sp act 3,000 Ci/mmol), and [3H]UTP (sp act 46.8 Ci/mmol) were from Amersham, and GeneScreen and GeneScreen Plus nylon membranes were from Du Pont-New England Nuclear. Nucleoside-5'-diphosphate kinase, yeast tRNA, glutamate dehydrogenase, restriction enzymes, and...
8-(4-chlorophenylthio)-cAMP (CPT-cAMP) were purchased from Boehringer Mannheim, and the oligolabeling kit was obtained from Pharmacia Biotechnology. RNasin and T3 and XbaI were purchased from Boehringer Mannheim, and the oligolabeling kit was obtained from ATCC. The isolated cDNA fragments derived from pPCK-10, pPFBPase, and pRLCGAP subcloned into pBluescript II SK(−) and transcription run-on experiments were performed as described. Insulin had a strong repressive effect on both mRNAs, whereas hypotonic conditions (405 mosmol/l) for the same time period decreased the mRNAs of PCK and FBP to 33% and 42% of normotonic (305 mosmol/l) control levels, respectively (Fig. 1, Table 1). Similar results were obtained when experiments were carried out with Hep G2 and Hep 3B human hepatoma cells or primary rat and human hepatocytes (Table 2). Incubation of the cells with CPT-cAMP (50 µM) or with dexamethasone (1 µM) increased PCK gene expression (Fig. 1, Table 1), whereas only CPT-cAMP was able to enhance FBP expression, in line with previous reports (12, 18). Insulin had a strong repressive effect on both mRNAs, even compared with basal levels. Hypotonicity was able to partially block the effects of the cyclic nucleotide or dexamethasone on the induction of the mRNAs; the decreased toxicity of 205 mosmol/l caused a 35% repression of the normally observed induction of PCK mRNA by CPT-cAMP and a 46% repression of the effect on FBP mRNA levels. Conversely, the effect of hypertonicity (405 mosmol/l) was synergistic with the effects of...
FBP mRNA levels, the mRNAs of GAPDH, tyrosine aminotransferase, liver-type glutaminase, and glutamine synthetase remained unchanged. Albumin mRNA levels were decreased after hypotonic exposure for more than 8 h by about one-half, in line with previous observations (26). Hypotonic exposure led to slight increases in β-actin and tubulin mRNA levels; however, these effects were not significant. Thus the effect of anisotonicity on PCK and FBP mRNA levels appeared to be specific, and hybridization with GAPDH cDNA was used for normalization of the mRNA on the blots.

Cell volume affects PCK and FBP mRNA levels. Treatment of the cells with 100 mM of the nonmetabolizable sugars raffinose or sucrose led to an induction of PCK and FBP mRNAs of a magnitude similar to that under exposure with increased sodium chloride (+50 mM) concentration. Furthermore, treatment with glycercol (100 mM) or urea (100 mM) at concentrations yielding hyperosmotic conditions (405 mosmol/l) but with no effect on cell volume did not change PCK and FBP mRNA levels. When normal osmotic pressure of hypertonic medium (205 mosmol/l) was restored by adding raffinose (100 mM) or sucrose (100 mM) instead of sodium ions, PCK and FBP mRNA levels remained unchanged (Table 1). These observations ruled out the possibility that the anisotonic-induced changes in PCK and FBP mRNAs were due to changes in ionic strength or osmolarity. However, the observations also strongly suggested that cell volume was the actual determinant of the observed effects. Furthermore, when the cell volume of H-4-II-E cells under the various experimental conditions was measured using a calibrated resistance cytometer, the cell volume determinations correlated well with the corresponding tonicity, hormone, or amino acid (Table 3). Also, these data are in line with previous estimations made with isolated rat liver perfu-

### Table 1. Effect of raffinose, glycerol, and urea on PCK and FBP mRNA levels in various human and rat liver cell lines

<table>
<thead>
<tr>
<th></th>
<th>Hypertonic (405 mosmol/l)</th>
<th>Hypotonic (205 mosmol/l)</th>
</tr>
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<tbody>
<tr>
<td><strong>PCK mRNA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (305 mosmol/l)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Hypertonic (405 mosmol/l)</td>
<td>6.72 ± 0.81*</td>
<td>5.36 ± 0.94*</td>
</tr>
<tr>
<td>Hypotonic (205 mosmol/l)</td>
<td>0.33 ± 0.04*</td>
<td>0.42 ± 0.07*</td>
</tr>
<tr>
<td>Raffinose (405 mosmol/l)</td>
<td>0.23 ± 0.11*</td>
<td>0.29 ± 0.18*</td>
</tr>
<tr>
<td>Glycerol (405 mosmol/l)</td>
<td>1.10 ± 0.12</td>
<td>1.03 ± 0.21</td>
</tr>
<tr>
<td>Urea (405 mosmol/l)</td>
<td>0.92 ± 0.22</td>
<td>1.05 ± 0.13</td>
</tr>
<tr>
<td>Raffinose + hypotonic (305 mosmol/l)</td>
<td>1.07 ± 0.21</td>
<td>1.12 ± 0.14</td>
</tr>
</tbody>
</table>

Values are means ± SD from 3 different experiments with control value of 305 mosmol/l taken as 1. Experimental conditions were as described in Table 1 legend. H-35 and Hep G2 hepatoma cells were cultured in DMEM-F12 medium and Hep 3B cells in MEM. Primary rat hepatocytes were prepared by collagenase treatment from livers of male Wistar rats and then cultured for 48–72 h in DMEM containing 2 mM glucose, 100 U/ml penicillin, 100 µg/ml streptomycin, thyroxine and trilodothyronine (10⁻⁵ mM), dexamethasone (10⁻⁶ mM), and 10% fetal calf serum. Before the experiments, cells were kept in DMEM-F12 medium for 6 h. Primary human hepatocytes were obtained from the Department of Surgery and treated the same way as rat hepatocytes. *Statistically different at P < 0.01. Values are means ± SD from 3 different experiments with control value of 305 mosmol/l taken as 1. Experimental conditions were as described in Table 1 legend. H-35 and Hep G2 hepatoma cells were cultured in DMEM-F12 medium and Hep 3B cells in MEM. Primary rat hepatocytes were prepared by collagenase treatment from livers of male Wistar rats and then cultured for 48–72 h in DMEM containing 2 mM glucose, 100 U/ml penicillin, 100 µg/ml streptomycin, thyroxine and trilodothyronine (10⁻⁵ mM), dexamethasone (10⁻⁶ mM), and 10% fetal calf serum. Before the experiments, cells were kept in DMEM-F12 medium for 6 h. Primary human hepatocytes were obtained from the Department of Surgery and treated the same way as rat hepatocytes. *Statistically different at P < 0.01.
Kinetics of the alterations of PCK and FBP mRNAs. All cells were grown to confluency in medium containing 5 mM glucose and 155 mM NaCl and then maintained at either 105 mM NaCl (520 mosmol/l) or 205 mM NaCl (5405 mosmol/l) for various times. The cell swelling induced by hypertonicity resulted in a biphasic response of PCK mRNA levels with an initial twofold increase, which was followed by a slight decrease and a second increase that reached a maximum induction of 6.7-fold at 6 h and a subsequent decrease plateauing at a fivefold induction. The mRNA of FBP increased constantly to reach a plateau of a fivefold induction at 6–12 h (Fig. 3). In contrast, the level of GAPDH mRNA remained unchanged when the cells were transferred to hypertonic medium. When the cells were transferred to hypotonic conditions, PCK and FBP mRNAs showed a rapid and coordinate decline to a minimum of 16% and 39%, respectively, of levels observed under control conditions. However, the decrease was transient and both mRNAs reached levels comparable to normotonic levels again by 12–24 h (Fig. 4). This response thus paralleled to a great extent the insulin-induced repression of PCK mRNA levels in the same cell line (19).

Effect of cell volume on transcription rates. To identify the possible mechanism responsible for the altered levels of PCK and FBP mRNAs under anisotonic conditions, transcription run-on assays were performed using nuclei isolated at different times after onset of

Table 3. Change in cell volume of H-4-II-E cells on exposure with hormones or changes in tonicity

<table>
<thead>
<tr>
<th>Change in Cell Volume, %</th>
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<tbody>
<tr>
<td>Control (305 mosmol/l)</td>
<td>100</td>
</tr>
<tr>
<td>Hypertonic (405 mosmol/l)</td>
<td>-17</td>
</tr>
<tr>
<td>Hypotonic (205 mosmol/l)</td>
<td>+21</td>
</tr>
<tr>
<td>Insulin (100 nM)</td>
<td>+19</td>
</tr>
<tr>
<td>cAMP (50 µM)</td>
<td>-12</td>
</tr>
<tr>
<td>Raffinose (100 mM)</td>
<td>-16</td>
</tr>
</tbody>
</table>

Values are means of 9 single determinations and are given as % increase or decrease in relation to normal control conditions (305 mosmol/l in DMEM-F12 at pH 7.4). Determination of cell volume of H-4-II-E cells using a calibrated pulse resistance cytometer (CASY 1, Schaef System, Reutlingen, Germany) under various experimental conditions. Cells were kept in DMEM-F12 medium in shaking water flasks to prevent attachment under conditions otherwise identical to the other experiments and then injected into a calibrated capillary (diam 0.5 mm) of the resistance cytometer, where the resistance is directly proportional to the medium inside the capillary, which is removed by a single cell. Thus the cell diameter is proportional to the resistance. The pulse amplitude obtained is analyzed regarding width, area, form, and time course and compared with standardized microparticles. Due to multiple measurements (~500 single measurements/cell) the whole volume of the cell can thus be calculated (31). cAMP, 8-(4-chlorophenylthio)-cAMP.
hypertonic (405 mosmol/l) or hypotonic (205 mosmol/l) conditions. The relative transcription rates of the PCK and FBP genes increased five- and fourfold, respectively, by 5 h after initiation of hypertonic conditions (Fig. 5). In contrast, hypotonic exposure resulted in a significant decrease in the transcription rate of the PCK gene to 11% and of the FBP gene to 28% of control levels by 6 h. The transcription of GAPDH was unaffected. The initial increase in transcription rates correlated with the increase in the respective mRNAs. Therefore, enhancement of transcription could account for the initial induction of the two mRNAs.

Effect of cell volume on mRNA half-life. H-4-II-E hepatoma cells were maintained under normotonic or anisotonic conditions for 6 h, after which the RNA synthesis inhibitor actinomycin D was added and the decrease in PCK and FBP mRNAs relative to that of GAPDH was determined. In all cases, the observed decrease occurred with first-order kinetics. The calculated half-lives for PCK mRNA under normotonic and hypertonic conditions were 4.4 and 3.7 h, respectively; the half-life under hypotonic conditions was 2.3 h (Fig. 6A). The corresponding half-lives for FBP mRNA were 5.4 and 4.8 h under normotonic and hypertonic conditions, respectively, with a decrease in half-life under hypotonic conditions to ~3.4 h (Fig. 6B). Thus destabilization could contribute to the decrease of PCK and FBP mRNAs under hypotonic conditions, while a slight but not significant destabilizing effect of hypertonic exposure on both mRNAs was observable. Such destabilization has already been shown to play a significant role in the repressive effect of PMA on renal PCK mRNA. However, the decline of both mRNAs under hypotonic exposure was due to both decreased transcription and mRNA stability.

Recovery from hypertonicity. When cells were maintained under hypertonic conditions for 24 h and then returned to normotonic conditions, the levels of PCK and FBP mRNAs returned to normal within 4–6 h. The decrease occurred with an apparent half-life of 30–40 min for both mRNAs (Fig. 7). This calculated half-life correlates well with the half-life of ~30 min obtained for liver PCK previously (16). However, this process occurred more rapidly than the apparent half-life of PCK and FBP mRNA measured in the presence of actinomycin D. Thus selective inactivation might also contribute to the rapid disappearance of the two mRNAs during recovery. Alternatively, the estimation of mRNA half-lives with actinomycin D might be erroneously high, since this drug has been shown to stabilize mRNA by itself.

Cell volume affects PCK and FBP gene expression independently of cAMP-dependent protein kinase and protein kinase C. The observation that both cell shrinkage and cAMP induced increases in PCK and FBP mRNA levels suggested that the effect of cell shrinkage may be mediated by cAMP. However, the response of both mRNAs to the two effectors was synergistic (Fig. 1). Furthermore, the cAMP-dependent protein kinase inhibitor HA-1004 (50 µM) completely blocked the inductive effect of cAMP but had only a minor effect on the adaptation caused by cell shrinkage (Table 4). These data thus largely rule out an involvement of the cAMP-dependent signal transduction pathway in mediating the inductive effect of cell volume decrease on PCK and FBP gene expression.

As both cell swelling and the phorbol ester PMA exhibit a repressive effect on PCK and FBP gene expression independently of cAMP-dependent protein kinase and protein kinase C, the question arose as to whether the repressive effect of cell swelling was transduced by a pathway involving protein kinase C. However, downregulation of protein kinase C by exposure of H-4-II-E cells to PMA for 24 h did not abolish the repressive effect of hypotonicity. Furthermore, the protein kinase C inhibitors chelerythrine (100 nM), staurosporine (25 nM), bisindolylmaleimide (100 nM), and Go 6976 (25 nM) were able to block the PMA effect but not the hypotonicity-induced repression of the two mRNAs (Table 4).

Pretreatment of H-4-II-E cells with the protein synthesis inhibitor cycloheximide (CHX) (200 µg/ml) did not abolish the increase in the levels of PCK or FBP mRNA after hypertonic exposure (405 mosmol/l) (PCK mRNA, 6.72 ± 0.81 vs. 6.43 ± 0.76 with CHX; FBP
mRNA, 5.35 ± 0.94 vs. 5.21 ± 1.23 with CHX). Also, the repressive effect of hypotonic conditions (205 mosmol/l) on the two mRNAs was unaffected by pretreatment with CHX (PCK mRNA, 0.33 ± 0.04 vs. 0.32 ± 0.06 with CHX; FBP mRNA, 0.42 ± 0.07 vs. 0.39 ± 0.12 with CHX). Thus ongoing protein synthesis did not appear to be required for the effects of anisotonicity on PCK or FBP gene expression in H-4-II-E cells.

**DISCUSSION**

Hepatic carbohydrate metabolism, more specifically gluconeogenesis, is known to be modulated by liver cell volume changes (1, 15, 24). However, the precise molecular mechanisms remain to be identified. In an attempt to provide insight into the putative cellular mechanisms involved, the effect of anisotonic-induced cell volume changes on gene expression of the rate-controlling enzymes of the gluconeogenic pathway was studied in H-4-II-E rat hepatoma cells. The present study shows that anisotonic exposure of H-4-II-E rat hepatoma cells resulted in profound changes in PCK and FBP mRNA levels. Hypertonic cell swelling increased whereas hypotonic cell shrinkage decreased both mRNAs. In addition, anisotonicity also affected PCK and FBP mRNAs in Hep G2 and Hep 3B human hepatoma cells and in primary rat and human hepatocytes. Moreover, hypotonic exposure was able to at least partially counteract the increased mRNA levels caused by cAMP or dexamethasone in H-4-II-E rat hepatoma cells. The repressive effect of hypotonicity by itself was similar in magnitude to that of insulin or phorbol esters such as PMA. However, the counteracting effect in the presence of cAMP or dexamethasone was less pronounced. The inductive effect of hypertonicity was comparable to that of both cAMP and dexamethasone and when administered simultaneously was synergistic.

The effect of hypertonicity on the mRNAs of the two genes could be mimicked by exposure to raffinose or sucrose at concentrations yielding the same osmolarity. When the osmolarity of hypertonic, sodium-chloride depleted medium was restored to normal by addition of a corresponding amount of raffinose, the levels of both mRNAs remained unchanged. Furthermore, increasing medium osmolarity by addition of urea or glycerol had no effect on PCK and FBP mRNAs. Thus the above-described effects appeared to be due to changes in liver cell volume. Furthermore, the inductive effect of cAMP on PCK and FBP gene expression was in line with the corresponding cell volume decrease exerted by this hormone (15) and the shrinkage-induced induction of the two genes. Conversely, the repressive effects of both insulin and hypotonicity on PCK and FBP gene expression correspond to the cell swelling properties of insulin (1).

The effects of anisotonicity on PCK and FBP mRNAs in hepatoma cells were osmolarity dependent. Even though changes of ±40 mosmol/l were already effective, stronger effects appeared with greater osmolarity changes. It is noteworthy that cell volume changes caused by osmolarity changes of ±40 mosmol/l are similar in extent to that caused by several hormones as well as amino acids that are taken up by cumulative substrate transport (15). At these changes in cell volume, effects on hepatic glucose release (15) and glycogen metabolism (24) are notable.

The changes in the levels of PCK and FBP mRNAs caused by anisotonicity were detected after 1 h of cell incubation. This rapid response to anisotonicity suggests that at least the initial phase was not caused by modification of mRNA stability. Nuclear run-on data demonstrated that an increased rate of transcription could account for the initial increases of both mRNAs on hypertonic exposure. Conversely, hypotonic conditions decreased the rate of transcription of the two genes. Surprisingly, the apparent half-lives of PCK and FBP mRNAs were even slightly, although not significantly, decreased by growth in hypertonic medium, whereas hypotonic exposure reduced the mRNA half-lives by ~50%. Thus increased PCK and FBP mRNA levels apparently resulted solely from increased transcription. In contrast, the decrease of both mRNAs during hypotonic exposure was due to both a decrease in the rate of transcription and a decrease in mRNA stability.
Changes in PCK and FBP gene expression could be related to modifications in hepatic glucose metabolism. In this regard, hepatocytes treated with anisotonic medium show corresponding changes in gluconeogenesis from different precursors (unpublished data), in that hypertonic exposure increases and hypotonicity decreases glucose production. The effect of hypotonicity thus closely mimicked the effect of insulin, suggesting that insulin-induced cell swelling might contribute to the well-known repressive effect of insulin on PCK and FBP gene expression. Furthermore, this concept would be in line with data showing that cell volume affects hepatic glycogen metabolism (24).

A major question arising from this study concerns the mechanism by which anisotonicity-induced cell volume changes exert their effects on PCK and FBP gene transcription. Jun has been shown to stimulate transcription from the PCK promoter in hepatoma cells, an effect which can be reversed by cotransfection of an expression vector containing the gene for c-Fos (14). In this context it is of interest to note that hypotonic

Fig. 6. Effect of anisotonicity on mRNA half-life. Disappearance of PCK (A) and FBP mRNAs (B) was observed under normal (305 mosmol/l), hypertonic (405 mosmol/l), and hypotonic (205 mosmol/l) conditions in the presence of actinomycin D (5 µg/ml). Cells were grown for 6 h under normal or anisotonic conditions, after which actinomycin D was added. Cells were harvested at the indicated time points after actinomycin D addition. Values represent means ± SD from 3 different experiments and are expressed relative to the mRNA level before addition of actinomycin D.

Table 4. Effect of various protein kinase activators and inhibitors on the effect of anisotonicity on levels of PCK and FBP mRNAs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PCK mRNA</th>
<th>FBP mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAMP (50 µM)</td>
<td>7.8 ± 1.5*</td>
<td>5.2 ± 1.9*</td>
</tr>
<tr>
<td>HA-1004</td>
<td>1.0 ± 0.3</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Hypertonic (405 mosmol/l)</td>
<td>6.9 ± 1.8*</td>
<td>5.4 ± 2.0*</td>
</tr>
<tr>
<td>cAMP + hypertonic</td>
<td>9.3 ± 1.6*</td>
<td>7.3 ± 1.8*</td>
</tr>
<tr>
<td>cAMP + HA-1004</td>
<td>1.6 ± 0.3</td>
<td>1.4 ± 0.7</td>
</tr>
<tr>
<td>Hypertonic + HA-1004</td>
<td>3.7 ± 1.9*</td>
<td>2.3 ± 1.7*</td>
</tr>
<tr>
<td>PMA (1 µM)</td>
<td>0.14 ± 0.11*</td>
<td>0.37 ± 0.32*</td>
</tr>
<tr>
<td>Chelerythrine</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>Hypotonic (205 mosmol/l)</td>
<td>0.28 ± 0.09*</td>
<td>0.36 ± 0.21*</td>
</tr>
<tr>
<td>PMA + chelerythrine</td>
<td>0.9 ± 0.2</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Hypotonic + chelerythrine</td>
<td>0.2 ± 0.2*</td>
<td>0.3 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SD of at least 3 determinations with the control set equal to 1. Total RNA was isolated from cells grown for 6 h in normotonic or anisotonic medium and simultaneously treated with 50 µM PPT-cAMP or 1 µM phorbol 12-myristate 13-acetate (PMA) in presence or absence of 50 µM HA-1004, 100 nM chelerythrine, 100 nM bisindolylmaleimide, or 25 nM Go-6976. In case of addition of inhibitors, these were added 15 min before. All data are calculated as specific hybridization relative to that of GAPDH. * Statistical significance at P < 0.01.
conditions increase the mRNA of c-Fos, whereas c-J un mRNA is increased under hypertonic exposure in H-4-II-E hepatoma cells (20). However, de novo protein synthesis is not required for the effects of anisotonicity on PCK gene expression, thus making the possibility that at least part of the cell volume-induced changes in mRNA levels may be mediated by changes in the levels of Fos and J un rather unlikely. It appears more likely that preexisting transcription factors bind to the PCK promoter due to an altered phosphorylation state induced by anisotonicity.

Protein kinase C has been shown to inhibit transcription from the PCK promoter and exert its effects at the CRE-1 element and to interfere with the binding and/or stimulation of transcription from the PCK promoter normally associated with the cAMP regulatory element binding protein (27). Likewise, okadaic acid (27), vanadate (4), and lithium (5) both decrease the basal rate of PCK gene transcription and block the positive effect of cAMP on transcription by interaction with the CRE-1 element. It remains a possibility that hypotonic-induced cell volume increase mediates its negative effect on PCK transcription via the same promoter sequence.

The signal transduction mechanism by which the anisotonicity-induced cell volume changes exert their effect on cellular processes in hepatocytes is not clear at the moment, whereas osmosensing pathways in yeast are better understood (6). It is known that cell swelling leads to a transient increase in intracellular calcium concentration (23), a hyperpolarization of the cell membrane (10), a decrease in intracellular pH (13), an increase in potassium conductance (29), and stimulation of inositol 1,4,5-trisphosphate formation (2). In addition, participation of the cytoskeleton in cell volume regulation by hepatocytes has been suggested. However, there are no data at present relating these mechanisms to the effects of cell volume on hepatic glucose metabolism and PCK and FBP gene expression. The repressive effect of hypotonicity on PCK and FBP mRNAs was not inhibited by protein kinase C inhibitors or prevented by downregulation of protein kinase C. Furthermore, the inductive effect of hypertonicity could not be completely blocked by the cAMP-dependent protein kinase inhibitor HA-1004 at concentrations sufficient to abolish cAMP-mediated induction. Preliminary experiments showed no difference in cAMP-dependent protein kinase activity between cells kept in normotonic (305 mosmol/l) and hypertonic medium (405 mosmol/l). Thus the effects of anisotonicity on PCK and FBP gene expression do not appear to be mediated by either of these two protein kinases. It is conceivable, however, that changes in cell volume and cell shape perturb the cytoskeletal network, which in turn alters the configuration of signal transduction systems across the plasma membrane. For example, this could lead to an agonist-independent activation of certain signal transduction pathways. Thus it has been demonstrated that osmotic changes may specifically alter the phosphorylation state of histone-like proteins (28). From the above results it is not possible to estimate whether such a mechanism could be operational in the signal transduction between cell volume and PCK and FBP gene expression. An alternative explanation could be postulated based on the observation that the transcriptional activity of certain genes is dependent on the association of chromatin with the nuclear matrix. Changes in cell volume and the resulting perturbation of cytoskeletal structures possibly alter the integrity of the nuclear matrix, which in turn modifies its interaction with chromatin, resulting in changes in gene activity. Interestingly, a recent study showed an inductive effect of H-4-II-E cells grown at high density on PCK mRNA levels (3). It appears possible that this observation is related to the effects observed in the present study and would strengthen the concept that the expression of the PCK gene is coupled to the liver cell shape.

In conclusion, the present study provides evidence in support for a role of cell volume in regulating PCK and FBP gene expression in the liver, regardless of whether volume changes are induced by anisotonicity, nonmetabolizable sugars, or hormones. The mechanism involves changes in transcription rates as well as mRNA stability of the two genes, is apparently independent of cAMP-dependent protein kinase and protein kinase C pathways, and does not require ongoing protein synthesis. The H-4-II-E rat hepatoma cells should provide a system that will make feasible the further characterization of this potentially novel type of signal transduction mechanism by which liver cells react to changes in cell volume and tranduce this to alter the expression of specific genes.

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