Response to hypertonicity in mesothelial cells: role of Na\(^+\)/myo-inositol co-transporter

Yasuko Matsuoka, Atsushi Yamauchi, Takeshi Nakanishi, Toshihiro Sugiyama, Hiroshi Kitamura, Masaru Hori, Yoshihiro Takamitsu, Akio Ando, Enyu Imai, and Masatsugu Hori

1First Department of Medicine, and Department of Clinical Laboratory Science, Osaka University School of Medicine, Suita, 2Department of Kidney and Dialysis, Hyogo College of Medicine, Nishinomiya, and 3School of Health and Sport Sciences, Osaka University, Toyonaka, Japan

Abstract

Background. During peritoneal dialysis, the peritoneal mesothelium is exposed continually to hypertonic dialysates. The purpose of this study is to see if rat mesothelial cells have an osmoregulatory mechanism to adapt to hypertonic environment.

Methods. The intracellular content of organic osmo-lytes was measured by HPLC methods. Myo-inositol transport activity was measured by Na\(^+\)-dependent uptake of \(^3\)H-myo-inositol. mRNA abundance for the Na\(^+\)/myo-inositol co-transporter (SMIT) was examined by Northern and slot-blot analyses.

Results. In isotonic mesothelial cells, only myo-inositol could be detected. After switching to hypertonic medium made by addition of NaCl, myo-inositol content gradually increased and peaked at 48 h after the switch. The myo-inositol content in hypertonic cells increased >7-fold over the value in isotonic cells. The contents of betaine and glycerophosphorylcholine (GPC) also increased but were less than that of myo-inositol. Sorbitol was not accumulated in this condition. When glucose was used to increase medium osmolality, all of the four osmolytes were increased by hypertonicity (myo-inositol > sorbitol > GPC > betaine). Thus, myo-inositol is the most abundant osmolyte in the mesothelial cells. Na\(^+\)-dependent myo-inositol uptake in hypertonic cells was ~7-fold the uptake in isotonic cells, reaching a maximum 16 h after switching to a hypertonic medium. The uptake rate increased as medium osmolality increased from 300 to 500 mosm/kg. SMIT mRNA rapidly increased after increasing medium osmolality, reaching a maximum 8 h after the switch. The relative increase in the mRNA abundance was ~11 times isotonic levels.

Conclusions. Mesothelial cells respond to extracellular hypertonicity by increasing SMIT mRNA abundance, myo-inositol transport activity and accumulating myo-inositol into the cells.

Key words: betaine; glycerophosphorylcholine; osmo-lyte; peritoneal dialysis; sorbitol

Introduction

Many types of cell respond to extracellular hypertonicity by the accumulation of high concentrations of small organic solutes that protect cells from the perturbing effects of high intracellular concentrations of electrolytes [1,2]. These solutes are referred to as ‘compatible osmolytes’, since they are compatible with enzyme function [1]. In mammals, organic osmolytes have been investigated most widely in renal tubular cells such as Madin–Darby canine kidney (MDCK) cells [3]. Organic solutes identified as osmolytes in tubular cells are myo-inositol, sorbitol, betaine and glycerophosphorylcholine (GPC).

The glucose concentration of dialysis fluids commonly used for peritoneal dialysis is very high. A high concentration of glucose has been shown to have similar effects to those NaCl in terms of osmolyte accumulation and transport activity of the osmolyte in kidney cells [4–6]. Since the peritoneal mesothelial cells are exposed continually to hypertonicity during peritoneal dialysis, it is possible that mesothelial cells accumulate organic osmolytes to adapt to extracellular hypertonicity as do renal medullary cells. There has been, however, no information available concerning organic osmolytes in mesothelial cells thus far.

The purpose of this research is to clarify whether mesothelial cells accumulate organic osmolytes in response to extracellular hypertonicity. We found that mesothelial cells predominantly accumulated myo-inositol in response to hypertonicity. To clarify the mechanism for accumulation of myo-inositol in the cells, we further examined the increase in myo-inositol transport activity as well as mRNA abundance for the Na\(^+\)/myo-inositol co-transporter (SMIT) by hypertonicity.
Measurement of intracellular organic osmolytes

Intracellular organic osmolytes were measured in perchloric acid (PCA) extracts of mesothelial cells grown on 10 cm dishes. The cells were rinsed twice with Dulbecco’s phosphate-buffered saline (PBS). For the cells in hypertonic medium, PBS was made hypertonic (500 mosm/kg) by adding NaCl. After aspirating the excess PBS, 1 ml of 7% PCA was added to each dish. The cells were scraped off the dish and centrifuged at 2400 × g for 10 min. The supernatants were used for determination of osmolyte concentrations. The pellets were then dissolved in 0.25 M NaOH and were used for measurement of protein content (Bio-Rad Protein Assay) and determination of osmolyte concentrations. The pellets were then dissolved in 0.25 M NaOH and were used for measurement of protein content (Bio-Rad Protein Assay; Bio-Rad Laboratories, Cleveland, OH). The supernatants were used for determination of osmolyte concentrations.

Concentrations of osmolytes in PCA extract were measured by high-performance liquid chromatography (HPLC) as described previously [8]. Briefly, the supernatants from the centrifugation were neutralized with 2.0 M KOH to pH 7 and centrifuged again; the supernatants were then passed through a Sep-Pack C18 cartridge (Waters, Milford, MA). Bovine serum albumin was treated in an identical fashion (PCA, NaOH) and used as a protein standard.

Concentrations of osmolytes in PCA extract were measured by high-performance liquid chromatography (HPLC) as described previously [8]. Briefly, the supernatants from the centrifugation were neutralized with 2.0 M KOH to pH 7 and centrifuged again; the supernatants were then passed through a Sep-Pack C18 cartridge (Waters, Milford, MA). Bovine serum albumin was treated in an identical fashion (PCA, NaOH) and used as a protein standard.

Concentrations of osmolytes in PCA extract were measured by high-performance liquid chromatography (HPLC) as described previously [8]. Briefly, the supernatants from the centrifugation were neutralized with 2.0 M KOH to pH 7 and centrifuged again; the supernatants were then passed through a Sep-Pack C18 cartridge (Waters, Milford, MA). Bovine serum albumin was treated in an identical fashion (PCA, NaOH) and used as a protein standard.

Concentrations of osmolytes in PCA extract were measured by high-performance liquid chromatography (HPLC) as described previously [8]. Briefly, the supernatants from the centrifugation were neutralized with 2.0 M KOH to pH 7 and centrifuged again; the supernatants were then passed through a Sep-Pack C18 cartridge (Waters, Milford, MA). Bovine serum albumin was treated in an identical fashion (PCA, NaOH) and used as a protein standard.

Concentrations of osmolytes in PCA extract were measured by high-performance liquid chromatography (HPLC) as described previously [8]. Briefly, the supernatants from the centrifugation were neutralized with 2.0 M KOH to pH 7 and centrifuged again; the supernatants were then passed through a Sep-Pack C18 cartridge (Waters, Milford, MA). Bovine serum albumin was treated in an identical fashion (PCA, NaOH) and used as a protein standard.

Concentrations of osmolytes in PCA extract were measured by high-performance liquid chromatography (HPLC) as described previously [8]. Briefly, the supernatants from the centrifugation were neutralized with 2.0 M KOH to pH 7 and centrifuged again; the supernatants were then passed through a Sep-Pack C18 cartridge (Waters, Milford, MA). Bovine serum albumin was treated in an identical fashion (PCA, NaOH) and used as a protein standard.
Data analysis

The results shown are means ± SD. When no error bar is shown in a figure, the SD is smaller than the symbol. For comparisons between two groups, the unpaired Student’s two-tailed t-test was used, and for multiple comparisons the one-way analysis of variance (ANOVA) was used. Statistical significance was set at P < 0.05. All experiments were performed more than twice with similar results.

Materials

[3H]myo-inositol was purchased from New England Nuclear. Unlabelled myo-inositol was purchased from Sigma Chemical (St. Louis, MO). Other chemicals were of the highest purity available from commercial sources.

Results

Effects of hypertonicity on osmolyte content

To see if mesothelial cells accumulate organic osmolytes in response to hypertonicity, we measured the concentration of organic osmolytes in isotonic mesothelial cells and the cells 24 h after the medium osmolality was increased (from 300 to 500 mosm/kg) by addition of NaCl or glucose. Figure 1 shows the content of myo-inositol, betaine, GPC and sorbitol, all of which have been identified as organic osmolytes in renal medulla. In isotonic cells, myo-inositol was the only osmolyte that could be detected by HPLC. When NaCl was used to increase medium osmolality, the myo-inositol content in hypertonic cells increased to ~7-fold the value in isotonic cells (Figure 1). Betaine and GPC also increased in hypertonic mesothelial cells so that these osmolytes could be detected in hypertonic cells. However, sorbitol was not detectable in this condition. When glucose was used to increase medium osmolality, all of the four osmolytes were increased in mesothelial cells. Although myo-inositol was the most abundant osmolyte in the cells, the magnitude of the increase was smaller than that in NaCl-induced hypertonicity. The increases in betaine and GPC were also smaller. In contrast, sorbitol content was markedly increased by glucose-induced hypertonicity. Thus, mesothelial cells accumulate myo-inositol, betaine, GPC and sorbitol in response to glucose hypertonicity.

Since myo-inositol is the most abundant of these osmolytes in hypertonic mesothelial cells, we focused on myo-inositol accumulation and its transport. Figure 2 shows the time course of myo-inositol accumulation in the cells after increasing extracellular osmolality. The cells were grown in isotonic medium, then switched to hypertonic medium at time 0. Myo-inositol content gradually increased after the cells were switched to hypertonic medium, reaching a maximum (~9-fold the value in isotonic cells) 48 h after the switch.

Effects of hypertonicity on myo-inositol transport

The accumulation of myo-inositol in renal medullary cells has been shown to be due to the increased uptake through Na+-coupled transporter [4,15]. To see if Na+-dependent myo-inositol transport in mesothelial cells is regulated by tonicity, we examined [3H]myo-inositol uptake in isotonic and hypertonic mesothelial cells. Na+-dependent myo-inositol uptake was ~60% of total uptake in isotonic mesothelial cells (data not shown). The Na+-dependent component was induced predominantly by hypertonicity so that Na+ dependency was >91% in hypertonic cells.

The time course of the hypertonicity-induced increase in the Na+-dependent uptake of myo-inositol is shown in Figure 3. Switching mesothelial cells to a hypertonic culture medium (from 300 to 500 mosm/kg) elicited a rapid increase in the rate of myo-inositol uptake. Sixteen hours after the switch, the peak rate was seven times that in isotonic cells, and then the uptake rate decreased.
Effects of hypertonicity on SMIT mRNA abundance

Northern analysis of RNA from mesothelial cells revealed several bands, with sizes ranging from 1.0 to 13.5 kb, among which the 10 kb band is the most intense (Figure 6). To quantify the mRNA abundance, we used slot-blot assay. Figure 7 shows the time course of the change of SMIT mRNA abundance after switching medium osmolality. The co-transporter mRNA abundance increased very rapidly after increasing osmolality, reaching a peak (>11-fold the isotonic level) at 8 h after the switch. As shown in the bottom of the figure, the intensity of ethidium bromide staining does not change, demonstrating equal loading. After the peak, the mRNA abundance fell to 6–8 times that in isotonic cells. To see if the different inducibility of the myo-inositol transport rate among various solutes is due to transcriptional regulation or not, we examined the co-transporter mRNA in mesothelial cells bathed in the various hypertonic media for 16 h. As shown in Figure 6, increases in mRNA abundance for SMIT showed a similar pattern to the transport rate.

**MMI inhibited cell survival and growth in a hypertonic environment**

To see the effects of myo-inositol depletion on survival and growth of mesothelial cells, we examined colony-forming efficiency as a quantitative measure of survival and growth of mesothelial cells with or without MMI, an inhibitor of SMIT (Table 1). After allowing 24 h for cell attachment in isotonic medium, the medium was switched to hypertonic medium with various concentrations of MMI. Results are expressed as the percentage of the colony-forming efficiency under control conditions (hypertonic medium without MMI). MMI
Organic osmolytes in mesothelial cells

Fig. 6. Northern blot analysis of RNA isolated from isotonic and hypertonic mesothelial cells. The left part of the figure shows the time course of expression of SMIT mRNA in mesothelial cells after changing osmolality. At time 0, cells cultured in isotonic medium (300 mosm/kg) were switched to hypertonic (500 mosm/kg) medium made by addition of NaCl. Total RNA was extracted from mesothelial cells at each time point. Samples of 20 μg of total RNA were separated by electrophoresis on a 1% agarose–formaldehyde gel. The right part of the figure shows the effects of different solutes on the SMIT mRNA abundance. Mesothelial cells cultured in isotonic medium were switched to the medium made hypertonic by addition of NaCl (N), glucose (G), raffinose (R) and urea (U). Eight hours after the switch, total RNA was extracted from the cells. The ethidium bromide-stained gel (EtBr stain) demonstrates equal loading.

Table 1. Effects of inhibition of myo-inositol transport on colony-forming efficiency of mesothelial cells in a hypertonic environment

<table>
<thead>
<tr>
<th>MMI (mM)</th>
<th>Myo-inositol (mM)</th>
<th>Colony-forming efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (~0.04)</td>
<td>100 ± 7</td>
<td></td>
</tr>
<tr>
<td>0.5 (~0.04)</td>
<td>69 ± 8*</td>
<td></td>
</tr>
<tr>
<td>1 (~0.04)</td>
<td>56 ± 4*</td>
<td></td>
</tr>
<tr>
<td>2 (~0.04)</td>
<td>18 ± 2*</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>86 ± 9*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>70 ± 5*</td>
<td></td>
</tr>
</tbody>
</table>

Mesothelial cells were seeded at a density of 200 cells per 10 cm dish in isotonic medium. One day later, the medium was replaced with hypertonic medium with or without addition of MMI at the concentration indicated. Hypertonic medium was made by addition of NaCl up to 500 mosm/kg. Growth medium contains ~40 μM myo-inositol. Myo-inositol was added to the medium at the concentration indicated. Colonies were allowed to grow for 1 week without feeding. The number of colonies with a diameter >1 mm were counted. Results are expressed as the percentage of the colony-forming efficiency without addition of MMI. Values are means ± SD of three independent experiments.

Discussion

This study demonstrates that mesothelial cells respond to hypertonic stress by accumulating organic osmolytes significantly decreased the colony-forming efficiency in a concentration-dependent manner. In contrast, no remarkable changes were observed in isotonic cells in the presence of MMI (data not shown). The addition of myo-inositol to the medium, at a concentration three times the value of MMI, mostly prevented the adverse effects of MMI. This suggests that the decrease in colony-forming efficiency is not due to the non-specific toxicity of MMI but to specific action on myo-inositol transport. These results suggest that myo-inositol play a crucial role in mesothelial cells regarding osmoregulation of the cells.

Fig. 7. SMIT mRNA abundance was quantitated by slot blot assay. Samples of 5 μg of RNA was spotted onto a nylon membrane using a slot-blot filtration manifold. Values were normalized to the isotonic level. Each point is the mean of three experiments. *P<0.001 vs time 0.

such as myo-inositol, betaine, GPC and sorbitol. Mesothelial cells predominantly accumulate myo-inositol in response to hypertonicity. We also found that the uptake of myo-inositol by mesothelial cells as well as the mRNA for SMIT was regulated by medium tonicity. The effect of hypertonicity on the transport and the mRNA abundance in mesothelial cells resembles that in MDCK cells, suggesting that myo-inositol transporter functions as an osmolyte transporter in mesothelial cells and participates in intracellular accumulation of myo-inositol. As far as we are aware, this is the first report concerning organic osmolytes in mesothelial cells.
The total osmolyte content we measured (the sum of myo-inositol, betaine, GPC and sorbitol) in hypertonic mesothelial cells was 900 nmol/mg protein in a hypertonic medium made by NaCl, and 700 nmol/mg protein for that made by glucose. These values are comparable with that in MDCK cells in similar conditions [16]. The difference in the content between isotonic and hypertonic cells was ~850 and 650 nmol/mg protein, respectively. If the water content of mesothelial cells is similar to that of MDCK cells, it corresponds to ~190 and 150 mM in terms of intracellular concentration. This intracellular concentration of organic osmolytes is almost enough to fill the gap in osmolality between isotonic and hypertonic cells.

The increase in myo-inositol transport activity in response to hypertonicity is preceded by an increase in the abundance of SMIT mRNA, suggesting that myo-inositol transport activity increases by changing the abundance of the mRNA for the myo-inositol transporter. This probably results in an increased number of transporters, increased uptake of myo-inositol into the cells and increased intracellular content of myo-inositol. These results suggest that enhanced transcription of the myo-inositol transporter gene is playing a key role in myo-inositol accumulation in mesothelial cells by hypertonicity, as shown in renal medullary cells [17] and mesangial cells [6].

It was reported that a significant number of patients on chronic peritoneal dialysis show deterioration in membrane transport performance with time [18]. The deleterious effects of frequent use of hypertonic exchanges have been reported [19]. There is evidence that in vitro toxicity of dialysates on mesothelial cells is mainly osmolality-mediated [20,21]. These results suggested that hyperosmolality and/or high glucose concentration have adverse effects on the peritoneal membrane. Previous studies have shown that hypertonicity has various effects on cells in tissue culture. High levels of hypertonicity generally depressed cell function. For example, DNA synthesis and cell growth are inhibited by hyperosmolality [22]. On the other hand, the expression of several genes, such as aldose reductase [23], Na\(^+\)/myo-inositol transporter [17], Na\(^+\)/Cl\(^-\) betaine transporter [24] and immediate early genes [25], has been shown to be increased. Hypertonicity also modulates the actions of some autacoids and cytokines [26–32]. These results raise the possibility that hypertonicity causes significant changes in structure and function of peritoneal membrane.

Since the peritoneal membrane exists essentially in an isotonic environment, we speculated that the peritoneal cells have only a poorly developed osmoregulatory mechanism. Unexpectedly, mesothelial cells accumulate organic osmolytes sufficiently to balance between the intracellular and extracellular osmolality. This means that the mesothelial cells have a well-developed osmoregulatory mechanism like renal medullary cells. If there are abundant osmolytes available in the extracellular fluid, the cells are able to accumulate enough osmolytes. There are, however, at least two problems. The first is that dialysis solution does not contain organic osmolytes such as myo-inositol and betaine. Although cells are able to synthesize sorbitol from glucose, it will not be enough to balance against the hyperosmolality of dialysis solution. The second problem is that the osmolality of the dialysis solution changes very rapidly. Although the mesothelial cells are exposed to an acute increase in osmolality several times a day, the cells accumulate organic osmolytes rather slowly (Figure 2). The deficiency of osmolytes has been shown to be harmful in a hypertonic environment [11,14]. In fact, survival and growth of the mesothelial cells under hypertonic conditions were markedly suppressed in the presence of an inhibitor of myo-inositol transport (Table 1). It is possible that the deleterious effect of hypertonic dialysates is at least partly due to the insufficient accumulation of the osmolytes during peritoneal dialysis. The findings of an in vitro study for osmolytes in the mesothelial cells have clinical importance, because these results support a possible therapeutic efficacy of supplementation of the osmolytes in peritoneal dialysis solution.

Acknowledgements. Rat peritoneal mesothelial cells were a gift from Dr T. R. Shockley and Dr Cathy Hoff, Baxter Healthcare Corporation (MacGaw Park, IL). We thank Dr Joseph S. Handler (The Johns Hopkins University, Baltimore) for helpful advice. This research was supported by a grant-in-aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan.

References


Received for publication: 9.9.98
Accepted in revised form: 8.1.99