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Intracellular sorbitol content in isolated rat inner medullary collecting duct cells

Regulation by extracellular osmolarity*

R. W. Grunewald and R. K. H. Kinne

Max-Planck-Institut für Systemphysiologie, Rheinlanddamm 201, D-4600 Dortmund 1, Federal Republic of Germany

Abstract. In order to study the mechanisms involved in the regulation of renal inner medullary sorbitol content, collecting duct cells were isolated from rat inner medulla and the effect of extracellular osmolarity on sorbitol synthesis and sorbitol content was investigated. Cells isolated at 300 mosmol/l and incubated up to 24 h as primary cultures in 300 mosmol/l media or in media made 600 mosmol/l by the addition of 150 mM NaCl showed no difference in total synthesis. Intracellular sorbitol content was, however, 2.3-fold higher in the cells kept in the higher osmotic medium. Cells isolated at 600 mosmol/l released sorbitol about 8 times faster when transferred into hypoosmotic medium (300 mosmol/l) than when transferred into isoosmotic (600 mosmol/l) media. Cells exposed to hyperosmotic media (900 mosmol/l with NaCl) maintained a higher intracellular sorbitol content than cells incubated in isoosmotic media. Changes of intracellular sorbitol content could not be attributed entirely to cell lysis – as demonstrated by determination of cellular content of lactate and lactate dehydrogenase. The alteration in sorbitol membrane permeability was reversible and was only observed when poorly permeable solutes (such as NaCl and sucrose) were used for the experiments, changes in urea elicited no effect. It is proposed that rapid changes in membrane permeability to sorbitol play an important role in the adjustment of intracellular sorbitol concentration in inner medullary collecting duct cells to changes in extracellular osmolarity.

Key words: Sorbitol – Organic osmolytes – Inner medullary collecting duct – Membrane permeability – Osmoregulation

Introduction

During recent years, various organic solutes have been implied to serve as "nonperturbing" osmolytes in the volume regulation of renal outer medullary and inner medullary cells. One of these solutes is sorbitol, which has been demonstrated to accumulate in papillary tissue of antidiuretic rats [22] and in inner medullary epithelial cell lines grown for extended periods of time in culture media with a high osmolarity [2]. In this inner medullary cell line it also has been demonstrated that high extracellular osmolarity induces synthesis of the enzyme aldose reductase [2, 5], which represents the major pathway of sorbitol synthesis in the inner medulla [9]. The changes observed in enzyme content are, however, slow compared to changes observed in inner medullary sorbitol content when in the intact animals the renal function changes acutely from antidiuresis to diuresis. The synthesis of aldose reductase requires days to reach significant levels [22], whereas for example in rat inner medulla a 4-fold reduction in inner medullary sorbitol content is observed within 4 h of furosemide infusion [22]. Thus, it seemed unlikely that in collecting duct cells intracellular sorbitol content is regulated solely by changes in sorbitol synthesis. We, therefore, hypothesized that also the plasma membrane permeability to sorbitol might be affected by extracellular osmolarity, similar to observations made in flounder erythrocytes with regard to the permeability to GABA, a nonperturbing osmolyte employed by this species [11, 12] and recently, with regard to sorbitol [3], in a cultured renal epithelial cell line. Since such studies in the intact animals are complicated by the difficulty to control and define the extracellular osmolarity and to distinguish between extracellular and intracellular sorbitol content, we used a model system of inner medullary collecting duct cells isolated from kidney inner medulla. This cell system has been shown previously to be morphologically, biochemically, and functionally very similar to the inner medullary collecting duct in situ [20]. The studies presented below indicate that indeed sorbitol permeability of inner medullary collecting duct cells can be modified rapidly and reversibly by changes in extracellular osmolarity and thus plays a major role in the short term regulation of intracellular sorbitol content. Part of the results has been presented in abstract form [13].

Materials and methods

Isolation of inner medullary collecting duct (IMCD) cells. Male Wistar rats (200–300 g) were sacrificed by cervical dislocation, the white inner medullae of the kidneys were removed rapidly and placed into an ice-cold HEPES Ringer's solution pH 7.4 (in mM: 118 NaCl, 16 HEPES, 16 Na HEPES, 14 glucose, 10 Na pyruvate, 2 Na acetate, 3.2 KCl, 2.5 $CaCl_2$, 1.8 $MgSO_4$, 1.8 KH_2PO_4). After mincing the inner medullae, the pieces were incubated in the same buffer with addition of 0.2% collagenase and 0.2% hyaluronidase. Incubation took place at 37° C in a water bath under gasing with room air for 75 min. DNAse (0.001%) was added after 45 min. IMCD cells were isolated by a three steps centrifugation procedure (28 × *g*, 2 min, about 30° C), as described previously [20]. The final cell preparation

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Offprint requests to: R. K. H. Kinne

contained 97% collecting duct cells. For some experiments the osmolarity of the isolation medium was raised from 300 to 600 mosmol/l by the addition of NaCl.

Measurement of sorbitol synthesis. Isolated IMCD cells were resuspended in HEPES Ringer's solutions of various osmolarities in the presence of 25 mM D-glucose as substrate. Osmolarity was adjusted by the addition of NaCl. The cells were then incubated for various periods of 1 – 24 h in sterile cell culture flasks at 37° C in a 5% CO_2/95% O_2 atmosphere. After incubation cells and supernatant were separated by centrifugation for 10 min at 3,000 × *g* and 4° C, the supernatants removed completely and the cell pellets lysed with 100 µl sodiumduodecylsulfate (SDS, 0.2%) and 900 µl aqua bid. Samples were incubated 15 min in an 80° C waterbath and subsequently centrifuged (12,000 × *g*, 4° C, 10 min). The deproteinized supernatant was used for sorbitol determination.

Measurement of sorbitol and lactate efflux. IMCD cells were incubated in media of various osmolarities and aliquots of the samples separated into pellet and supernatant by centrifugation, as noted above. Pellet and supernatant were then used for the determination of sorbitol content or lactate content and lactate dehydrogenase activity.

Protein determination. Protein was measured in triplicate by the method described by Lowry et al. after precipitation of the protein with 10% ice-cold trichloroacetic acid [17]. Bovine serum albumin (Boehringer, Mannheim, FRG), in concentrations between 0.3 and 0.7 mg/ml was used as standard.

Determination of sorbitol. Sorbitol was determined by a commercially available test kit (Boehringer, Mannheim, FRG), where sorbitol is oxidized in the presence of sorbitol dehydrogenase and NAD to fructose and $NADH + H^+$. NADH is then oxidized by iodonitrotetrazolium chloride in the presence of diaphorase to formazan. The samples (100 µl) and assay mixture (400 µl) were incubated for 45 min at room temperature in the dark. Then, the increase of absorbance was measured at 492 nm. Sorbitol from Fluka (Neu-Ulm, FRG) was used as external and internal standard [6, 7].

Determination of lactate. Lactate was determined by a commercially available test kit (Behringwerke, Marburg, FRG). Lactate is oxidized by NAD in the presence of lactate dehydrogenase to pyruvate and $NADH + H^+$. In a second step, pyruvate is converted in the presence of glutamate pyruvate transaminase and glutamate to alanine. The increase of NADH is measured at 340 nm after an incubation for 45 min at room temperature [18].

Determination of lactate dehydrogenase activity. Lactate dehydrogenase (LDH) activity was determined in freshly prepared and homogenized IMCD cells (by ultrasonication for 2 min at 4° C) and in the supernatants of IMCD cells used for efflux studies. The test was performed with a commercially available kit (Boehringer, Mannheim FRG) in which the reduction of pyruvate by NADH in the presence of LDH to lactate and NAD is monitored by the rate of decrease in absorbance at 340 nm. Absorbance changes were recorded at 37° C every minute over a total period of 6 min in a Beckman DU 6 spectrophotometer [10, 21].

Statistical analysis. For statistical analysis paired or unpaired Student's *t*-test was employed, a difference was considered as significant at $p < 0.05$. Mean values with their respective standard deviation are given throughout the paper.

Materials. All chemical reagents were of the highest purity commercially available. The aldose reductase inhibitor AL 01576 was a generous gift of Alcon Laboratories, Fort Worth, TX, USA.

Results

Effect of osmolarity on sorbitol content and synthesis in isolated IMCD cells

In order to establish whether isolated cells of the inner medullary collecting duct responded to changes in extracellular osmolarity in a way similar to the inner medulla in vivo we first investigated the intracellular sorbitol content in IMCD cells exposed in vitro to different osmolarities. After 2 h of incubation, intracellular sorbitol content was significantly higher in cells incubated at 600 mosmol/l (103 ± 13 mmol/kg protein) than in cells incubated at 300 mosmol/l (73 ± 5 mmol/kg protein, $n = 3$, $p < 0.025$). After 24 h of incubation, the sorbitol content in the high osmolarity cells had increased from initially 49 to 221 mmol/kg protein. Since in the same experiments also the sorbitol content of the extracellular medium was determined, the total sorbitol synthesis could be calculated. It amounted to 78 ± 6 mmol/kg · h and was slightly lower in the high osmolarity cells (74 ± 5 mmol/kg · h, not significant, $n = 3$), than in the low osmolarity cells. These studies demonstrate that isolated IMCD cells, as IMCD cells in vivo, adjust their intracellular sorbitol content to the osmolarity of the extracellular medium. The results indicate furthermore that sorbitol synthesis appears not to be the regulating factor underlying this adjustment. From the data presented above also the transmembrane gradient for sorbitol under the various experimental conditions can be estimated. The transmembrane gradient in high osmolarity cells increased 5-fold during the 24 h incubation period suggesting that the membrane permeability for sorbitol at high osmolarity is lower than at low osmolarity. We, therefore, attempted in the following to study the effect of osmolarity on the sorbitol permeability of the plasma membrane directly[1].

Effect of osmolarity on sorbitol and lactate efflux from IMCD cells

In the experiments described below IMCD cells were isolated in a 600 mosmol/l medium and then subjected to the identical osmolarity, to hypoosmotic conditions (300 mosmol/l) or hyperosmotic conditions (900 mosmol/l) for various time intervals. Pyruvate was used as substrate in order to reduce the synthesis of sorbitol. The effect of exposure to different

[1] The calculation of the transmembrane sorbitol gradient is based on the assumption of an intracellular volume of 9 l per kg protein. This value was derived from the results that 1 g of wet tissue is [20] equivalent to 100 mg of protein and yields 10% dry mass

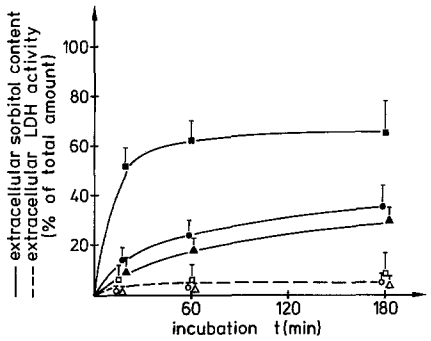

Fig. 1. Effect of extracellular osmolarity on sorbitol and LDH release from IMCD cells. IMCD cells were isolated in 600 mosmol/l buffer. Incubation of the cells for 20 min up to 3 h at 37° C took place in different buffers: 300 mosmol/l (*squares*), 600 mosmol/l (*circles*) and 900 mosmol/l (*triangles*). 10 mM pyruvate were used as substrate and osmolarity was increased by addition of NaCl. Extracellular sorbitol content (*closed symbols*) and extracellular LDH activity (*open symbols*) are shown in percent of the total content (131 ± 17 μmol/g protein). Mean values ± SD derived from 9 experiments are given

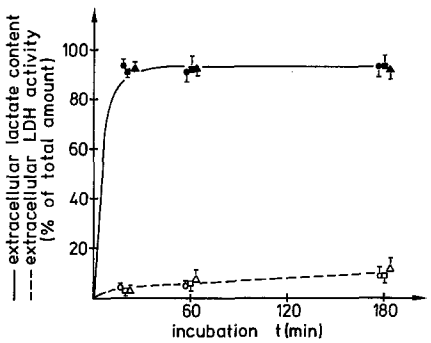

Fig. 2. Lactate efflux from IMCD cells. IMCD cells were isolated in 600 mosmol/l buffer. Incubation of the cells for 20 min up to 3 h at 37° C took place in different buffers: 300 mosmol/l (*squares*), 600 mosmol/l (*circles*) and 900 mosmol/l (*triangles*). 10 mM pyruvate were used as substrate and osmolarity was increased by addition of NaCl. Extracellular lactate content (*closed symbols*) and extracellular LDH activity (*open symbols*) are shown in percent of the total content. Mean values ± SD derived from 9 experiments are given

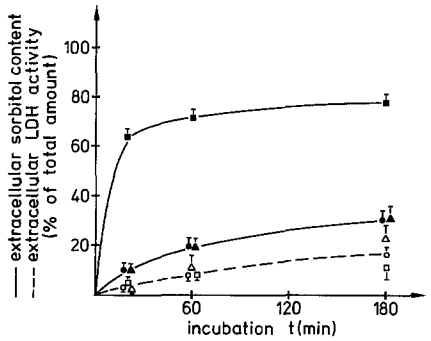

Fig. 3. Sorbitol efflux from IMCD cells in the presence of sucrose. IMCD cells were resuspended in buffers with different osmolarities after isolation at 600 mosmol/l: 300 mosmol/l (*squares*), 600 mosmol/l (*circles*), 900 mosmol/l (*triangles*). The extracellular sorbitol content (*closed symbols*) and the extracellular LDH activity (*open symbols*) are shown as a percentage of the total amount as mean values ± SD ($n = 3$). 10 mM pyruvate as substrate were used during the incubation and the osmolarity was changed by sucrose added to the 300 mosmol/l buffer

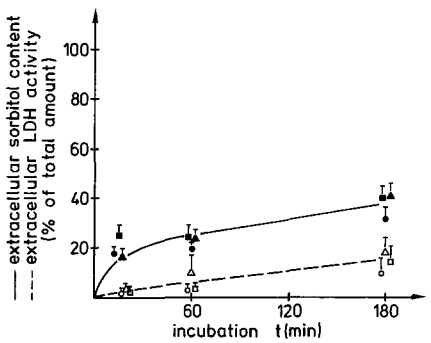

Fig. 4. Sorbitol efflux from IMCD cells in the presence of urea. IMCD cells were resuspended in buffers with different osmolarities after isolation at 600 mosmol/l: 300 mosmol/l (*squares*), 600 mosmol/l (*circles*), 900 mosmol/l (*triangles*). The extracellular sorbitol content (*closed symbols*) and the extracellular LDH activity (*open symbols*) are shown as a percentage of the total amount as mean values ± SD ($n = 3$). 10 mM pyruvate as substrate were used during the incubation and the osmolarity was changed by urea added to the 300 mosmol/l buffer

osmolarities on the release of sorbitol from IMCD cells is shown in Fig. 1.

Cells exposed to hypoosmotic media release sorbitol at a much faster rate than cells exposed to isoosmotic or hyperosmotic media. The corresponding $t_{1/2}$ estimated from the first three values is 13 min for the hypoosmotic conditions, 100 min for isotonic conditions and 170 min for the hypertonic conditions.

Considering the intracellular sorbitol content, transfer into the isoomotic medium leads to a loss of 30% after 3 h. This loss is dramatically accelerated when cells are transferred into a hypoosmotic medium. Already after 20 min about 50% of intracellular sorbitol have been released; after 1 h about 60%. On the contrary, when exposed to high osmolarity 75% of the initial intracellular sorbitol are still present after 3 h of incubation.

Since the drastic changes observed when transferring IMCD cells into the hypoosmotic medium could be caused by cell rupture rather than changes in membrane permeability, we also investigated the appearance of lactate dehydrogenase (LDH) in the supernatant under the different incubation conditions. As also shown in Fig. 1, transfer of the cells into the hypoosmotic medium does not lead to a significantly different release of LDH into the medium. The total LDH activity was 3047 U/g protein in the cells.

Further evidence for a specific response of the cell membrane to changes in medium osmolarity can be derived from Fig. 2, where the effect of changes in extracellular osmolarity on lactate efflux is depicted. Under all experimental conditions, lactate efflux proved to be unaffected by different osmolalities.

Effect of sucrose and urea on sorbitol efflux

In order to test whether the effect of addition of 300 mosmol/l NaCl on sorbitol permeability was due to an increase in salt

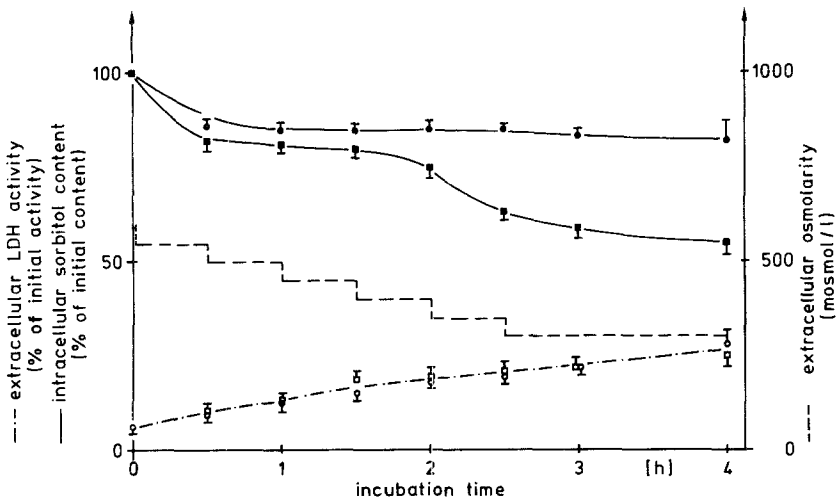

Fig. 5
Sorbitol efflux from IMCD cells when osmolarity is slowly decreased. IMCD cells were isolated in a 600 mosmol/l buffer. Osmolarity was decreased in steps of 50 mosmol/l and per 30 min by addition of water (*closed squares*) while the osmolarity in the control remained at 600 mosmol/l (*closed circles*). Presented are the mean values ± SD of the intracellular sorbitol content (corrected for LDH leak) and the extracellular LDH activities (corresponding *open symbols*) in percent of total amount ($n = 2-3$)

Table 1. The effect of the aldose reductase inhibitor AL 01576 on sorbitol production in IMCD cells

	Incubation time (h)	Total sorbitol amount mmol/kg protein	*n*	Significance
Begin of incubation	–	185±12	3	
Control	3	347±18	3	$p < 0.0025$
AL 01576 10^{-6} M	3	191±12	3	n.s.

Three incubations of freshly isolated IMCD cells were performed for 3 h at 37° C in 600 mosmol/l buffer with 14 mM glucose. Total sorbitol amount = content inside IMCD cells plus culture medium. Values are means ± SD. (n.s., not significant; *n*, number of experiments)

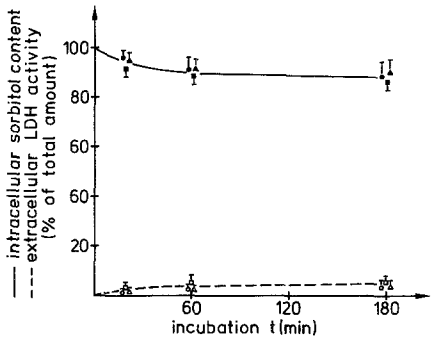

Fig. 6. Temperature dependence of sorbitol efflux. IMCD cells were incubated at 4° C in buffers with different osmolarities after isolation at 600 mosmol/l: 300 mosmol/l (*squares*), 600 mosmol/l (*circles*), 900 mosmol/l (*triangles*). The intracellular sorbitol content (*closed symbols*) and the extracellular LDH activity (*open symbols*) (mean values ± SD) are presented in percent of total amount ($n = 3$). Osmolarity was changed by addition of NaCl to the 300 mosmol/l buffer

concentration or due to the increase in the transmembrane osmotic gradient, the efflux studies were repeated under conditions where the extracellular osmolarity was increased by sucrose or urea instead of NaCl. The results obtained in these experiments are compiled in Fig. 3. When the osmolarity of the incubation medium was varied by the addition of sucrose, data similar to the ones with NaCl were obtained. When, however, urea, which readily permeates membranes was used, no significant difference in sorbitol efflux was observed (Fig. 4). These results suggest that it is the osmotic difference between the intra- and extracellular fluid which triggers the permeability changes.

In vivo, changes of extracellular osmolarity are normally not as drastic and fast as those applied to the cells in the experiments reported above. We, therefore, decreased osmolarity in smaller steps in order to study whether under more "physiological" conditions sorbitol permeability of IMCD membranes also changed. As shown in Fig. 5, also under these conditions an increase in sorbitol efflux after the reduction in osmolarity could be demonstrated. Sorbitol loss was low at the beginning when the medium osmolarity dropped from 600 to 500 mosmol/l, at osmolarities below 400 mosmol/l the sorbitol efflux was markedly stimulated. LDH loss under these experimental conditions was identical in the two cell preparations, indicating the specificity of the change in sorbitol permeability.

Effect of substrate and temperature on sorbitol efflux

In order to exclude that the permeability changes observed were due to an impairment of cellular energy metabolism since only pyruvate had been used as substrate the experiments were repeated in the presence of D-glucose. The aldose reductase inhibitor (AL 01576) was added in order to prevent sorbitol synthesis from D-glucose, which would have complicated the interpretation of the data. In preliminary experiments the concentration of aldose reductase inhibitor required to inhibit sorbitol synthesis was determined. Since, as shown in Table 1, a concentration of 1 μM completely inhibited sorbitol synthesis, this concentration was used in the following. Also under these experimental conditions changes in sorbitol efflux by medium osmolarity were observed suggesting that pyruvate as substrate for IMCD cells has no negative effect on cell metabolism or membrane permeability. LDH leakage was also not different from the pyruvate experiments (Table 2).

When the incubation temperature was lowered to 4° C (see Fig. 6), the decrease in intracellular sorbitol under isoosmotic conditions was already lower than at 37° C, pointing to a role of the plasma membrane in this process. More interestingly, also the modulation of sorbitol release

Table 2. Substrate influence on sorbitol efflux

Incubation time (min)	Osmolarity (mosmol/l)	Substrate	Total sorbitol amount (mmol/kg protein)	Extracellular sorbitol (% of total amount)	LDH activity (% of total activity)	*n*
Start	600	Glucose	120± 6	3±2	1±1	3
20	600	Pyruvate	115± 8	13±3	2±1	3
20	600	Glucose + ARI	117± 3 n.s.	11±2 n.s.	2±2 n.s.	3
20	300	Pyruvate	120± 7	54±4	3±2	3
20	300	Glucose + ARI	119±13 n.s.	54±2 n.s.	2±1 n.s.	3

IMCD cells were isolated in a 600 mosmol/l buffer and incubated for 20 min in a 600 or 300 mosmol/l buffer with either pyruvate (10 mM) or glucose (14 mM) plus the aldose reductase inhibitor (10 μM) as substrates. The osmolarity was varied by addition of NaCl to the 300 mosmol/l buffer. Mean values ± SD are given. (n.s., not significant; *n*, number of experiments; ARI, aldose reductase inhibitor)

Table 3. Reversibility of changes in sorbitol permeability

Incubation time (min)	Osmolarity (mosmol/l)	Total sorbitol amount (mmol/kg protein)	Intracellular sorbitol content (mmol/kg protein)	Intracellular sorbitol concentration (mM)	Extracellular sorbitol content (mmol/kg protein)	Extracellular sorbitol concentration (mM)	Transmembrane gradient	*n*
Start	600	122±19	119±19	13.2	4± 3	0.002	6600	4
20	300	117±16	66±17	7.3	51±14	0.026	281	4
80	300 (control)	158±16	74±15	8.2	84± 2	0.042	195	3
	600	156±13 (+1%) n.s.	90±15 (+22%) $p < 0.05$	10.0	66± 4 (−21%) $p < 0.05$	0.033	303	3
200	300 (control)	261±23	128± 6	14.2	134±28	0.067	211	3
	600	246±55 (−6%) n.s.	169±21 (+32%) $p < 0.05$	18.7	77±35 (−43%) $p < 0.05$	0.038	492	3

After the isolation of IMCD cells in 600 mosmol/l and exposure to 300 mosmol/l for 20 min, the osmolarity was raised again to 600 mosmol/l by addition of NaCl. A control was further incubated at 300 mosmol/l. Glucose (14 mM) was added in order to increase intracellular sorbitol synthesis to normal levels. The intracellular sorbitol concentration is calculated assuming a constant intracellular volume of 9 l per kg protein [20]. The extracellular sorbitol concentration is calculated taking into account that each sample contained in the average 0.5 g protein/l. The transmembrane gradient is the ratio between intra- and extracellular sorbitol concentration. (n.s., not significant, *n*, number of experiments)

by changes in extracellular osmolarity was completely abolished. Sorbitol content in hypoosmotic and hyperosmotic cells was identical to that observed in isoosmotic cells.

Reversibility of changes in sorbitol permeability in IMCD cells

If changes in membrane permeability were to play a role in volume regulation of IMCD cells in vivo, such changes should be reversible to enable the cells to adapt to a reversal of osmotic gradients. Therefore, in the next series of experiments cells isolated at 600 mosmol/l were first exposed to a 300 mosmolar buffer for 20 min without glucose. Then, osmolarity was raised by addition of 300 mosmol/l NaCl and glucose was added as substrate. Control cells were kept at 300 mosmol/l, but received also D-glucose. The results of these experiments are shown in Table 3. In both conditions total sorbitol recovered in supernatant and pellet increased to the same extent, confirming the data obtained previously. Most importantly, however, intracellular sorbitol content in the cells exposed to 600 mosmol/l increased much faster than in control cells. As a consequence, the calculated transmembrane gradient was 1.6-fold higher after 1 h and 2.3-fold higher after 3 h of incubation. These results suggest that the increase of sorbitol membrane permeability induced by reducing the osmolarity of the extracellular medium is reversible and cannot be attributed solely to unspecific effects on cell viability.

Discussion

Sorbitol content and metabolism in isolated IMCD cells

The sorbitol content found in the current studies in isolated IMCD cells (about 100 mmol/kg protein) compares favorable with the values reported by Wirthenson et al. for isolated collecting tubules [22]. This value is slightly lower than the value found in the inner medullary tip of diuretic rats of 180 mmol/kg protein and higher than the sorbitol content in conditioned inner medullary epithelial cells reexposed for 3 weeks to 300 mosmol/l culture medium. The sorbitol content is, however, much lower than the one found by Bagnasco et al. in inner medullary epithelial cells cultured for 3 years in a medium with 600 mosmol/l of 830 mmol/kg

protein and the one found in the inner medullary tip in antidiuretic rats [1].

The discrepancy with the latter values can be explained by at least two factors. First, the preparation used in our experiments contains cells derived from the whole inner medulla, where it has been demonstrated that the sorbitol content increases from about 30 mmol/kg protein at the base of the inner medulla to 900 mmol/kg protein at the tip of the inner medulla [22]. Assuming that the sorbitol content is constant during the preparation only an average value significantly lower than at the tip would be expected in the isolated cells. In view of the results presented above a second factor which might reduce the intracellular sorbitol content is efflux during the isolation of the cells. Since the cells are isolated from normal rats (average inner medullary sorbitol content 74 ± 22 mmol/kg), in media with an osmolarity lower than the osmolarity in the interstitium stimulation of sorbitol efflux can be expected. That such efflux occurs during the cell isolation is also borne out by the fact that cells isolated in 600 mosmol/l media have a higher sorbitol content than cells isolated at 300 mosmol/l.

The rate of sorbitol synthesis observed in our experiments (73 mmol/h · kg protein) is similar to the one found by Wirthenson (54.6 mmol/h · kg protein) at the D-glucose concentration of 25 mM used in our experiments and slightly higher than the rate found by Bagnasco in the conditioned inner medullary epithelial cell cultures [2, 22]. Thus, both with regard to intracellular sorbitol content and sorbitol synthesis the isolated IMCD cells seem to be comparable to other cell lines or preparations and the inner medullary collecting duct in situ. The same holds for the response of these cells to increased extracellular osmolarity. Similar to the situation in vivo intracellular sorbitol content increases markedly under hyperosmotic conditions.

Effect of extracellular osmolarity on sorbitol release

After having established that at least during short time exposure sorbitol synthesis is not altered by augmenting extracellular osmolarity, the increased intracellular sorbitol content could be either due to a decreased rate of metabolic breakdown or decreased release from the cell. The former possibility seems unlikely, since according to preliminary enzymatic studies and investigations using C^{13} NMR the activity of the sorbitol dehydrogenase is low in isolated IMCD cells compared to the rate of synthesis [14]. A change in breakdown is also improbable in view of the fact that in the efflux studies the intracellular sorbitol was recovered unaltered in the extracellular medium.

A difference in membrane permeability depending on medium osmolarity was first suggested from the calculation of the transmembrane sorbitol gradients after incubation of the cells for 24 h in the different media. Under these conditions it seems to be relatively safe to assume that the cells had sufficient time to adjust their volume to an identical value. Therefore, conversion of the content per kg cell protein to intracellular concentrations appeared to be justified. The studies of Bagnasco et al. [1] involving longer time periods show that no difference in intracellular volume exists between conditioned cells and non conditioned cells. Electron microprobe analysis data, however, suggest differences in collecting duct cell volume in diuresis and antidiuresis [4, 8]. Thus, direct measurements of the cell volume have to be performed in order to substantiate the above made assumption. Further evidence for the regulation of sorbitol release by the osmolarity of the extracellular medium was derived from experiments, where at a given sorbitol gradient sorbitol efflux was determined. The rapid release of sorbitol into the hypoosmotic medium was carefully scrutinized with regard to the possibility of cell lysis and unspecific decrease in cell viability. Therefore, LDH release was determined. When the change in osmolarity was performed in smaller steps, also significant effects on sorbitol release were observed. Interestingly, these studies revealed that a certain osmolarity gradient had to be present across the membrane in order to elicit the increased sorbitol release. The reason for this apparent threshold remains to be determined.

In addition, reversibility of the permeability changes was demonstrated. Based on this compounded evidence we conclude that reducing the extracellular osmolarity increases the membrane permeability for sorbitol. Since this regulation was also observed in the opposite direction i.e. decrease in permeability when the medium osmolarity was increased, we suggest that the IMCD cells regulate their intracellular osmolarity mainly by changes in their membrane permeability.

Nature of the osmotically regulated sorbitol permeability

Previous to these studies the permeability of plasma membranes to polyols was considered to be relatively low. In hypoosmotic medium, however, this seems not to be the case. From our results some clues about the nature and the specificity of the osmotically induced permeability can be derived. The permeability is temperature-dependent, i.e. seems to be controlled by the fluidity of the membrane. Membrane permeation, therefore, probably occurs via a carrier mechanism rather than by simple diffusion or a channel – the latter processes show usually a low temperature sensitivity. The translocation system is not inhibited by the aldose reductase inhibitor, thus, a difference in substrate specificity between the aldose reductase and the translocator can be predicted. The translocator probably also does not accept sucrose, since efflux in equiosmolar sucrose and salt solution was similar. From the same experiments it can be concluded that the transport system does not require the presence of sodium or chloride at the outside of the cell. It also seems to differ from the bacterial transport system for polyols, since it operates downhill, probably without the involvement of metabolic energy [16, 19].

The nature of the regulatory signal for the permeability remains obscure. The only fact that can be presented at the present time is that a certain transmembrane gradient of poorly permeable solutes i.e. probably an initial change in cell volume is required to activate the system. Therefore, urea is unable to elicit the response. The next steps in the chain of signal transduction could include posttranslational modifications of membrane proteins, as described in flounder erythrocytes [15] and bacteria [23]; further studies are required to elucidate these questions.

Physiological role of osmotically induced changes in sorbitol permeability

The current studies provide the first experimental basis to understand the mechanism underlying the changes in sorbitol content in the inner medulla of rats subjected to various diuretic states. According to studies by Wirthenson et al. the

inner medullary sorbitol content of initially antidiuretic rats decreases by about 75% during a 4 h period of furosemide diuresis [22]. Such a decrease is easily explainable from our results, where within 1 h the sorbitol content in isolated IMCD cells decreased by 60%, when incubated in hypoosmotic media. If one takes into account that probably the transmembrane gradients for sorbitol in the above reported in vivo experiments are higher than in vitro, the slightly larger decrease observed in vivo can readily be accounted for.

The recovery rate in vivo can be estimated from experiments with Brattleboro rats, which received an infusion of ADH. Within 3 h, the inner medullary sorbitol content increased from 50 to 100 mmol/kg protein, while urinary osmolarity (but not necessarily inner medullary osmolarity) increased from 113 mosmol/l to 943 mosmol/l [22]. The rate of sorbitol synthesis found in the present study of 78 mmol/kg protein · h is sufficient to achieve this increase even if the rate of synthesis might be lower under in vivo conditions.

In summary, the mechanism described herein is fully compatible with the view that one of the early events in adaptation of collecting duct cells to changes in interstitial (and urinary) osmolarity is a rapid change – within minutes – in sorbitol permeability of the plasma membrane. Thus, a fast release of this organic osmolyte from the cells is possible. Recovery of the intracellular sorbitol content is a slower process governed by the rate of synthesis of the compound. Under optimal metabolic conditions the sorbitol released from the cells during transition from the antidiuretic to the diuretic state can be replaced within 1 h. Intracellular sorbitol concentration could be adjusted, however, much faster, if, in addition, transient shrinkage of the cells occurred.

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