Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 6002–6006, August 1989
Physiological Sciences

Osmoregulatory changes in *myo*-inositol transport by renal cells

(kidney medulla/cell culture/high NaCl/osmolytes/MDCK cells)

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Communicated by Robert W. Berliner, May 1, 1989

ABSTRACT **Renal medullary cells contain high concentrations of *myo*-inositol, sorbitol, betaine, and glycerophosphocholine, whose levels vary with urinary osmolality. Accumulation of these "compatible" organic osmolytes is believed to help the cells osmoregulate in response to the high extracellular osmolality that occurs as part of the urinary concentrating mechanism. MDCK cells (a line from dog kidney) were previously shown to accumulate *myo*-inositol in response to increased medium osmolality. We demonstrate here that this accumulation requires the presence of *myo*-inositol in the medium, implying that the *myo*-inositol is not synthesized by the cells but rather is transported into them from the extracellular solution. The MDCK cells contain sodium-dependent *myo*-inositol transporters. Relative to isotonic controls, sodium-dependent *myo*-inositol uptake is higher in cells exposed to increased osmolality either acutely (1–7 days) or chronically (>1 year). Transport is further enhanced when the cells are cultured in *myo*-inositol-free medium. The transport has both high- and low-affinity components. The observed changes in transport involve changes in maximal velocity of the high-affinity component but not in its K_m. We conclude that renal cells can osmoregulate by changing the number (or, less likely, the transport turnover rate) of functioning sodium-dependent *myo*-inositol transporters.**

It is generally accepted that cell membranes are not able to sustain sizable osmotic differences and thus that the osmolarity of the cytoplasm is close to that of the extracellular solution. Consequently, when the extracellular salt concentration rises, cells shrink due to osmotic water loss. Many osmotically shrunken cells regulate back toward their normal volume by taking up sodium and potassium salts; however, this is only a temporary response. Comparative studies of a wide variety of organisms chronically subjected to high-salt environments indicate that it is typically the intracellular concentrations of organic rather than inorganic solutes which are increased to balance increased extracellular osmolarity (1). It is particularly interesting that the concentrations of only certain types of solutes are elevated. These are osmotically active, compatible organic solutes, or "organic osmolytes."

The organic osmolytes fall mainly into three groups: polyols (such as sorbitol, *myo*-inositol, and sucrose), methylamines (such as glycerophosphocholine and betaine), and amino acids (such as glycine and proline). Yancey *et al.* (1) have reviewed the theoretical basis for understanding why cells use these particular organic compounds to adjust their intracellular osmolarity. The distinction is between perturbing and nonperturbing solutes. Whereas high concentrations of perturbing solutes (including NaCl, KCl, and urea) inhibit cellular enzymes and affect nucleotides and nonenzyme proteins, high concentrations of nonperturbing solutes do not.

Cells in the renal inner medulla contain much higher levels of osmolytes (predominantly sorbitol, *myo*-inositol, betaine, and glycerophosphocholine) than normally present in other mammalian tissues, including the renal cortex (2). A major role of these osmolytes is presumably to balance the osmotic pressure of extracellular NaCl, which is normally high in the renal inner medulla and varies with urine osmolarity as part of the urinary concentrating mechanism.

The high levels of sorbitol observed in the renal medulla are accompanied by correspondingly high levels of the enzyme aldose reductase, which catalyzes the synthesis of sorbitol from glucose (2, 3). A line of renal medullary cells (GRB-PAP1) in which aldose reductase activity is induced by extracellular hyperosmolarity has been identified (4). Thus in these cells, and presumably also in the inner medulla, the increased levels of intracellular sorbitol which balance the osmotic pressure of elevated extracellular salt arise from intracellular synthesis. Little is known, however, about the source or regulation of the high levels of the other medullary organic osmolytes.

We have recently reported that the renal epithelial cell line MDCK (Madin–Darby canine kidney) accumulates high concentrations of intracellular *myo*-inositol when it is grown in hypertonic medium, consistent with a role of *myo*-inositol in osmoregulation (5). We demonstrate here that this accumulation is the result of increased sodium-dependent *myo*-inositol transport. Our data are consistent with synthesis or induction of sodium-dependent *myo*-inositol transporters by these cells in response to increased extracellular osmolarity.

METHODS AND MATERIALS

Cell Culture. MDCK cells were purchased from the American Type Culture Collection and grown in either serum-supplemented [315 milliosmoles (mosmol)/kg] or defined medium (320 mosmol/kg). The serum-supplemented medium (DMEM/S) was Dulbecco's modified Eagle's medium with 5 mM D-glucose plus 10% fetal bovine serum (GIBCO), 4 mM L-glutamine, penicillin at 100 international units (IU)/ml, and streptomycin at 100 μg/ml. The defined medium was a 50:50 mixture of Dulbecco's modified Eagle's medium (without D-glucose) and Coon's modified Ham's F-12 medium (6) containing 10 mM Hepes (GIBCO), transferrin at 5 μg/ml, insulin at 5 μg/ml, 5 pM triiodothyronine, 50 nM hydrocortisone, 10 nM $Na_2SeO_3 \cdot 5H_2O$, prostaglandin E_1 at 25 ng/ml, 2 mM L-glutamine, penicillin at 100 IU/ml, and streptomycin at 100 μg/ml (7). The defined medium was employed with or without 120 μM *myo*-inositol, as indicated in the table and figure legends. MDCK cells adapted to a hypertonic medium (5) and grown continuously in this medium for more than 1 year (>20 passages) were also used in some experiments. These cells were grown in DMEM/S made hypertonic (915

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Abbreviation: osmol, osmole.

mosmol/kg) by the addition of equal osmolal amounts of NaCl and urea. All cells were maintained at 37°C in a 5% CO_2 atmosphere.

Cells were grown for at least 10 days after splitting before use in transport or other experiments. In experiments where the effects of increased extracellular osmolarity were investigated, cells were switched in a single step from isotonic medium (320 mosmol/kg) to hypertonic medium (500 mosmol/kg, by addition of the solutes indicated, or 700 mosmol/kg, by addition of equal osmolal amounts of NaCl and urea).

Measurement of Intracellular *myo*-Inositol. Intracellular *myo*-inositol was measured in trichloroacetic acid extracts of MDCK monolayers growing in Cluster 6 dishes (Costar). The cells were rinsed with isosmotic ice-cold calcium- and magnesium-free phosphate-buffered saline and drained. Then 1 ml of 7% trichloroacetic acid was added and the cells were scraped off the dish and centrifuged at 2400 × *g* for 10 min at 5°C. Trichloroacetic acid was extracted from the supernatant by mixing with 2.5 ml of a solution of 26% tri(*n*-octyl)amine and Freon-113 (Fisher) and recentrifugation (8). *myo*-Inositol was measured in the upper phase by the method of MacGregor and Matschinsky (9).

Measurement of *myo*-Inositol Transport. *myo*-Inositol uptake into intact MDCK monolayers growing in Cluster 12 dishes (Costar) was measured as follows. The cells were rinsed twice with Dulbecco's phosphate-buffered saline (with the same osmolality as the growth medium), then incubated for 15 min at 37°C in transport medium (10 mM Tris/Hepes‡ containing 150 mM NaCl or 150 mM LiCl, 5 mM KCl, 2 mM $CaCl_2$, 1.2 mM $MgCl_2$, and other additions as noted). This medium was then removed and replaced by the same solution containing appropriate concentrations of *myo*-[^{3}H]inositol (0.25–2.0 μCi/ml; 1 Ci = 37 GBq). Fifteen minutes later the cells were rinsed four times with 2 ml of ice-cold stop solution (10 mM Tris/Hepes containing 0 or 300 mM mannitol, 300 mM NaCl, and 1 mM phlorizin) and dried in air at room temperature. The cells were then solubilized in 1 ml 0.25 M NaOH and aliquots were taken for liquid scintillation counting and protein determination (Bio-Rad protein assay kit). In control experiments where the time the cells were left in the stop solution was prolonged, we have established that there is no significant loss of intracellular *myo*-[^{3}H]inositol during the stopping and washing procedure.

myo-Inositol uptake by MDCK monolayers was found to be linear with time for at least 20 min under the experimental conditions assayed here (data not shown). Accordingly, uptake measured after 15 min of incubation was taken as a measure of initial uptake rate. Uptake of 10 μM *myo*-inositol in the presence of 150 mM extracellular sodium was typically an order of magnitude or more greater than that measured with lithium replacing sodium (see below). Uptake measured in the presence of lithium was linear with *myo*-inositol concentrations up to 500 μM (not shown) and was subtracted from uptake measured in the presence of sodium to give the sodium-dependent component of transport.

Calculations and Data Presentation. The results shown are means ± SEM for three or more experiments carried out under the same conditions. Nonlinear least-squares analysis of the data was carried out by using the program NONLIN (Systat, Evanston, IL). Student's *t* test was used in the statistical evaluation of data and *P* values less than 0.05 were taken to indicate statistically significant differences.

Materials. *myo*-[^{3}H]Inositol was obtained from New England Nuclear. Phlorizin and unlabeled *myo*-inositol were from Sigma.

‡Hepes (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) at 10 mM titrated to pH 7.4 with Tris.

RESULTS

As already mentioned, it has been demonstrated that the MDCK cells accumulate *myo*-inositol in response to chronic increased extracellular osmolarity. Since both intracellular *myo*-inositol synthesis from glucose by inositol synthase (10) and active *myo*-inositol accumulation by transport from the extracellular medium (11–17) have been documented in a number of cell types, we first investigated which (if either) of these two mechanisms was involved in the response of the MDCK cells to hypertonicity. Fig. 1 illustrates the time course of *myo*-inositol accumulation in MDCK cells switched from isotonic (320 mosmol/kg) to hypertonic (700 mosmol/kg) medium. Since *myo*-inositol is present in the tissue culture medium which we use routinely and is normally present in serum (typical concentrations are 25–100 μM) used to supplement media, defined medium with and without *myo*-inositol was employed in these experiments to control for the effects of its presence. Cells were initially grown in isotonic medium containing *myo*-inositol. Relative to control cells left in the original isotonic medium, cells switched to hypertonic medium still containing *myo*-inositol showed a dramatic increase in myo-inositol content over the first 2–7 days in culture. In contrast, cells switched to hypertonic *myo*-inositol-free medium showed decreased *myo*-inositol content with time. This experiment demonstrates that *myo*-inositol accumulation by MDCK cells in response to hypertonicity is dependent on the presence of extracellular *myo*-inositol and thus strongly implies that this accumulation is the result of *myo*-inositol transport rather than intracellular synthesis.

Preliminary experiments indicated that *myo*-inositol uptake by MDCK monolayers was sodium dependent and inhibited by phlorizin with half-maximal inhibition at approximately 100 μM (data not shown). The sodium dependence of *myo*-inositol transport raises the possibility that the increased *myo*-inositol accumulation observed with hypertonicity in Fig. 1 can be explained simply as the result of increased transport due to an increased sodium chemical gradient. However, a continuously more favorable sodium chemical gradient cannot account for the sustained increase in *myo*-inositol in the cells during chronically elevated osmolarity because we previously demonstrated that their intracellular sodium concentration is approximately twice that of isotonic cells, matching the increase in extracellular sodium (5). Further, the additional experiments shown in Table 1 also

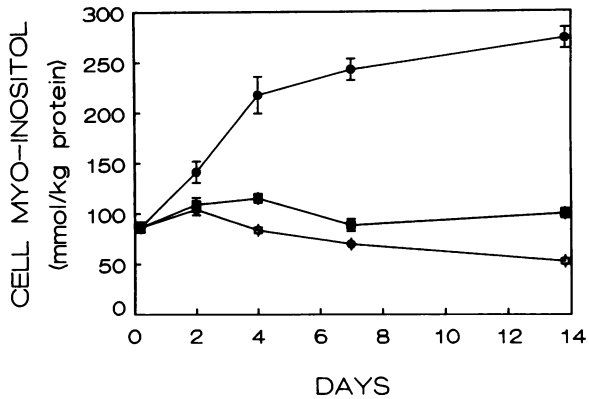

FIG. 1. Intracellular *myo*-inositol content after switching MDCK cells to hypertonic medium. On day 0 cells growing in defined medium containing 120 μM *myo*-inositol (320 mosmol/kg) were switched to the same medium made hypertonic (700 mosmol/kg) by the addition of equal osmolal amounts of NaCl and urea, with (●) or without (○) 120 μM *myo*-inositol. Control cells (■) were maintained in isotonic defined medium with *myo*-inositol. All cells were fed on days 0, 2, 4, 7, 9, and 11. Error bars indicate SEM.

Table 1. Intracellular *myo*-inositol content of MDCK cells 4 days after switching to various hypertonic media

Solute added	Medium osmolality, mosmol/kg	*myo*-Inositol content, mmol/kg of protein
None (control)	320	93.6 ± 3.5
NaCl plus urea	700	284.6 ± 19.0
Urea	500	123.4 ± 8.9
NaCl	500	319.8 ± 17.5
Raffinose	500	295.7 ± 2.7
Sorbitol	500	270.9 ± 2.5
Dextrose	500	286.7 ± 10.7

MDCK cells growing in defined medium containing 120 μM *myo*-inositol were switched to media made hypertonic by the addition of the solutes indicated. Control cells were maintained in isotonic medium. Four days later intracellular *myo*-inositol content was assayed. Results are presented as mean ± SEM. All test values are significantly greater than control ($P < 0.02$; $n = 3$ for all points).

argue against this. Here we demonstrate that increasing the extracellular osmolality by the addition of raffinose, sorbitol, or dextrose is just as effective in inducing *myo*-inositol accumulation as the addition of NaCl plus urea (as in Fig. 1) or NaCl alone. Table 1 also shows that increasing the osmolality of the culture medium by the addition of urea alone has only a small effect on myo-*inositol* accumulation.

The kinetics of sodium-dependent *myo*-inositol uptake into MDCK monolayers growing in DMEM/S is illustrated in Fig. 2. Uptakes from control (isotonic) cells, measured in the presence of 150 mM NaCl, and from cells adapted to hypertonic medium (see *Methods and Materials*), measured in the presence of both 150 mM and 300 mM NaCl, are shown. The data are presented in Eadie–Hofstee plots. The plots are clearly curvilinear, indicating the existence of at least two sodium-dependent *myo*-inositol transport systems. Attempts to fit these data to a theoretical equation representing two saturable sites, each obeying Michaelis–Menten kinetics, were unsuccessful owing to the inability of the nonlinear least-squares regression procedure to converge on reliable estimates for the kinetic parameters of the low-affinity site. Accordingly, the data were fit to the equation

$$\text{Uptake} = \frac{V_{max}\cdot[myo]}{(K_m + [myo])} + k\cdot[myo],$$

in which [*myo*] represents *myo*-inositol molar concentration and k is a rate constant. That is, the high-affinity site was fitted to the Michaelis–Menten equation while the low-affinity site was approximated by a diffusion-like term. Note that this is only a mathematical convenience, since the low-affinity site is a component of sodium-dependent *myo*-inositol transport and therefore unlikely to function simply by diffusion. The nonlinear least-squares fits to the uptake data measured at 150 mM NaCl (solid lines; see Fig. 2 legend) confirm the impression given by visual inspection of Fig. 2, namely, that the magnitude of both components of sodium-dependent *myo*-inositol uptake is increased in the hypertonic cells without a significant change in the K_m of the high-affinity site.

The effect of increased extracellular sodium concentration (300 mM during the measurement of transport) on the kinetics of *myo*-inositol transport in the hypertonic cells is to increase the V_{max} of the high-affinity transport site without affecting its K_m; no significant increase in transport via the low-affinity site due to increased sodium concentration is detectable under these experimental conditions (cf. least-squares fits in legend to Fig. 2).

Given the increase in V_{max}, it is tempting to ascribe the increased sodium-dependent *myo*-inositol transport exhibited by hypertonic MDCK cells in Fig. 2 to an increase in

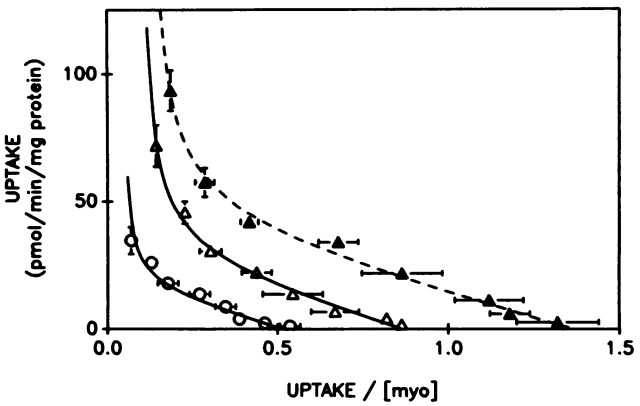

FIG. 2. Kinetics of sodium-dependent *myo*-inositol uptake by MDCK cells grown in isotonic (○) DMEM/S or in hypertonic (△, ▲) DMEM/S. *myo*-Inositol uptake was measured over the concentration range 2–500 μM. The transport medium for the hypertonic cells was supplemented with 300 mM mannitol plus 300 mM urea (▲) or 150 mM NaCl plus 300 mM urea (△). Nonlinear least-squares fits of the data to the equation given in the text yield $K_m = 38.9 \pm 12.8$ μM, $V_{max} = 18.3 \pm 5.8$ pmol/min per mg of protein, and $k = 0.042 \pm 0.021$ pmol/min per mg per μM (○); $K_m = 39.5 \pm 4.7$ μM, $V_{max} = 30.7 \pm 2.9$ pmol/min per mg, and $k = 0.088 \pm 0.008$ pmol/min per mg per μM (▲); and $K_m = 35.9 \pm 3.4$ μM, $V_{max} = 45.3 \pm 3.7$ pmol/min per mg, and $k = 0.101 \pm 0.11$ pmol/min per mg per μM (△). The lines drawn through the data points were calculated from these least-squares fits.

myo-inositol transporter number or capacity induced by hyperosmolarity. However, it is also necessary to exclude the possibility that increased transport is due to increased trans-stimulation of unidirectional *myo*-[^{3}H]inositol uptake by unlabeled intracellular *myo*-inositol, since the intracellular *myo*-inositol concentration of the hypertonic cells is 6 times that of isotonic controls (5). This possibility was explored by studying the effects of hypertonicity on *myo*-inositol transport by cells switched to *myo*-inositol-free media. This prevents the increase in intracellular *myo*-inositol which would otherwise occur (cf. Fig. 1). Fig. 3 shows the results of a series of experiments in which sodium-dependent *myo*-inositol uptake was measured at various times after cells growing in defined medium containing *myo*-inositol were switched to hypertonic or isotonic media without *myo*-inositol. Results have been normalized to the uptake observed in unswitched controls. Cells switched to isotonic medium without *myo*-inositol showed somewhat increased uptake relative to controls, possibly due to induction of transport resulting from *myo*-inositol removal. Cells switched to hypertonic medium without *myo*-inositol, on the other hand, showed a much more dramatic increase in uptake, reaching levels approximately 4 times those of controls over the 7 days of the experiment. This increase in sodium-dependent *myo*-inositol uptake by hypertonic cells could not possibly be due to trans-stimulation by increased intracellular *myo*-inositol because these cells actually show decreased *myo*-inositol content relative to controls (Fig. 1). Thus, these data provide strong evidence for a direct effect of hypertonicity on the capacity of MDCK cells to transport *myo*-inositol.

The increase in *myo*-inositol transport capacity induced by switching MDCK cells to hypertonic medium is explored in more detail in Fig. 4. Here the kinetics of sodium-dependent *myo*-inositol uptake into cells growing for 7 days in isotonic and hypertonic *myo*-inositol-free medium are compared. In contrast to chronically adapted hypertonic cells, which show increased *myo*-inositol uptake by both the high- and low-affinity sites (Fig. 2), both visual inspection and nonlinear least-squares analysis of Fig. 4 (see figure legend) indicate that only transport via the high-affinity site is significantly

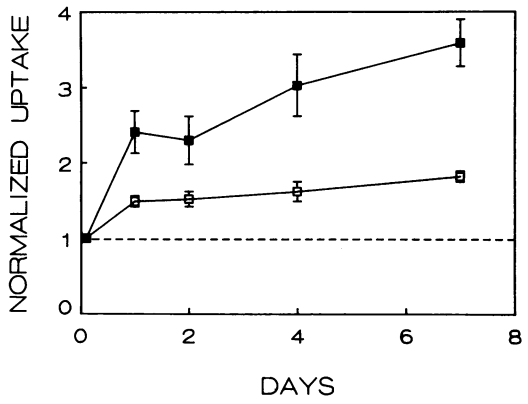

FIG. 3. Time dependence of sodium-dependent *myo*-inositol (10 μM) uptake into MDCK cells switched into isotonic and hypertonic *myo*-inositol-free media. On day 0 MDCK cells growing in defined medium containing 120 μM *myo*-inositol were switched into defined medium without *myo*-inositol (□) or defined medium made hypertonic (700 mosmol/kg) by the addition of equal osmolal amounts of NaCl and urea without *myo*-inositol (■). Uptake has been normalized to that observed in cells maintained in defined medium containing *myo*-inositol. The transport medium for the hypertonic cells contained an additional 200 mM mannitol plus 200 mM urea to balance intracellular osmolarity.

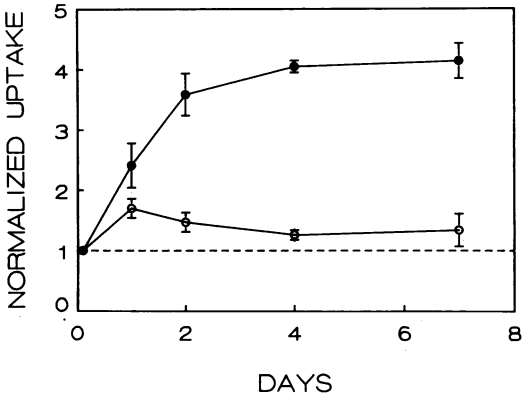

FIG. 5. Time dependence of sodium-dependent *myo*-inositol (10 μM) uptake into MDCK cells switched into hypertonic media containing *myo*-inositol. On day 0 MDCK cells growing in defined medium containing 120 μM *myo*-inositol were switched into the same medium made hypertonic by the addition of equal molal amounts of NaCl and urea (700 mosmol/kg, ○) or by the addition of raffinose (500 mosmol/kg, ●). Uptake has been normalized to that observed in cells maintained in isotonic medium. The transport medium for the hypertonic cells contained an additional 200 mM mannitol (●) or 200 mM mannitol plus 200 mM urea (○) to balance intracellular osmolarity.

increased after 7 days in hypertonic medium. Additional experiments are required to establish the significance of this apparent difference in the rate at which these two sodium-dependent *myo*-inositol transport sites are affected by hypertonicity.

Fig. 5 shows the effects of switching cells to hypertonic conditions when *myo*-inositol is not removed from the culture medium. In these experiments extracellular osmolality was increased either with NaCl plus urea or with raffinose. When osmolality was increased with NaCl plus urea, sodium-dependent *myo*-inositol uptake increased after 1 day then decreased to levels close to those observed in unswitched controls by 4 days. In contrast, cells switched to hypertonic medium containing raffinose showed a monotonic increase in sodium-dependent *myo*-inositol uptake over the period of the experiment. In spite of these differences in uptake, however, cells grown in the two media have almost identical intracellular *myo*-inositol contents (Table 1). This result suggests that the cells are regulating their *myo*-inositol transport capacities in such a way that the amount of intracellular *myo*-inositol they accumulate depends on the magnitude but not the origin of the extracellular osmotic perturbation. Thus, cells grown in hypertonic NaCl plus urea may require fewer *myo*-inositol transporters to produce a given intracellular accumulation, since these cells may have a larger extracellular-to-intracellular sodium gradient.

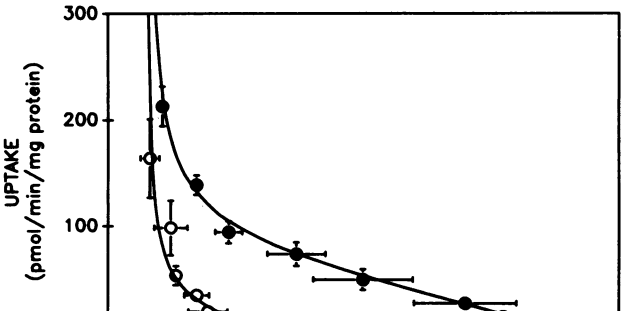

FIG. 4. Kinetics of sodium-dependent *myo*-inositol uptake by MDCK cells in *myo*-inositol-free isotonic or hypertonic serum-free medium. MDCK cells growing in defined medium containing 120 μM *myo*-inositol were switched into isotonic defined medium without *myo*-inositol (○) or defined medium made hypertonic (700 mosmol/kg) by the addition of equal osmolal amounts of NaCl and urea without *myo*-inositol (●). Seven days later *myo*-inositol uptake was measured over the concentration range 2–500 μM. The transport medium for the hypertonic cells contained an additional 200 mM mannitol plus 200 mM urea to balance intracellular osmolarity. Nonlinear least-squares fits of the data to the equation given in the text yielded $K_m = 31.5 \pm 10.9$ μM, $V_{max} = 31.0 \pm 10.0$ pmol/min per mg of protein, and $k = 0.285 \pm 0.049$ pmol/min per mg per μM (○); and $K_m = 27.9 \pm 2.1$ μM, $V_{max} = 95.4 \pm 6.2$ pmol/min per mg, and $k = 0.254 \pm 0.020$ pmol/min per mg per μM (●). The lines drawn through the data points were calculated from these least-squares fits.

DISCUSSION

High levels of *myo*-inositol have been reported in the renal medullas of a variety of species (2, 18, 19). Renal medullary *myo*-inositol content has been observed to vary significantly with hydration state in the dog and rat (18, 20) and rabbit (21), consistent with a role in osmoregulation [however, the decreases with diuresis were not statistically significant in wild rodents (19)]. Also, Lohr *et al.* (22) observed increased kidney *myo*-inositol in chronically hypernatremic rats. In all species studied to date, levels of *myo*-inositol were higher in the outer medulla than in the inner medulla (18, 19, 21), leading Yancey (19) to suggest that this compound may serve functions in addition to osmoregulation.

Little is known about the source or regulation of renal *myo*-inositol levels. We previously demonstrated that the MDCK cells (a line from dog kidney) accumulate high levels of *myo*-inositol in response to increased medium osmolarity (5). The purpose of the present studies was to determine the mechanism of this response. The results presented here provide good evidence that the increased levels of intracellular *myo*-inositol observed in MDCK cells growing in hypertonic media are due to increased sodium-dependent *myo*-inositol transport from the extracellular solution. We find two components of sodium-dependent *myo*-inositol transport in the MDCK cells, a high-affinity site with $K_m \approx 35$ μM and a lower-affinity site whose K_m we were unable to estimate. Similar sodium-dependent *myo*-inositol transport systems have been observed in a variety of tissues (11–17); however,

the reason for the existence of these concentrative mechanisms for *myo*-inositol and the elevated intracellular *myo*-inositol levels seen in many nonrenal tissues is still not clear. Both acute (7 days) and chronic (>1 year) exposure to increased osmolarity resulted in an increase in the maximal velocity (V_{max}) of the high-affinity sodium-dependent *myo*-inositol transport site without significantly changing its K_m. Increased sodium-dependent transport via the low-affinity site was associated with chronic but not acute exposure to hypertonic medium, possibly due to different rates of induction of these two transporters in response to hypertonicity.

As discussed earlier, the increased uptake of *myo*-inositol by MDCK cells in hypertonic medium cannot be accounted for by an increased chemical gradient for sodium. From the present studies we are unable to exclude the possibility that this effect is due to an increased electrical driving force (membrane potential) for sodium-dependent *myo*-inositol uptake; however, Roy and Sauve (23) have recently demonstrated that acute changes in osmolality have no sustained effect on the intracellular potential of these cells. An increase in the sodium/*myo*-inositol coupling stoichiometry of the sodium-dependent transporter could also lead to increased uptake and increased concentrating capacity for *myo*-inositol. But one would expect such a dramatic effect on the functional properties of the transporter to result in a change in its K_m as well. Thus, our observation of an increased V_{max} of sodium-dependent *myo*-inositol transport, with no change in K_m, is most easily explained by an increase in the number (or, less likely, the turnover rate) of high-affinity sodium-dependent *myo*-inositol transporters expressed by MDCK cells in hypertonic medium.

Although it seems reasonable to conclude that the increased sodium-dependent uptake of *myo*-inositol we observe in MDCK cells adapted to hypertonic medium is sufficient to account for their increased concentrating capacity for this osmolyte, it is difficult for us to make any quantitative evaluation of this hypothesis. This concentrating capacity will depend on the number and coupling stoichiometry of the sodium-dependent transporters as well as on the sodium electrochemical gradient and magnitude of the *myo*-inositol leak pathways. We can, however, confirm that this increased concentrating capacity is not due to a reduced *myo*-inositol leak pathway in the hypertonic cells. A comparison of the sodium-independent component of *myo*-inositol uptake by MDCK cells grown in isotonic medium with that found in cells grown and assayed in high-salt medium (Fig. 2) indicates that this component of uptake is approximately 40% larger in the hypertonically adapted cells (data not shown).

The results shown in Table 1 may offer a clue to the cellular signal that mediates between changes in extracellular osmolality and changes in cellular inositol transport and accumulation. Note that addition of NaCl (with or without urea), raffinose, sorbitol, or dextrose caused a large increase in *myo*-inositol accumulation, whereas urea alone had hardly any effect. This pattern is the same as that observed for induction of aldose reductase activity, which is responsible for osmoregulatory accumulation of sorbitol by renal medullary cells (24). In the case of aldose reductase the intracellular signal apparently is ionic strength, based on a very close correlation between aldose reductase activity and the sum of intracellular sodium plus potassium concentration. The same conclusion was previously reached with regards to bacteria (25). When the osmolarity of the medium surrounding *Escherichia coli* cells is elevated, their intracellular ionic strength rises, which in turn causes increased transcription of the gene coding a high-affinity betaine transporter. It will be of interest to see if intracellular ionic strength also mediates increased *myo*-inositol transport in MDCK cells.

It also remains to be established whether *myo*-inositol transport in renal medullas *in vivo* is similar to that in MDCK cells in tissue culture and whether transport *in vivo* changes in response altered osmolarity. Nevertheless, extrapolation of the present findings to the intact kidney would imply that transport may account for the osmoregulatory accumulation of *myo*-inositol by renal medullary cells in intact kidneys.

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