Na+/myo-inositol transport is regulated by basolateral tonicity in Madin-Darby canine kidney cells.

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Research Article

We investigated the effects of change in basolateral osmolality on Na(+)-dependent myo-inositol uptake in Madin-Darby canine kidney cells to test our hypothesis that the Na+/myo-inositol transporter (SMIT), an osmolyte transporter, is mainly regulated by osmolality on the basolateral surface. A significant osmotic gradient between both sides of the epithelium persisted at least 10 h after basolateral osmolality was increased. [3H]myo-inositol uptake increased in a basolateral osmolality-dependent manner. The magnitude of the increase is comparable to that for making both sides hypertonic. Apical hypertonicity also increased the uptake on the basal side, but the magnitude of the increase was significantly smaller than the basolateral or both sides hypertonicity. Betaine-gamma-amino-n-butyric acid transporter activity, measured by [3H]gamma-amino-n-butyric uptake, showed a pattern similar to SMIT activity in response to basolateral hypertonicity. The most plausible explanation for the polarized effect of hypertonicity is that the basal membrane is much more water permeable than the apical membrane. These results seem to be consistent with the localization and regulation of the SMIT in vivo.

Na^+/*Myo*-inositol Transport Is Regulated by Basolateral Tonicity in Madin-Darby Canine Kidney Cells

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Abstract

We investigated the effects of change in basolateral osmolality on Na^+-dependent *myo*-inositol uptake in Madin-Darby canine kidney cells to test our hypothesis that the Na^+/*myo*-inositol transporter (SMIT), an osmolyte transporter, is mainly regulated by osmolality on the basolateral surface. A significant osmotic gradient between both sides of the epithelium persisted at least 10 h after basolateral osmolality was increased. [^{3}H]*myo*-inositol uptake increased in a basolateral osmolality–dependent manner. The magnitude of the increase is comparable to that for making both sides hypertonic. Apical hypertonicity also increased the uptake on the basal side, but the magnitude of the increase was significantly smaller than the basolateral or both sides hypertonicity. Betaine-γ-amino-*n*-butyric acid transporter activity, measured by [^{3}H]γ-amino-*n*-butyric uptake, showed a pattern similar to SMIT activity in response to basolateral hypertonicity. The most plausible explanation for the polarized effect of hypertonicity is that the basal membrane is much more water permeable than the apical membrane. These results seem to be consistent with the localization and regulation of the SMIT in vivo. (*J. Clin. Invest.* 1996. 97: 263–267.) Key words: osmolytes • betaine • γ-amino-*n*-butyric acid • polarity • water permeability

Introduction

Many kinds of cells respond to extracellular hypertonicity by the accumulation of high concentrations of small organic solutes, which are referred to as "compatible osmolytes," that protect cells from the perturbing effects of high intracellular concentrations of electrolytes (1, 2). It has been shown that *myo*-inositol and betaine function as organic osmolytes in various tissues and types of cells (3–5). Madin-Darby canine kidney (MDCK)[1] cells accumulate these osmolytes through specific Na^+-coupled transporters when cultured in a hypertonic medium (6, 7). Cloning of the cDNAs encoding osmolyte transporters such as Na^+/*myo*-inositol transporter (SMIT) (8) and Na^+/Cl^-/betaine-γ-amino-*n*-butyric acid (GABA) transporter (BGT-1) (9) offers insight into understanding of molecular mechanism for accumulation of these osmolytes. The abundance of the mRNAs for these transporters and the transcription rate of the genes increased when the cells were cultured in a hypertonic medium (10, 11), suggesting that transcription is the primary step in regulation of these transporters by hypertonicity. The canine BGT-1 gene has been recently cloned (12), and a hypertonic stress–responsive element has been identified from the 5′-flanking region of the BGT-1 gene (13).

Myo-inositol is the only osmolyte found in substantial amounts in the cortex and outer medulla in addition to the inner medulla (14). Our previous report (15) showed that SMIT mRNA abundance was highest in the outer medulla rather than in the inner medulla, and a small but significant amount of SMIT mRNA was present in the cortex. SMIT mRNA in the cortex as well as that in the medulla was significantly induced by dehydration. We have recently shown using in situ hybridization (16) that SMIT mRNA is predominantly present in the thick ascending limb of Henle's loop (TALH) and macula densa cells, and that NaCl loading rapidly increases the signals. Basal expression and magnitude of the induction by NaCl administration seemed to be uniform throughout the TALH as well as macula densa cells. The fact that furosemide markedly reduced the signals indicates that SMIT is not regulated by luminal osmolality. Our question is what is the signal for the expression of SMIT.

Although we cannot deny the idea that SMIT is regulated by unknown factor(s) other than osmolality, there has been no report regarding such a factor in spite of extensive studies of this transporter. We speculate that SMIT expression may change with osmolality on the basolateral surface, which is supposed to be dependent on NaCl reabsorption of the tubular cells. To investigate this issue, we examined the effects of the change in basolateral osmolality on Na^+-dependent *myo*-inositol uptake in MDCK cells cultured on a porous support. We used MDCK cells because a large amount of data regarding osmolyte transporters and their regulations has been accumulated and established for MDCK cells. There are no other cell

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1. *Abbreviations used in this paper:* ADH, antidiuretic hormone; BGT-1, betaine GABA transporter; GABA, γ-amino-*n*-butyric acid; MDCK, Madin-Darby canine kidney; SMIT, Na^+/*myo*-inositol transporter; TALH, thick ascending limb of Henle's loop.

lines available which clearly express SMIT and BGT-1. The results obtained demonstrate that SMIT and BGT-1 activities are predominantly regulated by basolateral osmolality in MDCK cells. This seems to be consistent with the localization and regulation of SMIT in vivo.

Methods

Cell culture. MDCK cells were a generous gift from the Japanese Cancer Research Resources Bank (National Institute of Health, Tokyo, Japan) and grown in DME supplemented with 10% FCS, 50 U/ml of penicillin, and 50 μg/ml of streptomycin equilibrated with 5% CO_2–95% air at 37°C. Cells were carried on tissue culture plastic dishes and seeded at confluent density on tissue culture–treated polycarbonate filters (Transwell™, Costar Corp., Cambridge, MA). Each 5-cm^2 Transwell™ filter (Nuclepore™, 3-μm pore) cup was placed in a 35-mm well of a Cluster Six tissue culture dish (Costar Corp.). 7 d after seeding, some were switched to the medium made hypertonic (350–450 mosmol/kg) by addition of raffinose on either side of the epithelium. Hypotonic medium was made by simple dilution of growth medium with water. Medium osmolality was measured with a freezing point osmometer (OM-6010; Kyoto-Daiichi-Kagaku, Kyoto, Japan). Others were maintained in isotonic (300 mosmol/kg) medium. *Myo*-inositol uptake was determined 6 h after basolateral osmolality increased. All experiments were performed using 25–30 passages of MDCK cells.

MDCK cells are tolerant of acute increase in osmolality up to 500 mosmol/kg when osmolalities of both sides are the same. In case of basolateral hypertonicity, however, the cells sometimes began to detach within 6 h over 500 mosmol/kg, so we used media up to 450 mosmol/kg. There were no significant differences in protein content between isotonic and hypertonic cells for at least 6 h after the switch.

Measurement of SMIT and BGT-1 activities. Because *myo*-inositol is taken up mostly on the basal side of MDCK cells (17), we measured basolateral uptake of *myo*-inositol in MDCK cells on a porous support. Na^+-dependent *myo*-inositol uptake was measured essentially as described previously with slight modifications (17). To measure uptake into MDCK cells grown on filters, the cells were rinsed twice on both surfaces with PBS and then incubated for 30 min at 37°C in preincubation solution (150 mM NaCl or 150 mM LiCl, 5 mM KCl, 2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM Hepes/Tris, pH 7.4). This solution was replaced by the same solution containing 10 μM *myo*-inositol (uptake medium). [^{3}H]*myo*-inositol (20 Ci/mmol; New England Nuclear, Boston, MA) was added (0.5 μCi/ml) to the solution on the basal side of the filter. To end the uptake period, the filter on its support was dipped into three beakers containing 100 ml ice-cold stop solution (PBS + 1 mM phlorizin) to stop the uptake and remove extracellular tracer. The filter with its attached epithelium was then cut out of the filter support with a surgical blade and put into a scintillation vial where the cells were solubilized overnight in 500 μl of 1 N NaOH. Then 500 μl of 1 N HCl was added to the solution. Duplicate aliquots of 25 μl were used for protein determination (Protein Assay kit; Bio-Rad Laboratories, Richmond, CA) using BSA as a standard. 10 ml of ACSII (Amersham Corp., Arlington Heights, IL) was added to the remainder of the sample for liquid scintillation counting. The uptake by MDCK cells was found to be linear with time for at least 60 min. Accordingly, uptake measured after 30 min of incubation was taken as the initial uptake rate.

We also examined the effects of basolateral hypertonicity on BGT-1 activity. BGT-1 activity was determined by measuring the initial rate of [^{3}H]GABA uptake into MDCK cells with the same procedures as *myo*-inositol uptake. The uptake buffer contained 10 μM of GABA and 0.5 μCi/ml of [^{3}H]GABA (40 Ci/mmol, New England Nuclear).

In every experiment, just before the last uptake period ended, aliquots were taken from the solution on each side of the filter to check for transepithelial leakage. Transepithelial leakage or transport of radiolabeled *myo*-inositol or GABA was negligible; tracer added to one surface was scarcely (< 0.1%) detected in the solution on the other surface of the epithelium, indicating that there was little transepithelial transport or leakage of *myo*-inositol or GABA. In experiments in which the cells were in hypertonic medium, PBS, preincubation medium, uptake medium, and stop solution were made hypertonic by adding mannitol. To study transport in the absence of sodium, lithium was used to replace all sodium.

Data analysis. The results shown are means±SD. When no error bar is shown in a figure, the SD is smaller than the symbol. The data were statistically analyzed by the one-way ANOVA. Statistical significance was set at $P < 0.05$.

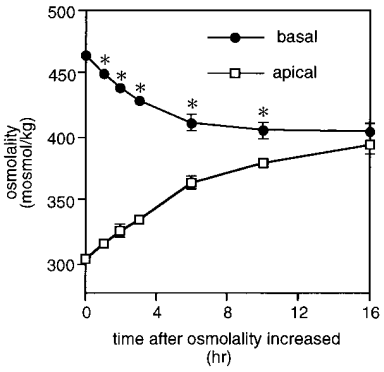

Figure 1. Time course of change in osmolality after basolateral medium osmolality increased to 450 mosmol/kg. On time 0, confluent cells cultured in isotonic medium (300 mosmol/kg) were switched to the same medium made hypertonic (450 mosmol/kg) by addition of raffinose on basal side. Medium osmolality of the sample from each side of the epithelium was measured successively. Results are means±SD of three independent experiments. *$P < 0.01$ vs apical medium.

Results

Previous reports have shown that monolayers of MDCK cells grown on a permeable support transport water at a low rate (18, 19). To examine how long a significant osmotic gradient between both sides of the epithelium persists, medium osmolality of the samples from each side of the epithelium was measured after basolateral osmolality was increased (Fig. 1). Osmolality of basal medium gradually decreased and that of apical medium gradually increased. A significant osmotic gradient persisted for at least 10 h. 16 h after the switch, osmolali-

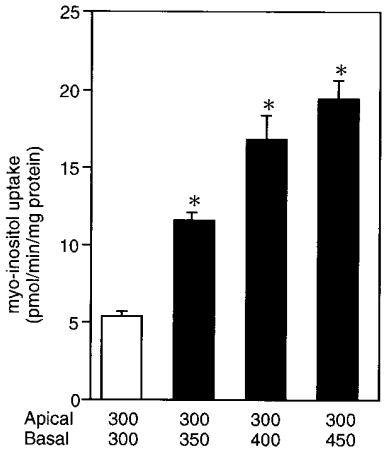

Figure 2. Basolateral hypertonicity increased *myo*-inositol uptake in an osmolality-dependent manner. Na^+-dependent *myo*-inositol uptake was measured 6 h after switching to different osmolalities on basal side. The medium osmolality ranged from 300 to 450 mosmol/kg and was made by addition of 0–150 mosmol/kg of raffinose. Number indicated below x-axis is osmolality of medium on each side (mosmol/kg). Each bar is mean of three independent experiments; error lines are SD. *$P < 0.01$ vs isotonic (300 mosmol/kg) cells.

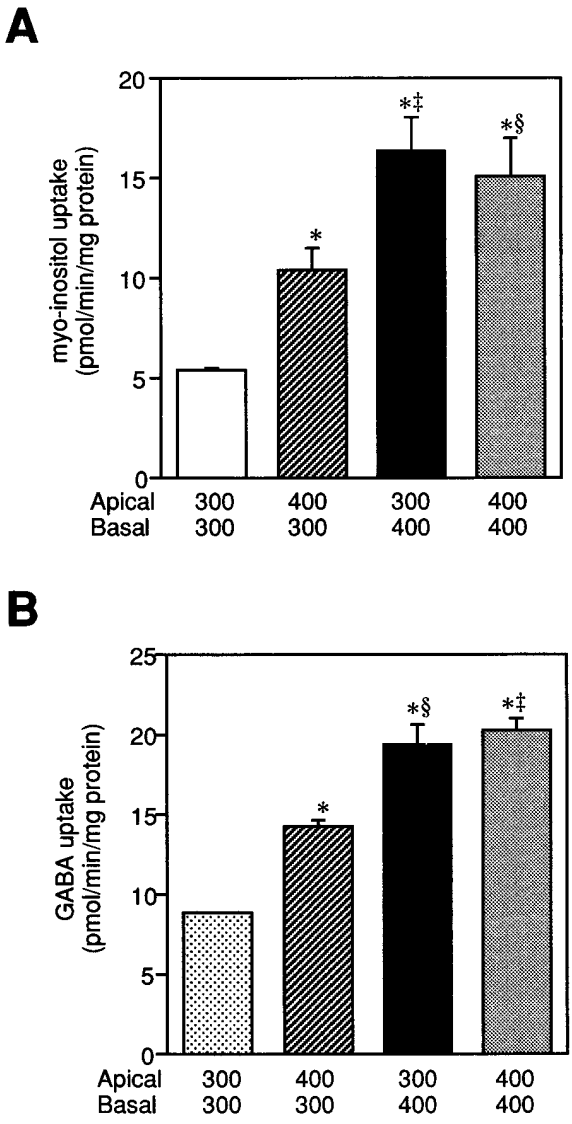

Figure 3. Basolateral hypertonicity increased *myo*-inositol (*A*) and GABA (*B*) uptake more than apical hypertonicity. Na^+-dependent *myo*-inositol (*A*) or GABA (*B*) uptake was measured 6 h after switching to hypertonic medium (400 mosmol/kg) on either side or both sides of the epithelium. Number indicated below x-axis is osmolality of medium on each side (mosmol/kg). Each bar is mean of three independent experiments; error lines are SD. $^*P < 0.01$ vs isotonic (300 mosmol/kg) cells; $^‡P < 0.01$ vs apical hypertonicity; $^§P < 0.05$ vs apical hypertonicity.

ties of both sides became very close. From this result, we decided that uptake experiments would be performed 6 h after the switch.

To examine whether the *myo*-inositol transport increases as basolateral osmolality increases, we measured Na^+-dependent *myo*-inositol uptake from basal side 6 h after basolateral medium was switched to media with different osmolalities (Fig. 2). The medium osmolality ranged from 300 to 450 mosmol/kg which were made by addition of 0–150 mosmol/kg of raffinose. The uptake rate increased as basolateral osmolality increased until 450 mosmol/kg.

To see if there is a difference in the increase in SMIT activity between apical and basal hypertonicity, we investigated the effect of hypertonicity on either side or both sides of the epithelium (Fig. 3 *A*). When basolateral osmolality increased, the magnitude of the increase was almost the same as that for making both sides hypertonic. Apical hypertonicity also increased the uptake, but the magnitude of the increase was significantly smaller than basolateral or both sides hypertonicity.

The polarized effect of hypertonicity may be restricted to induction of SMIT activity or may be common to induction of other osmoregulatory transporters. To clarify this issue, we measured BGT-1 activity, another osmolyte transporter in MDCK cells. BGT-1 activity, measured by [^{3}H]GABA uptake, showed a pattern similar to SMIT in response to basolateral hypertonicity. It increased in a basolateral osmolality–dependent manner (data not shown). Basolateral hypertonicity increased BGT-1 activity significantly more than apical hypertonicity (Fig. 3 *B*). Thus, the predominant effect of basolateral hypertonicity is common to osmolyte transporters.

We measured *myo*-inositol uptake from the apical side under different conditions. There was some *myo*-inositol uptake from the apical side that was induced by hypertonicity as well (17). Either apical or basal hypertonicity increased both apical and basal transports. Although basal hypertonicity was more effective than apical hypertonicity, the ratio of basal transport to apical transport was almost constant under different conditions ($\sim$ 20–30 times). These results confirmed that apical uptake was always negligible compared to basal uptake.

In situ hybridization study indicated that SMIT predominantly expressed TALH (16), where a large transepithelial osmotic gradient exists (Fig. 4) (20). So we examined the possibility that the transepithelial osmotic gradient might induce SMIT in MDCK cells. Hypotonic media were made simply by dilution of the growth medium with water. When the isotonic cells were switched to hypotonic medium (100 mosmol/kg) on

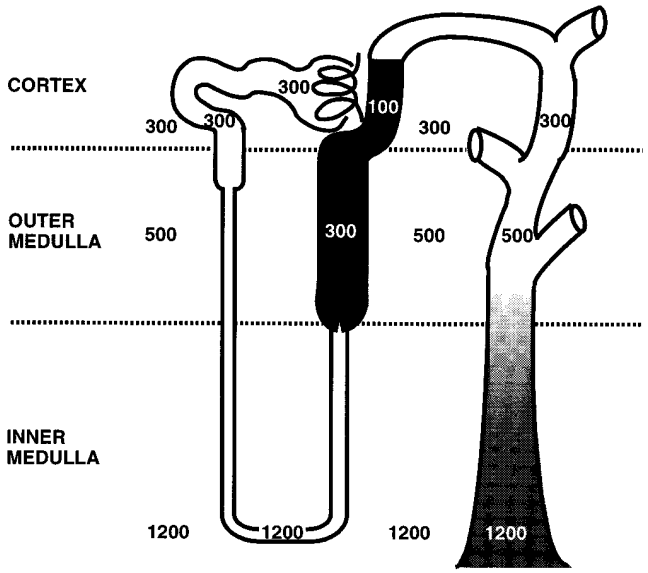

Figure 4. Expression of SMIT in rat kidney (16). The thickness of the dark sections indicate the level of the expression of SMIT mRNA. SMIT is predominantly present in the TALH and macula densa cells. NaCl loading rapidly induces SMIT throughout this segment. Less intense signals are seen in the inner medullary collecting duct cells where SMIT expression seems to correspond to the corticomedullary osmotic gradient. The numbers indicates putative local osmolality during antidiuretic state (27).

apical side, no increase was observed in *myo*-inositol transport (Fig. 5). We also examined the effect of apical hypotonicities in case of basal hypertonicity. The isotonic cells were switched to hypertonic medium (400 mosmol/kg) on basolateral membrane and to the media with different osmolalities, ranging from 100 to 300 mosmol/kg on the apical side. As shown in Fig. 5, osmotic gradient per se did not accelerate SMIT activity. It is apparent, however, that basolateral hypertonicity predominantly affects SMIT activity rather than apical hypotonicity because SMIT activity significantly increased even when apical osmolality was only 100 mosmol/kg. When the osmolality of each side was reversed in this experiment, apical hypertonicity did not significantly increase the transport activity in case of basolateral hypotonicity (data not shown). This result further suggests the predominance of basolateral hypertonicity for inducing osmolyte transporters.

Discussion

In the present study, SMIT activity as well as BGT-1 activity is predominantly regulated by basolateral osmolality in MDCK cells. These results could be explained by the difference in water permeability between apical and basolateral plasma membranes. The fact that the transepithelial gradient holds for several hours (Fig. 1) indicates that one or both of the membranes is water impermeable. This result is consistent with a previous report showing that water permeability of MDCK cell monolayers was in the lower range of the values reported for biological membranes (19). Generally, the basolateral plasma membrane of epithelia is water permeable, and the apical plasma membrane water permeability varies among epithelia. In case of the antidiuretic hormone (ADH)-responsive epithelia, apical membranes have a low osmotic water permeability, whereas basolateral membranes are relatively permeable to water (21). Since addition of vasopressin or dibutyryl cAMP to MDCK cell monolayers induced an increase in water permeability (19), they apparently belong to ADH-responsive epithelia. These results suggest that the rate-limiting site for water and solute transport in MDCK cells is at the apical (luminal) epithelial surface, at least in the absence of ADH. Furthermore, it has been shown that plasma membrane lipid order is asymmetrical in MDCK cells (22), and ADH modulates lipid order of apical plasma membrane (23). Taken together, it is very likely that water permeability of the apical membrane is lower than that of basal membrane in MDCK cells.

When the basolateral membranes are water permeable and the apical membranes are water impermeable, the high basolateral osmolality is supposed to be rapidly balanced with the intracellular osmolality (or ionic strength), which is considered to be a trigger for stimulation of osmoregulatory genes (24). In contrast, apical (luminal) tonicity does not affect the intracellular osmolality so much. This hypothesis seems to be consistent with the results of the present study for SMIT and BGT-1 transports in MDCK cells.

In case of the TALH cells in vivo, where SMIT expresses most predominantly (Fig. 4) (16), apical membranes have an extremely low osmotic water permeability, whereas basolateral membranes are relatively permeable to water (25). Thus, the same notion could be applicable to TALH cells in vivo. SMIT expression did not correspond to luminal osmolality, since SMIT highly expressed throughout the TALH and was universally induced by acute NaCl administration. When NaCl loading to TALH cells increases, the primary event to stimulate NaCl reabsorption is considered to be the activation of the Na^++K^+-pump located in the basolateral membrane. In this case, it is possible that the osmolality close to basolateral plasma membrane of the tubular cells, that is peritubular osmolality (20), may be very high throughout the TALH. There is direct evidence of peritubular hypertonicity in case of macula densa cells. The chloride concentration or osmolality of juxtaglomerular interstitium, that is, the area located on basal side of macula densa cells, has been shown to be very high and to change with the tubular flow rate in Amphiuma (26). NaCl transport into the interstitium at the glomerular vascular pole proceeds through the water impermeable tubular epithelium, which results in basolateral hypertonicity during elevated flow rates. Because the mechanism of NaCl absorption across macula densa cells is at least qualitatively similar to that of TALH cells, we assume that the osmolality close to the TALH cells is also hypertonic.

As shown in Fig. 4, there is an osmotic gradient between apical and basal sides of this nephron segment (20). We first assume that the transepithelial osmotic gradient may be a signal for the induction of SMIT. Osmotic gradient, however, did not accelerate the increase in SMIT activity in MDCK cells (Fig. 5). In this experiment, it is possible that the dilution of some component on the apical fluid may be important in regulating *myo*-inositol uptake because we made hypotonic media by simply diluting the growth media. To clarify this issue, we tried to do the experiments in Fig. 5 using the isotonic media (300 mosmol/kg) made by addition of 100 mosmol/kg of raffinose to hypotonic (200 mosmol/kg) media. Comparing apical hypotonicity to both sides isotonic, there is no significant difference in the transport rate (data not shown). Thus, transepithelial osmotic gradient per se seemed not to induce the transport activity in MDCK cells.

In summary, we investigated the effects on SMIT activity of the change in osmolality on either side of MDCK cells. SMIT activity increased in a basolateral osmolality–dependent manner, and basolateral hypertonicity increased the activity significantly more than apical hypertonicity. BGT-1 activity showed a similar pattern as SMIT. These results were consistent with our hypothesis that SMIT is mainly regulated by osmolality on the basolateral surface. The polarized effect of hypertonicity might be due to the difference in water permeability between

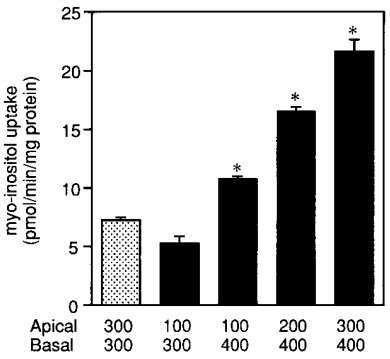

Figure 5. Osmotic gradient between both sides of the epithelium did not induce *myo*-inositol uptake. Na^+-dependent *myo*-inositol uptake was measured 6 h after switching to isotonic or hypertonic medium (400 mosmol/kg) on basolateral side and to different osmolalities on apical side of the epithelium. Number indicated below x-axis is osmolality of medium on each side (mosmol/kg). Each bar is mean of three independent experiments; error lines are SD. $*P < 0.01$ vs isotonic (300 mosmol/kg) cells.

basal and apical membranes. Further studies will be required to clarify whether SMIT is regulated in vivo by the same mechanism in TALH cells.

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