Osmotic Water Permeability and Regulatory Volume Decrease of Rat Thymocytes

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Abstract. Rat thymocytes displayed robust regulatory volume decrease (RVD) when suspended in NaCl-based hypotonic Ringer solutions. The RVD of thymocytes was completely abolished upon replacement of external Na^+ ions with K^+, indicating a role of coupled efflux of K^+ and Cl^- ions as a driving force of regulatory volume decrease. Osmotic water permeability (P_f) measured in KCl-based hypotonic solutions was $(1.3 \pm 1.0 \times 10^{-4}$ cm/s at 25°C and was temperature-dependent with low activation energy ($E_a = 4.65 \pm 0.77$ kcal/mol) characteristic to water transport through pores. $HgCl_2$ and a sulfhydryl-blocking reagent, methyl methanethiosulphonate (MMTS), modulated the water permeability of thymocytes in a biphasic manner: inhibited at low dose (0.1–1 μmol/l) and restored or even enhanced at higher (10–100 μmol/l) concentrations. RVD paralleled the P_f: it was greatly suppressed at low dose of MMTS (sufficient to attenuate the water transport), but recovered at higher dose, when the water movement was restored. Therefore we suggest that thymocytes require the effective water transport for functional regulatory volume decrease.

Key words: Thymocytes — Cell volume regulation — Water permeability

Introduction

Virtually all cells go through osmotic transitions during lifetime, since both intracellular metabolism and membrane transport are expected to produce fluctuations in concentrations of osmotically active constituents. Cell volume regulation is a widespread phenomenon and enables cells to maintain their normal volume. In most cells, hypotonic stimulation activates potassium and chloride transporting pathways resulting in efflux of both ions and osmotically obligated water from the cells, a process termed regulatory volume decrease (RVD) (Strange et al. 1996;

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Nilius et al. 1997; Okada 1997; Rouzaire-Dubois et al. 1999; Bostel et al. 2001). Recent studies have shown that RVD machinery in general and volume-activated ion channels in particular are involved not only in volume regulation, but also in cell proliferation (Voets et al. 1995, 1997; Rouzaire-Dubois and Dubois 1998; Wondergem et al. 2001) and apoptosis (Maeno et al. 2000; Okada et al. 2001; Okada and Maeno 2001). During cells swelling and shrinkage, one may anticipate massive transport of water through the cell plasma membrane. Recent studies have shown that basal lipid matrix water permeability is not sufficient for effective volume regulations, and expression of specialized water channels is required for effective RVD process in epithelial cells (Kida et al. 1998; Morishima et al. 2001).

Thymocytes are immature lymphocytes that undergo negative selection by T-cell-receptor (TCR)-induced apoptosis in thymus before being released to the bloodstream. Developing T-cells proliferate in thymus during maturation and later upon antigen-stimulation (Savino and Dardenne 2000). Although it is expected that RVD machinery is involved in these processes, cell volume regulation of thymocytes and water movement during their volume changes is poorly studied. In the present paper we have shown that the osmotic water permeability (P_f) of rat thymocytes and its activation energy is similar to water channel-mediated transport. The water permeability was suppressed by the water channel blockers, $HgCl_2$ and metyl methanethiosulphonate (MMTS), suggesting involvement of specialized water channels. The RVD efficiency paralleled the water permeability modulation by MMTS suggesting the necessity of effective water transport for thymocyte cell volume regulation.

Materials and Methods

Solutions

Normal NaCl-based Ringer solution contained (mmol/l): 140 NaCl, 6 KCl, 15 HEPES, 1 $CaCl_2$, 1.5 $MgCl_2$, pH = 7,4. Osmolality of this solution was 300 ± 2 mosmol/kg H_2O as measured by a freezing-point depression osmometer (OM802, Vogel, Germany). In KCl-based Ringer solution, all NaCl was replaced with KCl. Hypotonic solutions were prepared by mixing these Ringer solutions with H-buffer of following composition (mmol/l): 15 HEPES, 1 $CaCl_2$, 1.5 $MgCl_2$, pH = 7,4 (34 ± 2 mosmol/kg H_2O). Osmolalities of hypotonic solutions were calculated from the formula:

$$\Pi = (\Pi_R V_R + \Pi_H V_H)/(V_R + V_H) \quad (1)$$

where Π_R and Π_H are osmolalities of Ringer and H-buffer solutions and V_R and V_H are respective volumes. The ratios and calculated final osmolalities of these solutions are given in Table 1. Glucose was not added in these solutions in order to exclude the effect of glucose transport in cell volume changes.

Table 1. Composition and tonicity of hypotonic solutions

No.	Ringer (ml)	H-buffer (ml)	Final osmolality (mosmol/kg H_2O)	Osmotic gradient (mosmol/kg H_2O)
1	7	0	300	0
2	6	1	262	38
3	5	2	224	76
4	4	3	186	114
5	3	4	148	152
6	2	5	110	190

The final osmolalities of hypotonic solutions were calculated according to the formula (1) as described in Materials and Methods.

Cells

Cell isolation was performed as described previously (Sabirov et al. 1993). Briefly, thymi were dissected from 100–150 g weighing Wistar rats (6–8 weeks old) according to a standard procedure (Hunt 1990). The cells were washed 2–3 times by NaCl-based Ringer solution containing in addition 5 mmol/l glucose and resuspended in this medium at a final concentration of 100×10^6 cell/ml. The suspension contained no more than 5% of damaged cells as assayed by trypan blue exclusion.

Cell volume measurement

Light transmittance measurement was used as an indicator of lymphocyte cell volume changes (Hempling et al. 1977; Sabirov et al. 1993). 900 μl of medium was added to the 1.5 cm^3glass cuvette thermo stated with a water jacket and equilibrated for 10 min. An aliquot (100 μl) of cell suspension was added to this medium and the light transmittance was measured at 610 nm (band-pass filter) using a photometer MKMF-01 (LOMO, St. Petersbourg, Russia). The output signal was continuously recorded using a chart recorder, scanned and digitized using Graph Digitizer 2.15 software (a shareware from N. Rodionov). In some experiments, cell diameter was determined by light microscopy using ocular micrometer with linear resolution of 0.1 μm.

Chemicals

Methyl methanethiosulphonate (MMTS) from Sigma (St. Louis, MO, USA) was diluted from 100 mmol/l stock solution to the final concentrations indicated. Vehicle dimethylsulfoxide (DMSO) at the same dose (no more than 0.1%) did not have any effect on the recorded signals. $HgCl_2$ was dissolved in water.

Data analysis

Data were analyzed with Origin 5.0 (MicroCal Software, Inc., Northampton, MA, USA). Pooled data are given as means ± SEM of n observations. If not indicated,

the data plotted in figures represent mean values of triplicate measurements from two different animals ($n = 6$).

Results

The light transmittance signal of thymocytes suspension in isotonic solution was stable during 20–30 minutes of recording indicating that cell sedimentation rate is low and does not contribute to the light transmittance change in these experimental conditions. Since osmotic cell swelling and following regulatory volume changes occurred within this time period, we did not use stirring throughout this study. Thymocytes suspended in NaCl-containing hypotonic medium displayed fast swelling followed by a robust regulatory volume decrease in osmotic gradient-dependent manner (Fig. 1A). At relatively low osmotic gradients (solution with final osmolality of 262 mosmol/kg H_2O) cell volume recovery was complete whereas at higher gradients cells remained partially swollen even after long period of recording. In general, this residual swelling was larger for higher osmotic gradients, although the absolute level was variable and in some instances we observed full recovery even in hypotonic 148 mosmol/kg H_2O solution. Substitution of Na^+ ions with K^+ ions in hypotonic solutions completely abolished the regulatory volume decrease and cells remained swollen for all along the experiments (Fig. 1B). This result suggests that potassium ion gradient is a driving force for the whole RVD process, consistent with previous findings of Soler et al. (1993) and Arrazola et al. (1993).

Since RVD may mask the passive osmotic response to hypotonic stress, we used KCl-based hypotonic solutions for further measurement of P_f. Fig. 2A shows the steady-state light transmittance (measured 10 min after osmotic challenge) as a function of medium osmolality. The signal was roughly linear in the range of 200–300 mosmol/kg H_2O, but deviated from linearity at very low osmolalities (seen

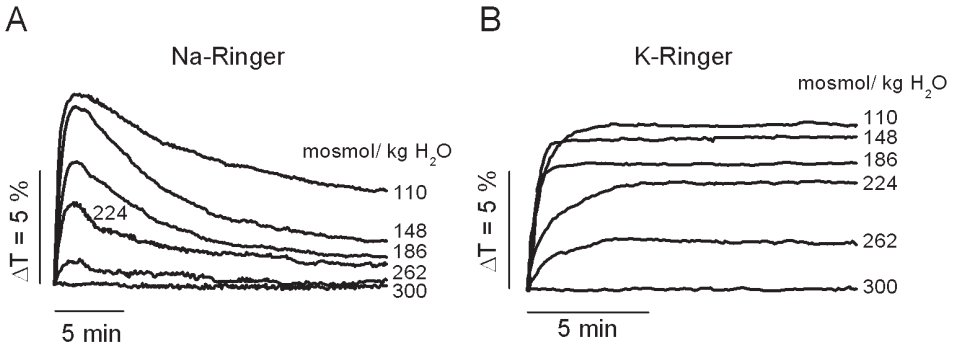

Figure 1. Representative records of light transmittance T of thymocyte suspension in the medium with different osmolality. **A.** Cells were suspended in NaCl-based Ringer solutions with indicated osmolalities. **B.** Cells were suspended in KCl-based Ringer solutions with indicated osmolalities.

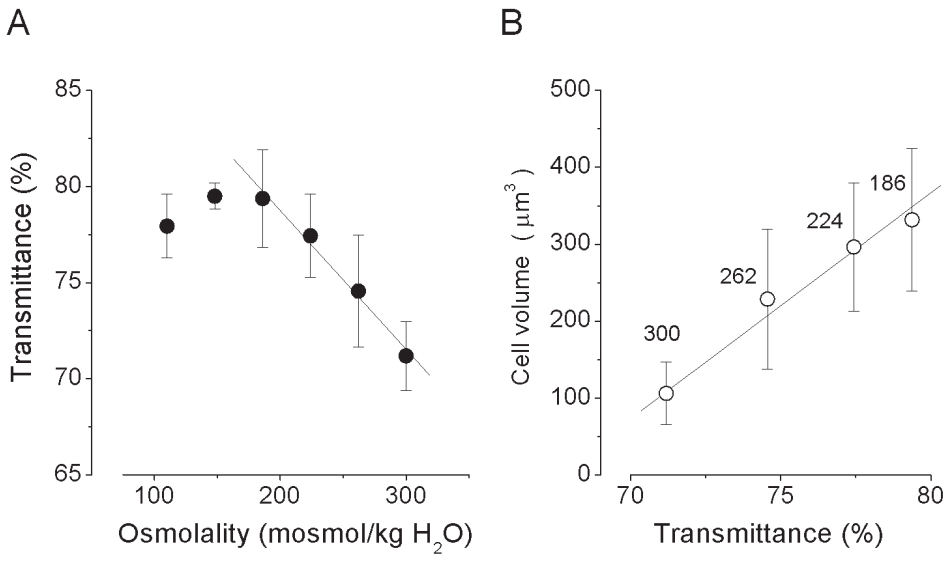

Figure 2. A. Relation between steady-state light transmittance and osmolality of KCl-based medium. **B.** Linear correlation between cellular volume measured by light microscopy and light transmittance. Numbers next to data indicate solution osmolality.

also in Fig. 1B) possibly due to cell damage upon sever osmotic shock. Cell volume calculated from the cell diameter measured by light microscopy (see Methods) was a linear function of light transmittance (Fig. 2B). The linear regression (solid line in Fig. 2B) was further used for calculation of cell swelling rate from initial slope of light transmittance changes upon hypotonic stress (0.5–3 min after osmotic challenge) in KCl-based solutions.

Cell swelling rate was a linear function of osmotic gradient in whole range of osmolalities and temperatures tested (Fig. 3A). This result suggests that even at very low osmolalities, the initial (within 1 min after osmotic challenge) stage of cell swelling was not deteriorated considerably. We suppose that prolonged application of severe osmotic stress (necessary for steady-state light transmittance measurements in KCl-based solutions) leads to cell damage and results in nonlinear dependence of light transmittance on medium osmolality shown in Fig. 2A.

P_f was obtained from the relationship (Zhang and Verkman 1991; Sabirov et al. 1998):

$$dV/dt = P_f S V_w \Delta\Pi \tag{2}$$

where dV/dt is the cell swelling rate; S is the lymphocyte surface area (cm^2) calculated from cell diameter d as $S = \pi \cdot d^2$, V_w is the partial molar volume of water (18 cm^3/mol) and $\Delta\Pi$ is the osmotic gradient (mosmol/kg H_2O).

Linear slope of the data at 25 °C yielded $P_f = (11.3 \pm 1.0) \times 10^{-4}$ cm/s. This

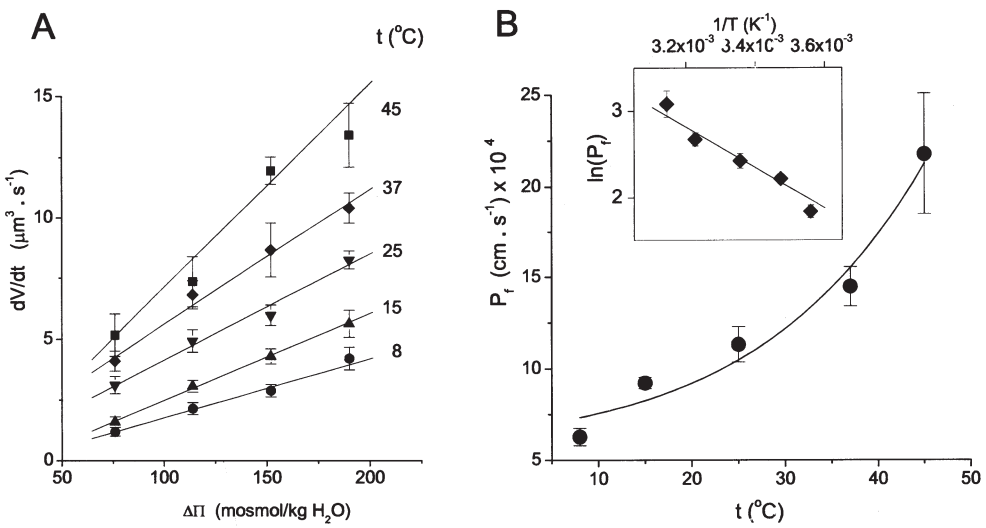

Figure 3. Temperature-dependence of water permeability of thymocytes. **A.** The rate of cell swelling as a function of osmotic gradient at different ambient temperatures. **B.** Osmotic water permeability of rat thymocytes was calculated from the linear slope of rate of cell swelling as a function of osmotic gradients ranged from 76 to 190 mosmol/kg H_2O (data from Fig. 3A; see Eq. 2) and plotted as a function of temperature. Inset: the same data are shown as an Arrhenius plot.

value is within the range found for various cell types (Verkman et al. 1996). P_f of artificial lipid bilayers can be as low as 2.3×10^{-4} cm/s for sphingomyelin/cholesterol membranes and as high as 48×10^{-4} cm/s for egg phosphatidylcholine at 25 °C (Fettiplace and Haydon 1980). Therefore, the absolute value of P_f is not informative in terms of mechanism of water transport.

The apparent activation energy is commonly used to distinguish between mechanisms by which water traverse the membrane. In our experiments, cell-swelling rate decreased when the ambient temperature was lowered down to 8 °C, and increased when the temperature was raised up to 45 °C (Fig. 3A). P_f was a hyperbolic function of temperature. The inset in Fig. 3B shows an Arrhenius plot for determination of the activation energy (E_a) of P_f. The slope of this linear function gave $E_a = 4.65 \pm 0.77$ kcal/mol. This value is lower than that typically found for water movement through artificial lipid bilayers and natural membranes without water channels (10–15 kcal/mol, Fettiplace and Haydon 1980; Verkman et al. 1996). The E_a in our experiments was close to the activation energy of viscose water flow and water movement through membranes containing aquaporins (4–5 kcal/mol, for review see Verkman et al. 1996). Therefore, our data suggest that thymocyte plasma membrane may contain specialised water channels, aquaporins, to facilitate osmotic water transport.

Aquaporins are sensitive to Hg^{2+} ions due to presence of highly conserved cystein residue in the water-permeating pathway (Kozono et al. 2002). In our experiments, relative cell swelling rate was inhibited by app. 20% in the presence of 1 μmol/l $HgCl_2$ (Fig. 4A). Increasing the concentration of $HgCl_2$ led to recovery of cell swelling rate to its initial level (10 μmol/l) or even slightly higher (100 μmol/l). A selective SH-modifying reagent, MMTS, can be alternatively used as a relatively specific inhibitor of aquaporin water transport (Kida et al. 1998; Morishima et al. 2001). In our experiments, low dose of MMTS (0.1 μmol/l) inhibited the P_f by about 20% (Fig. 4B). This result indicates that a sulfhydryl group (presumably of a specific water channel) is involved in water transport through thymocyte plasma membrane. At higher concentrations of MMTS (1–10 μmol/l), the cell-swelling rate restored to its initial value and even increased when higher dose of 100 μmol/l was applied. Recent studies have shown that some aquaporins (AQP6) can be activated, not inhibited, by Hg^{2+} (Yasui et al. 1999). We suppose that MMTS effect at high concentrations may reflect opening of AQP6-like water transporting pathway in thymocytes plasma membrane.

Cell volume regulation is associated with massive flux of water during swelling and recovering phases of RVD, and inhibition of water transporting pathways can greatly suppress the whole RVD process (Kida et al. 1998; Morishima et al. 2001). Our attempts to test the effect of $HgCl_2$ on thymocytes cell volume regulation were unsuccessful. The poor reproducibility of cell volume recovery observed in the presence of $HgCl_2$ might be due to known toxicity of this drug. In contrast, less toxic MMTS produced more reproducible results. We found that MMTS at low dose completely abolished the volume recovery of rat thymocytes after swelling upon hypotonic stimulation in NaCl-based Ringer solution (Fig. 4C). Consistent with the increase in water permeability at higher dose (Fig. 4B), further increase in MMTS concentration restored the regulatory volume decrease of thymocytes (Fig. 4C). Therefore, we conclude that P_f is one of key determinants of regulatory volume decrease machinery of rat thymocytes. Degree of volume recovery estimated 15 min after the hypotonic challenge is summarized in Fig. 4D. Note that RVD restoration required higher dose of MMTS than cell swelling rate: at 1 μmol/l MMTS water permeability was already close to the control value (Fig. 4B) while no signs of volume recovery could be observed on Fig. 4C. Such quantitative discrepancy might be related to the different time scale of the experiments: volume recovery needed much longer observation during which the effective local concentration of the drug could change. MMTS at 100 μmol/l not only restored the initial cell volume but also induced cell shrinkage after about 5 min (Fig. 4C and D). Such overshoot might be related to the function of AQP6-like channels as poorly selective anion channels (Yasui et al. 1999; Hazama et al. 2002) that may mediate additional efflux of cytosolic osmolytes. Neither $HgCl_2$ nor MMTS affected the thymocyte cell volume in isotonic conditions at the concentrations used, excluding a possible non-specific effect of these drugs on plasma membrane permeability.

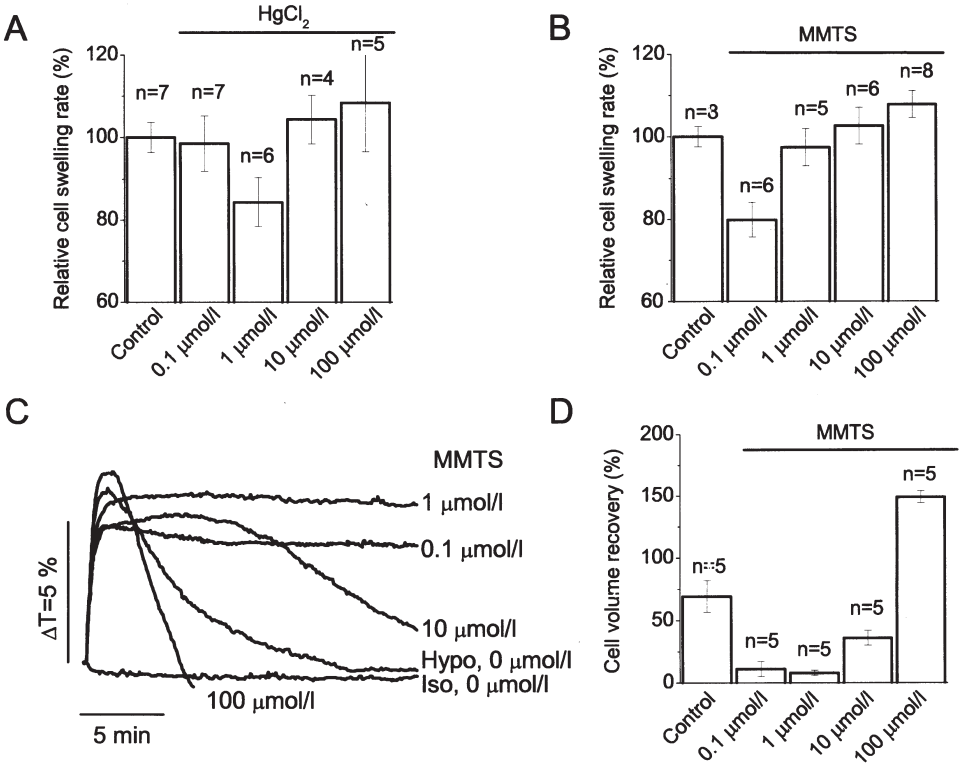

Figure 4. Effect of water channel blockers, $HgCl_2$ and MMTS, on water permeability (A and B) and cell volume regulation (C and D) of thymocytes. **A.** and **B.** KCl-based Ringer solution (148 mosmol/kg H_2O) was used to assess the water permeability in the presence of $HgCl_2$ and MMTS, respectively. Relative cell swelling rate is expressed as percentage of cell swelling rate measured in control medium without the drugs. **C.** Representative traces of light transmittance change for cells suspended in NaCl-based Ringer hypotonic solution (Hypo) (148 mosmol/kg H_2O) in the absence (Hypo, 0 μmol/l) and presence of MMTS at indicated concentrations. The time course of light transmittance in isotonic solution (ISO) (300 mosmol/kg H_2O) is shown as Iso, 0 μmol/l. Vertical scale bar represents light transmittance change (ΔT). **D.** Cell volume recovery was estimated as $(T_{max} - T_0)/(T_{max} - T_{15}) \times 100\,\%$, were T_0 and T_{max} are the light transmittances before the hypotonic challenge and at the peak of cell swelling, T_{15} is the light transmittance measured 15 min after the hypotonic challenge.

Discussion

Bone marrow-derived T-cell precursors rapidly proliferate in thymus. Most of them eventually die by apoptosis during positive and negative selection for self-reactive cells, and this culminates in the production of correctly selected, non-autoreactive, peripheral T lymphocytes (Savino and Dardenne 2000). Both cell proliferation and

apoptosis require fully functional cell volume regulation system, as impairing RVD by blocking volume-sensitive channels was shown to inhibit cell proliferation and differentiation (Voets et al. 1995, 1997; Rouzaire-Dubois and Dubois 1998; Wondergem et al. 2001), as well as apoptotic cell death (Maeno et al. 2000; Okada and Maeno 2001; Okada et al. 2001). Consistent with previous findings (Arrazola et al. 1993), we observed robust regulatory volume decrease of thymocytes suspended in NaCl-based hypotonic Ringer solutions. The RVD of thymocytes was completely abolished upon replacement of external Na^+ ions with K^+, indicating a role of coupled efflux of KCl as a driving force of regulatory volume decrease. The pathway for KCl extrusion was suggested to be K^+-Cl^- cotransporter based on its sensitivity to a specific K^+-Cl^- cotransport inhibitor, DIOA (Arazola et al. 1993; Soler et al. 1993). This feature differentiates the immature thymic T-cells from peripheral lymphocytes, where a role of separate potassium and chloride channels was proposed (Grinstein and Foskett 1990; Cahalan et al. 2001).

Feray et al. (2000) observed extensive magnesium efflux associated with cell swelling when thymocytes were placed in 150 mmol/l KCl solution. In contrast, we did not detect any swelling in isotonic high potassium Ringer solution (Fig. 1B). This difference appears to be due to difference in divalent cation content of extracellular solutions: no divalents were added by Feray et al. (2000), whereas in our experiments high-K^+ solutions contained millimolar concentration of Ca^{2+} and Mg^{2+}, potent regulators of multitude of cell physiological functions (Chattopadhyay and Brown 2000).

Cell volume changes are associated with a massive movement of water in and out of the cells. Physiologically meaningful water transport usually occurs *via* specialised water channels, aquaporins (Verkman et al. 1996; Kozono et al. 2002). In our experiments, thymocytes displayed a moderate osmotic water permeability with low activation energy characteristic to water transport through pores. Moreover, the sulfhydryl-blocking reagents, $HgCl_2$ and MMTS, modulated the water permeability of thymocytes in a biphasic manner: inhibited at low dose and enhanced at higher concentrations. This result may suggest that different types of water transporting proteins are expressed on the plasma membrane of thymocytes.

Recent findings showing that inhibition of water permeability of human intestinal epithelial cells results in complete abolishing of regulatory volume decrease (Kida et al. 1998; Morishima et al. 2001) prompted us to verify this hypothesis in case of thymocytes. Indeed, in our experiments, RVD was coupled to the P_f : MMTS at low dose attenuated water transport and greatly suppressed the RVD, whereas at higher dose the water movement was restored, as well as regulatory volume decrease. Therefore we suggest that thymocytes resemble epithelial cells in their requirement for the effective water transport during RVD. It remains to be verified whether such requirement is a common property of regulatory volume decrease machinery in other cell types.

It should be noted, that neither Hg^{2+} nor MMTS are highly selective for aquaporins. Thus, Hg^{2+} inhibits ROMK and HERG potassium channels as well as various transporters (Moschen et al. 2001), whereas MMTS was reported to affect

ryanodine receptors (Quinn and Ehrlich 1997) and K-Cl fluxes in sheep erythrocytes (Lauf 1988; Ryu and Lauf 1990). As potassium and chloride channels as well as K-Cl cotransport system are known to contribute to RVD in variety of cells, direct effect of $HgCl_2$ and MMTS on channels and transporters functionally expressed in thymocytes needs to be assessed in separate electrophysiological and ion flux experiments.

Most of the water channel expression studies focus on specialised water transporting tissues, such as kidney and intestinal epithelium, etc. Erythrocytes were the first cells shown to express aquaporins (Verkman et al. 1996; Kozono et al. 2002). Leukocytes are expected to experience same osmotic perturbations as erythrocytes, and water channels AQP3 (Ishibashi et al. 1995) and AQP9 (Ishibashi et al. 1998) were reported to present in peripheral leukocytes. The molecular nature and physiological regulation of water channels in thymocytes is not studied at present and remains to be elucidated.

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References

Arrazola A., Rota R., Hannaert P., Soler A., Garay R. P. (1993): Cell volume regulation in rat thymocytes. J. Physiol. (London) **465**, 403—414

Bostel S., Rouzaire-Dubois B., Dubois J. M. (2001): Background osmolyte current involved in cell volume regulation of neuroblastoma × glioma hybrid NG108–15 cells. Gen. Physiol. Biophys. **20**, 281—291

Cahalan M. D., Wulff H., Chandy K. G. (2001): Molecular properties and physiological roles of ion channels in the immune system. J. Clin. Immunol. **21**, 235—252

Chattopadhyay N., Brown E. M. (2000): Cellular "sensing" of extracellular calcium (Ca_0^{2+}): emerging roles in regulating diverse physiological functions. Cell. Signal. **12**, 361—366

Feray J. C., Guerrouache K., Garay R. P. (2000): Dramatic magnesium efflux induced by high potassium in rat thymocytes. Biochem. Biophys. Res. Commun. **268**, 673—676

Fettiplace R., Haydon D. A. (1980): Water permeability of lipid membranes. Physiol. Rev. **60**, 510—550

Grinstein S., Foskett J. K. (1990): Ionic mechanisms of cell volume regulation in leukocytes. Annu. Rev. Physiol. **52**, 399—414

Hazama A., Kozono D., Guggino W. B., Agre P., Yasui M. (2002): Ion permeation of AQP6 water channel protein. Single channel recordings after Hg^{2+} activation. J. Biol. Chem. **277**, 29224—29230

Hunt S. V. (1990): Isolation of lymphocytes and accessory cells. In: Lymphocytes. A practical approach (Ed. G. G. Klaus), pp. 16—68, MIR, Moscow (in Russian)

Hempling H. G., Thompson S., Dupre A. (1977): Osmotic properties of human lymphocyte. J. Cell. Physiol. **93**, 293—302

Ishibashi K., Sasaki S., Saito F., Ikeuchi T., Marumo F. (1995): Structure and chromosomal localization of a human water channel (AQP3) gene. Genomics **27**, 352—354

Ishibashi K., Kuwahara M., Gu Y., Tanaka Y., Marumo F., Sasaki S. (1998): Cloning and functional expression of a new aquaporin (AQP9) abundantly expressed in the peripheral leukocytes permeable to water and urea, but not to glycerol. Biochem. Biophys. Res. Commun. **244**, 268—274

Kida H., Morishima S., Ueda S., Miyoshi T., Chiba T., Okada Y. (1998): The role of water channels in osmotic water flow and cell volume regulation in human small intestinal epithelial cell line. Jpn. J. Physiol. **48**, (Suppl.) S49

Kozono D., Yasui M., King L. S., Agre P. (2002): Aquaporin water channels: atomic structure molecular dynamics meet clinical medicine. J. Clin. Invest. **109**, 1395—1399

Lauf P. K. (1988): Kinetic comparison of ouabain-resistant K : Cl fluxes (K : Cl cotransport) stimulated in sheep erythrocytes by membrane thiol oxidation and alkylation. Mol. Cell. Biochem. **82**, 97—106

Maeno E., Ishizaki Y., Kanaseki T., Hazama A., Okada Y. (2000): Normotonic cell shrinkage because of disordered volume regulation is an early prerequisite to apoptosis. Proc. Natl. Acad. Sci. U.S.A. **97**, 9487—9492

Morishima S., Kida H., Konno T., Ueda S., Okada Y. (2001): Water channel as a requisite to cell volume regulation in a human epithelial cell line. In: XXXIV International Congress of Physiological Science Abstract CD-ROM, Christchurch, New Zealand, Abstract ID Number: 827

Moschen I., Schweizer K., Wagner C. A., Geis-Gerstorfer J., Lang F. (2001): Effects of gallium and mercury ions on transport systems. J. Dent. Res. **80**, 1753—1757

Nilius B., Eggermont J., Voets T., Buyse G., Manolopoulos V., Droogmans G. (1997): Properties of volume-regulated anion channels in mammalian cells. Prog. Biophys. Mol. Biol. **68**, 69—119

Okada Y. (1997): Volume expansion-sensing outward-rectifier Cl^- channel: fresh start to the molecular identity and volume sensor. Am. J. Physiol. **273**, C755—789

Okada, Y., Maeno E. (2001): Apoptosis, cell volume regulation and volume-regulatory chloride channels. Comp. Biochem. Physiol., A: Mol. Integr. Physiol. **130**, 377—383

Okada Y., Maeno E., Shimizu T., Dezaki K., Wang J., Morishima S. (2001): Receptor-mediated control of regulatory volume decrease (RVD) and apoptotic volume decrease (AVD). J. Physiol. (London) **532**, 3—16

Quinn K. E., Ehrlich B. E. (1997): Methanethiosulfonate derivatives inhibit current through the ryanodine receptor/channel. J. Gen. Physiol. **109**, 255—264

Rouzaire-Dubois B., Dubois J. M. (1998): K^+ channel block-induced mammalian neuroblastoma cell swelling: a possible mechanism to influence proliferation. J. Physiol. (London) **510**, 93—102

Rouzaire-Dubois B., Bostel S., Dubois J. M. (1999): Evidence for several mechanisms of volume regulation in neuroblastoma × glioma hybrid NG108–15 cells. Neuroscience (Oxford) **88**, 307—317

Ryu K. H., Lauf P. K. (1990): Evidence for inhibitory SH groups in the thiol activated K : Cl cotransporter of low K sheep red blood cells. Mol. Cell. Biochem. **99**, 135—140

Sabirov R. Z., Manjosova M. A., Tadjibaeva E. T., Krasilnikov O. V. (1993): The Interaction of amphotericin B with cell membrane of rat thymocytes. Gen. Physiol. Biophys. **12**, 249—257

Sabirov R. Z., Morishima S., Okada Y. (1998): Probing the water permeability of ion channels using Xenopus oocytes. Biochim. Biophys. Acta **1368**, 19—26

Savino W., Dardenne M. (2000): Neuroendocrine control of thymus physiology. Endocr. Rev. **21**, 412—443

Soler A., Rota R., Hannaert P., Cragoe E. J. Jr, Garay R. P. (1993): Volume-dependent K^+ and Cl^- fluxes in rat thymocytes. J. Physiol. (London) **465**, 387—401

Strange K., Emma F., Jackson P. S. (1996): Cellular and molecular physiology of volume-sensitive anion channels. Am. J. Physiol. **270**, C711—730

Verkman A. S., van Hoek A. N., Ma T., Frigeri A., Skach W. R., Mitra A., Tamarappoo B. K., Farinas J. (1996): Water transport across mammalian cell membranes. Am. J. Physiol. **270**, C12—30

Voets T., Szücs G., Droogmans G., Nilius B. (1995): Blockers of volume-activated Cl^- currents inhibit endothelial cell proliferation. Pflügers Arch. **431**, 132—134

Voets T., Wei L., De Smet P., Van Driessche W., Eggermont J., Droogmans G., Nilius B. (1997): Downregulation of volume-activated Cl^- currents during muscle differentiation. Am. J. Physiol. **272**, C667—674

Wondergem R., Gong W., Monen S. H., Dooley S. N., Gonce J. L., Conner T. D., Houser M., Ecay T. W., Ferslew K. E. (2001): Blocking swelling- activated chloride current inhibits mouse liver cell proliferation. J. Physiol. (London) **532**, 661—672

Yasui M., Hazama A., Kwon T. H., Nielsen S., Guggino W. B., Agre P. (1999): Rapid gating and anion permeability of an intracellular aquaporin. Nature **402**, 184—187

Zhang R. B., Verkman A. S. (1991): Water and urea permeability properties of Xenopus oocytes: expression of mRNA from toad urinary bladder. Am. J. Physiol. **260**, C26—34

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