ISSN 0006-2979, Biochemistry (Moscow), 2025, Vol. 90, No. 10, pp. 1366-1375 © Pleiades Publishing, Ltd., 2025.
Russian Text © The Author(s), 2025, published in Biokhimiya, 2025, Vol. 90, No. 10, pp. 1463-1473.

Transmembrane Transport of Water and Urea in Rat Corneal Endothelial Cells

Lyubov E. Katkova[1,2,a#], Galina S. Baturina[1,2#], Igor A. Iskakov[3], and Evgeny I. Solenov[1,4,b*]

[1]*Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, 630090 Novosibirsk, Russia*

[2]*Novosibirsk National Research State University (NSU), 630090 Novosibirsk, Russia*

[3]*Federal State Autonomous Institution "National Medical Research Center "Interbranch Scientific and Technical Complex "Eye Microsurgery" named after Academician S. N. Fedorov" of the Ministry of Health of the Russian Federation, Novosibirsk Branch, 630096 Novosibirsk, Russia*

[4]*Novosibirsk State Technical University (NSTU), 630087 Novosibirsk, Russia*

[a]*e-mail: ile@academposter.ru* [b]*e-mail: eugsol@bionet.nsc.ru*

Received July 3, 2025
Revised September 12, 2025
Accepted September 25, 2025

Abstract—This study investigated permeability of the apical and basolateral membranes of rat corneal endothelial cells to water and urea. We demonstrated that the apparent water permeability of the basolateral membrane of endothelial cells (4.43E−05 ± 7.57E−07 cm/s) is more than three times higher than that of the apical membrane (1.21E−05 ± 1.03E−07 cm/s). Permeability of the basolateral membrane to urea (1.23E−04 ± 1.56E−06 cm/s) was statistically significantly higher than that of the apical membrane (9.52E−05 ± 1.02E−06 cm/s) by approximately 30%. We examined contribution of the phloretin-inhibited urea transport across the apical and basolateral membranes in these cells. Phloretin at concentration of 0.1 mM significantly reduced urea permeability by more than 20% through both the apical and basolateral membranes. The results suggest that the compositions of transporters involved in water transport in the apical and basolateral membranes differ significantly. It is hypothesized that high apparent water permeability of the basolateral membrane of endothelial cells is due to contribution of the concomitant water transport with ions involved in active transport processes. Presence of the phloretin-sensitive urea transporters in the plasma membrane of endothelial cells, likely involved in its transcellular transport, has been demonstrated. The results indicate potential significance of urea for corneal function.

DOI: 10.1134/S0006297925601881

Keywords: corneal endothelium, transmembrane transport, water permeability, urea permeability, phloretin

INTRODUCTION

Corneal transparency in many animals and humans is achieved through mechanisms that maintain osmotic balance in the stroma, which contains hydrophilic proteoglycans [1, 2]. Disruption of the balance between water and osmotically active substances can cause stromal swelling, leading to uneven spacing between collagen fibers, increased light scattering, and reduced corneal transparency. Corneal edema is counteracted by the multilayered epithelium, which exhibits low water permeability and low rate of ion transport, and the endothelium, which is characterized by higher permeability and active ion transport processes in the cells. Corneal endothelium is a monolayer of tightly connected cells that separates stroma from anterior chamber of the eye. Endothelium can maintain stromal thickness and hydration levels by transporting water and ions. This function is typically

* To whom correspondence should be addressed.
# These authors contributed equally to this study.

described in terms of the "pump-leak" model, according to which the flow of water into the stroma, determined by osmotic and hydrostatic pressure gradients, is balanced by active transport (pump) of ions and associated water into the anterior chamber of the eye [3].

Permeability of endothelium to water is largely determined by the presence of water channels in the apical and basolateral cell membranes, represented by aquaporin 1 (AQP1) [4]. Immunohistochemical studies indicate presence of AQP3 in the plasma membrane, but functional contribution of this channel in the corneal endothelial cells remains unexplored [5]. AQP3 water channels are permeable to water, glycerol, urea, and hydrogen peroxide [6, 7].

Transport of water and solutes across a monolayer with tight junctions between the cells, as in the case of the corneal endothelium, depends on permeabilities of both basolateral and apical cell membranes, which could differ significantly. For example, studies of anion transport across the corneal endothelium have shown that permeability of the basolateral membrane to HCO_3^- is significantly higher than that of the apical membrane [8]. However, transport of water and electrically neutral molecules in the corneal endothelial cells remains poorly understood.

Urea appears to be an important and well-regulated agent necessary for eye function. Disruption of its metabolism, particularly decrease in the urea concentration in tear fluid in dry eye syndrome [9], indicates its possible role in regulating osmotic balance of the cornea. However, current research on the role of urea transport in the mechanism of stromal osmoregulation is virtually absent in the literature. Expression of the *SLC14a1* gene, which encodes the urea transporter UT-A (UT, urea transporters), has been demonstrated in the corneal endothelial cells [10], which is present in significant amounts in the basolateral membrane of endothelial cells, indicating its possible importance for urea transport and maintenance of stromal osmotic balance [11]. Studies of mice with knockout of the *SLC14a1* gene have revealed abnormalities in the corneal endothelium leading to its edema [12]. Phloretin (2′,4′,6′-trihydroxy-3-p-hydroxyphenylpropiophenone) is a plant-derived flavonoid widely used in research as a pharmacological agent that inhibits various transport pathways, including urea transporters [13, 14]. In particular, UT-B and UT-A exhibit high sensitivity to phloretin, even at low concentrations [15, 16]. Several studies have also shown modulating effect of phloretin on certain aquaglyceroporins [17-19].

In this study, we investigated permeabilities of the apical and basolateral surfaces of rat corneal endothelial cells to water and urea. Given wide variety of the molecular forms of potential urea transporters, the study of urea transport was limited to examining possible contribution of their phloretin-sensitive forms.

MATERIALS AND METHODS

Animals. Experiments were conducted on isolated corneal endothelial cells from Wistar rats. Animals aged 3 months were obtained from the vivarium of conventional animals at ICG SB RAS (Novosibirsk).

Preparation of corneal endothelium. To obtain a preparation of cells with an accessible basolateral surface, the endothelium was applied to a coverslip covered with a poly-D-lysine solution (0.1% (w/v), Sigma-Aldrich, USA) using the imprint method [20]. The coverslip with endothelial cells was then placed in a flow chamber of a microscope. To obtain preparation of cells with an accessible apical surface, a fragment of a Descemet's membrane with cells oriented with the apical surface toward the perfusing medium was placed on a coverslip and placed in the flow chamber of the microscope, where the fragment was mechanically held using a nylon mesh.

Measurement of cell volume. In the microscope chamber with stopped flow, cells were loaded with a fluorescent dye calcein (Calcein AM, 10^{-5} M, 20 min, 37°C). Changes in cell volume were determined using a method based on the quenching effect of calcein fluorescence by cytoplasmic proteins [21-23].

Design of the flow chamber has been described previously [22]. The experimental setup was based on a fluorescence microscope Observer-Z1 (objective Fluar 25/0.8 M27, Zeiss, Germany). The chamber volume was ~50 μL, and solution flow rate was 15 mL/min, ensuring rapid ($t_1/_2$ < 70 ms) medium exchange. Temperature was maintained at 36.8 ± 0.2°C. To determine the rates of changes in cell volume reflecting permeabilities to water and urea, cells were equilibrated in a PBS medium (280 mOsm/kg H_2O), the medium was changed to a "PBS + mannitol" (560 mOsm/kg H_2O) (mannitol, Sigma-Aldrich), and the cells were equilibrated in this hypertonic medium. The medium was then changed to an isotonic "PBS + urea" (560 mOsm/kg H_2O), returned to PBS medium (280 mOsm/kg H_2O), and increase in fluorescence was recorded. Emitted light was detected using a photodetector based on a photomultiplier tube (PMT) equipped with a field diaphragm to measure fluorescence intensity in the region of interest. Signal recording was performed using a digital oscilloscope AKTAKOM ASK-3102 with an interval of 10 ms throughout the experiment at low excitation light intensity, which prevented photobleaching of the fluorophore during the experiment. The signal was recorded with 8-bit resolution and saved to a computer.

Calculation of membrane permeability coefficients. Water permeability was calculated based on the flow equation (1):

$$dV/dt = -S \cdot V_w \cdot Pf \cdot grad\Phi, \quad (1)$$

where Pf is osmotic permeability coefficient (cm/s), S is surface area, Vw is molar volume of water, and Φ is osmotic pressure.

Fluorescence profile F(t) in the initial section was approximated by a linear function $F(t) = A + Kr \cdot t$. The value of Kr was taken as an approximate estimate of dV/dt, initial rate of change in cell volume. Given that, as we have previously shown, $V/V_0 = F/F_0$ [23], where V_0 is the initial cell volume and F_0 is the initial fluorescence signal level, Pf (osmotic permeability coefficient) corresponds to:

$$dV/dt = V_0 \cdot Kr = Pf \cdot S \cdot V_w \cdot \Delta C, \quad (2)$$

$$Pf = V_0 \cdot Kr/(S \cdot V_w \cdot \Delta C), \quad (3)$$

where Kr is linear regression coefficient of the initial section of the fluorescence profile (F/F_0), and ΔC is difference in osmotic concentrations (Osm/kg H_2O) across the plasma membrane of the cell.

Permeability coefficient for urea was determined by analyzing kinetics of the cell volume increase in a hypertonic medium during the isotonic entry of urea. The increase in cell volume in such system can be approximately described by the equation (4):

$$V/V_0 \approx 1 + C_u/\Pi_{out} \approx 1 + [(S \cdot P_u \cdot \Delta C)/(V_0 \cdot \Pi_{out})] \cdot t; \quad (4)$$

from which it follows:

$$\Delta C = C_{out} - C_u; \quad (5)$$

$$(V/V_0 - 1)/t \Rightarrow (F/F_0)/t = Kr_u; \quad (6)$$

$$P_u \approx Kr_u \cdot (V_0 \cdot \Pi_{out})/(S \cdot C_{out}). \quad (7)$$

where Kr_u is regression coefficient of the cell volume in the presence of urea, S is surface area, C_u is urea concentration in the cell, C_{out} is urea concentration in the medium, Π_{out} is osmotic concentration of the medium, V_0 is initial cell volume, and P_u is permeability coefficient for urea.

Experimentally, the area of the plasma membrane surface of the cell open for exchange (S) was determined by the area in an image of the endothelium stained with calcein, since thickness of these cells (size along the Z-axis) (3-5 μm) is significantly smaller than the dimensions along the XY axes. The cells were considered flat, and no correction for the surface curvature was made [24].

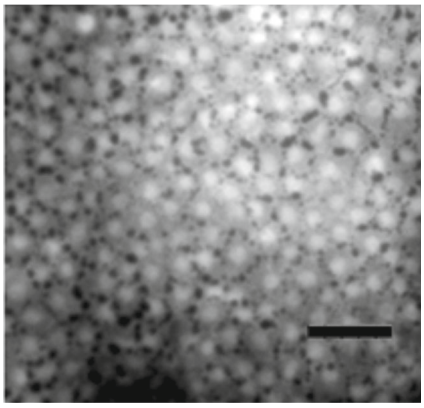

Fig. 1. Rat corneal endothelium preparation stained with calcein. Scale bar: 50 μm.

Permeability coefficients were calculated using the average value of S = 2.5E−06 ± 1.3E−08 cm^2 (M ± standard error of the mean (SEM), n = 100) (Fig. 1).

Statistical analysis. Statistical analysis of fluorescence profiles was performed using Origin 5.0. Statistical calculations were performed using the Statistica 6.0 software package for Windows. The obtained data were analyzed using Student's *t*-test. Results are presented as M ± SEM. Results were considered significant at $p < 0.05$. Normality of distribution was tested using the Shapiro–Wilk test in the Statistica 6.0 program.

RESULTS

Averaged osmotic permeability coefficients and urea permeability in the intact preparations of corneal endothelial cells were as follows: for the apical membrane (M ± SEM) – Pf = 1.2E−05 ± 1.03E−07 cm/s, n = 30; P_u = 9.5E−05 ± 1.02E−06 cm/s, n = 30; for the basolateral membrane – Pf = 4.4E−05 ± 7.57E−07 cm/s, n = 36; P_u = 1.2E−04 ± 1.56E−06 cm/s, n = 36.

In this study, to avoid the influence of errors arising from determining cell surface area (S) in the calculations of permeability coefficients (Pf, P_u), the linear regression coefficient of the initial section of the fluorescence profile (Kr) was used as an experimental estimate of the initial rate of change in cell volume (dV/dt) to assess the rate of transmembrane fluxes of water and urea.

As we have previously shown, rapid osmotic entry of water into the cell, on the one hand, initially causes the effect of increased water permeability, which is not reproduced upon repeated shocks, and on the other hand, activates the mechanism of regulatory volume decrease [23, 25]. To avoid such effects, which significantly distort the results of determining the value of water permeability, in this study,

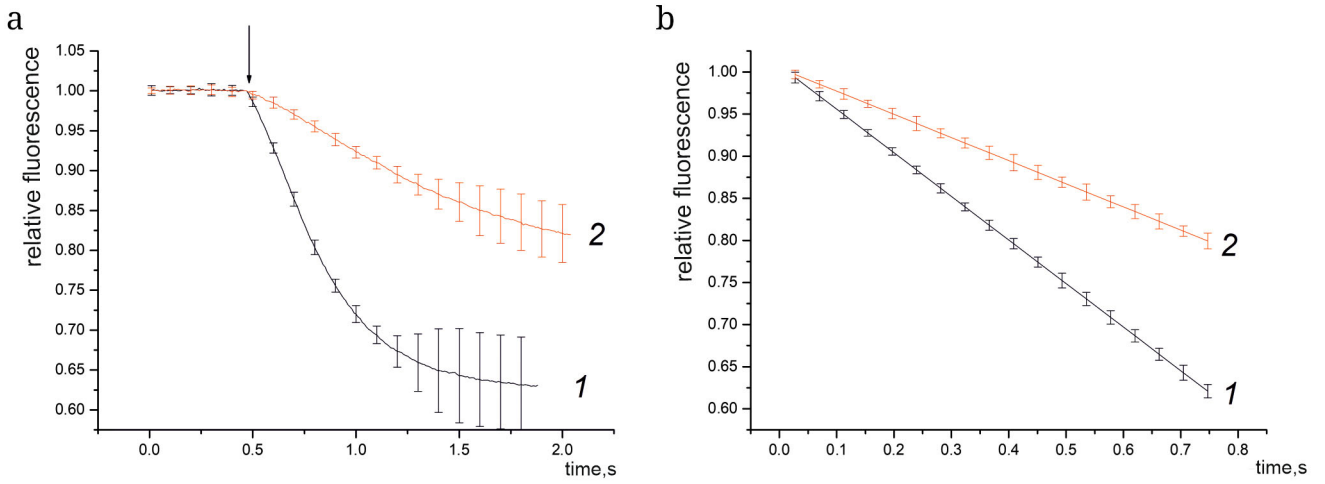

Fig. 2. Dynamics of cell volume decrease during water outflow through the basolateral (*1*) and apical (*2*) surfaces into a hypertonic medium. a) Fluorescence decrease profile; b) initial sections of the profile. The arrow indicates the moment of switching from isotonic PBS solution (280 mOsm/kg H_2O) to hypertonic solution (560 mOsm/kg H_2O).

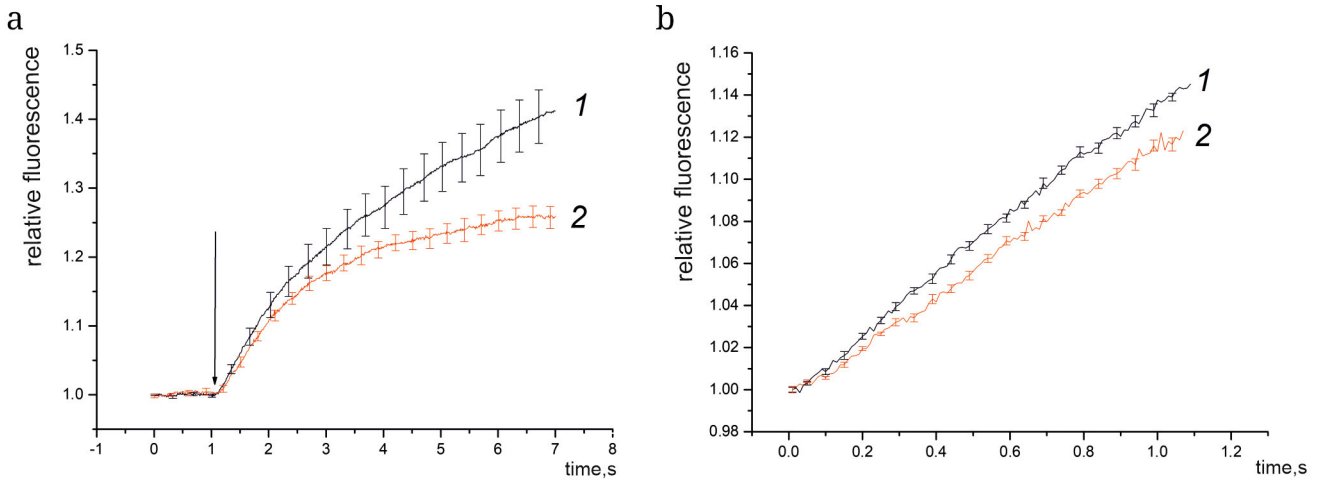

Fig. 3. Dynamics of cell volume increase during urea entry through the basolateral (*1*) and apical (*2*) surfaces under isotonic urea gradient conditions. a) Fluorescence increase profile; b) initial sections of the profile. The arrow indicates the moment of isotonic switching from hypertonic PBS solution (PBS + mannitol, 560 mOsm/kg H_2O) to hypertonic urea solution (PBS + urea, 560 mOsm/kg H_2O).

we used hypertonic exposure, which allows obtaining more reproducible results. According to the obtained results, the intensity of osmotic water outflow from cells with increase in osmotic pressure in the external environment through the apical and basolateral membranes differed significantly. With the same osmotic gradient, the flow through the basolateral surface was more than 3-fold higher than through the apical surface (Table 1, Fig. 2).

During the isotonic entry of urea and associated water into the corneal endothelial cells, the rate of volume increase was higher when water entered through the basolateral cell surface compared to the same flow through the apical surface.

Intensity of urea transport through the basolateral surface in these experiments exceeded transport through the apical surface less significantly than for water, being only 30% higher (Table 1, Fig. 3).

Phloretin present in the medium at concentration of 0.1 mM significantly reduced the rate of urea transport by more than 20% through both the apical and basolateral membranes (Table 2, Fig. 4).

From the obtained results, one can see that with the same osmotic gradients, the resulting water flows through the basolateral membrane are several times greater compared to the flows through the apical surface of the cell. Such asymmetry of flows apparently indicates significant difference in the composition of transporters involved in water transport in the apical and basolateral membranes. The more intense total water flow through the basolateral membrane is obviously explained by the

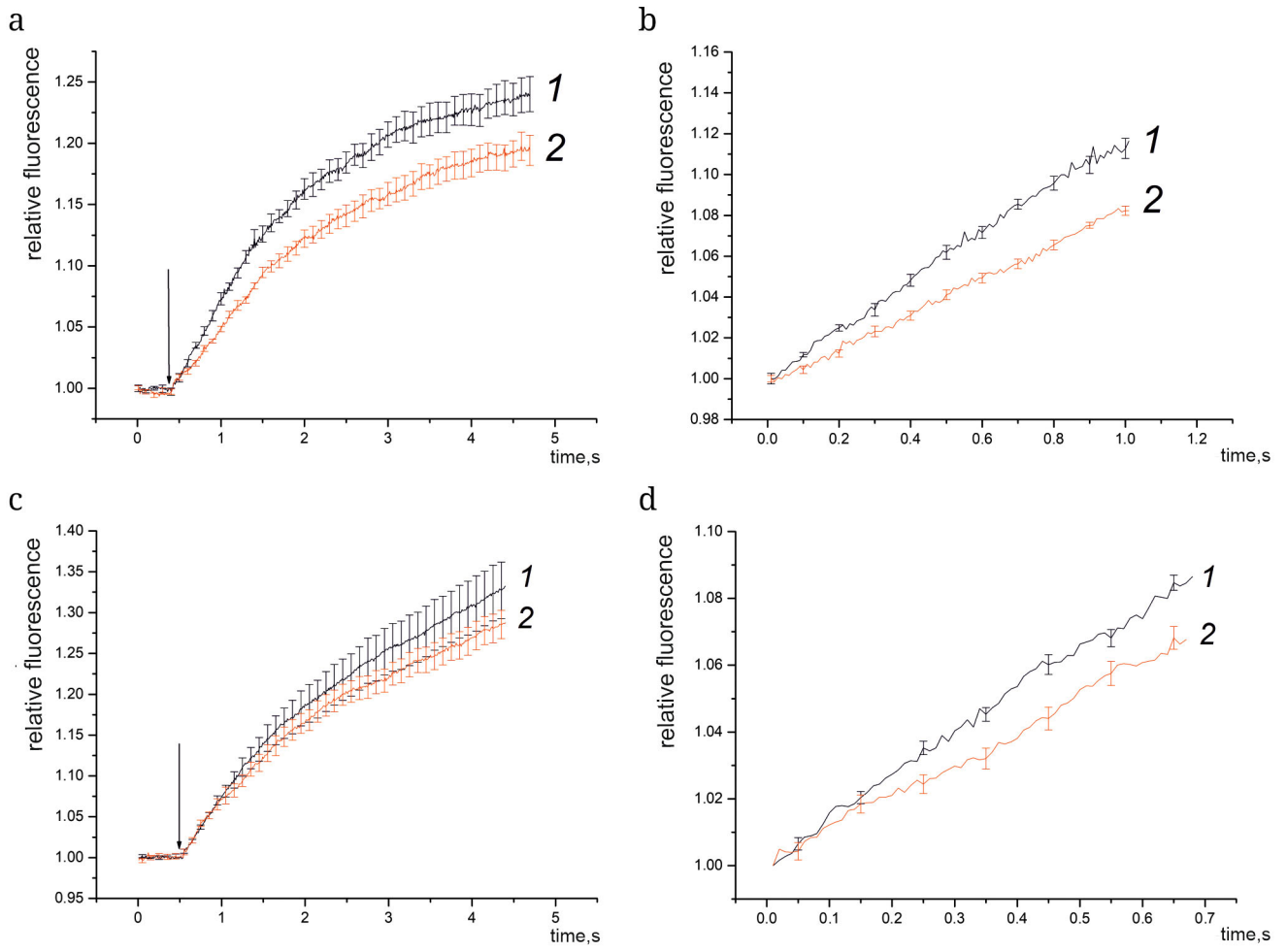

Fig. 4. Effect of phloretin on the dynamics of cell volume increase during urea entry through the basolateral (c, d) and apical (a, b) surfaces under isotonic urea gradient conditions. *1* – Control; *2* – effect of phloretin (0.1 mM). a, c) Fluorescence increase profile; b, d) initial sections of the profile.

Table 1. Linear regression coefficients of the initial sections of the profiles of cell volume dynamics during osmotic water outflow (Kw) or urea entry under its isotonic gradient conditions (Ku)

	Apical membrane (n = 23)	Basolateral membrane (n = 29)	p
K_w	−0.15 ± 5.1E−04	−0.56 ± 6.7E−03	p = 8.06E−03
K_u	0.12 ± 6.6E−04	0.15 ± 1.2E−03	p = 1.18E−02

Table 2. Effect of phloretin on the dynamics of urea entry

	Apical membrane (n = 23)	p	Basolateral membrane (n = 29)	p
K_u	0.12 ± 6.6E−04	6.72E−05	0.15 ± 1.2E−03	4.86E−03
K_u (0.1 mM phloretin)	0.08 ± 4.7E−04		0.12 ± 1.3E−03	

significant water flows associated with the ions involved in the active transport processes in this part of the cell. At the same time, urea flows through the apical and basolateral membranes differ by less than 30% and have a similar degree of inhibition by phloretin (Table 1).

These results allow us to assume existence of the phloretin-sensitive urea transporters in the endothelial cells that carry out its transcellular transport; molecular organization of these transporters and significance of the urea flow through the endothelium are currently unknown and require further investigation.

DISCUSSION

In this study, we determined the differences in the integral values of osmotic water and urea fluxes across the apical and basolateral membrane of corneal endothelial cells based on the kinetics of changes in cell volume during rapid changes in osmotic pressure in the perfusing solution. To the best of our knowledge, this is the first study of urea transport across the apical and basolateral membranes of corneal endothelial cells.

According to the obtained results, under the same osmotic gradient, the water flux through the basolateral surface of corneal endothelial cells exceeded the flux through the apical surface by more than threefold. In many cell types, such as epithelial cells, the area of the basolateral membrane significantly exceeds the area of the apical membrane, and due to this, the apical surface acts as a rate-limiting barrier for transcellular transport. However, the corneal endothelium consists of a monolayer of flat hexagonal cells, and in this cell type, there is likely no significant excess of basolateral membrane area over the apical membrane. According to our data, similar to the water transport, intensity of urea transport through the basolateral surface of corneal endothelial cells exceeded the flux through the apical surface. The obtained results suggest that the pathways for water, urea, and presumably ion transport across the apical and basolateral plasma membranes of corneal endothelial cells have different conductivities and different compositions of transporters.

Water transport across the corneal endothelium largely depends on the presence of water channels, as well as HCO_3^- and Cl^- transporters [26-28]. The structure of water transport pathways in the mammalian eye, according to various authors, may include six [4, 29, 30] or nine [31] types of aquaporins (AQP). AQP1, AQP4, and AQP7 are expressed in the corneal endothelium [31-33]. The recent data obtained by immunohistochemical methods indicate presence of the aquaglyceroporin AQP3 in the corneal endothelial cells, which, in addition to water, can conduct some neutral molecules such as urea and glycerol [5]. AQP1 is widely present on both apical and basolateral membranes of endothelial cells [34], although its functional contribution remains largely unexplored. The models with knockout of the AQP1 gene showed almost no phenotypic differences from the wild-type mice, except that the cornea thickness in the knockout animals was reduced [32]. In the later works, the authors conclude that the function of endothelial AQP1 and AQP4 is minimal under physiological conditions and is, likely, manifested during edema or other pathological conditions [33, 35].

In addition to the transport through water channels, water can be transported across the plasma membrane in a bound state, such as during ion transport or with flows of electroneutral molecules, for example, urea. Thus, high permeability of the basolateral membrane to water could be associated with high metabolic activity of the corneal endothelial cells, which requires intense ion flows generated in the reactions during both ATP synthesis and its hydrolysis [36, 37]. The literature data on composition and expression of basolateral membrane transporters in the corneal endothelial cells support the assumption of their role in the transport of bound water and increased apparent water permeability of this part of the cell plasma membrane. High expression and activity of the cotransporters NBC-1 (Na^+/HCO_3^-) and NKCC1 ($Na^+/K^+/2Cl^-$), as well as of the cAMP-activated chloride ion transporter that is not a CFTR channel, have been demonstrated on the basolateral membrane [38, 39].

High expression of the SLC4A11 protein, which has been shown to play a role in the transport of H^+, NH_3, and water, has been demonstrated in the basolateral membrane of corneal endothelial cells [40-42]. Mutations in the SLC4A11 cause congenital hereditary endothelial dystrophy and, in some cases, Fuchs endothelial corneal dystrophy [43-47]. However, due to the wide variety of transporters present in the basolateral membrane, determining their functional contribution will require further investigations.

As with water permeability, urea flux through the basolateral membrane of the corneal endothelial cells is higher compared to the apical membrane under the same gradients. The difference between the urea fluxes is less significant, which could indicate differences in the number of molecular structures involved in urea transport. It should be noted that the plasma membrane of these cells has high permeability to urea. As can be seen from our results, permeability of the corneal endothelial cells to urea is comparable to permeability of the collecting duct epithelium of the kidney [48]. It could be assumed that such high permeability indicates a similar set of transporters and that intensive urea exchange in the cornea, as in the kidney, could be associated with its role in maintaining the level of osmotic pressure, in this case in the stroma. Since the degree of inhibition of urea fluxes through the apical and basolateral membranes by phloretin is similar, it is most logical to assume that

molecular structure of these transporters could be the same. It is possible that aquaporins are involved in urea transport. For example, it has been shown that deletion of the aquaporin genes AQP3 and/or AQP4 disrupts urea transport in the kidney [49].

Phloretin is considered as a potential inhibitor of some aquaporins, particularly aquaglyceroporins [50]. Studies have been conducted on the inhibition by phloretin (0.1 mM) of water and urea permeability of the plasma membrane of *Xenopus* oocytes injected with RNA encoding aquaporins 3 or 9. It has been shown that phloretin inhibits 86% of water transport and about 20% of urea transport in the case of AQP9. In the case of AQP3, suppression of both water and urea transport by about 10% has been demonstrated [17, 18]. Functional study of the effect of phloretin on the model of oocytes expressing AQP4 did not reveal its effect on water permeability [19]. Thus, phloretin is ineffective as a blocker of aquaporins expressed in the corneal endothelium.

Urea transporters (UT) facilitate water diffusion across the cell membranes, as shown in the experiments with expression of UT-B, UT-A2, and UT-A3 transporters in the *Lithobates* oocytes [51]. The issue regarding the pathways of urea transport in the corneal endothelial cells is complicated. Currently, there is little information about the molecular transporters capable of urea transport expressed in the corneal endothelium on the apical and basolateral membranes. The most significant indication of the involvement of urea transporters in the regulation of stromal osmotic balance is expression of the *SLC4a1* gene, which encodes the urea transporter UT-A in the corneal endothelial cells. Expression of UT-A in mice results in the synthesis of several proteins – UT-A, UT-A1, and UT-A3 – due to alternative splicing, but knockout of the gene results in complete suppression of the phloretin-sensitive urea transport. In the UT-A1/3$^{-/-}$ mice, low cell permeability to urea, which was not inhibited by phloretin, was observed, allowing the authors to suggest that this flux was not due to any known urea transporter [52].

The results of this study indicate a significantly higher permeability of the basolateral membrane compared to the apical membrane for water, and suggest that high permeability to urea indicates its intensive transport in the rat corneal endothelial cells, which is mediated through the transporters with similar permeability in the apical and basolateral membranes and sensitive to inhibition by phloretin. The results obtained in this study suggest that investigations of the urea transporters in endothelial cells could improve diagnosis of the functional state of endothelium and help to identify molecular mechanisms that are promising as targets for therapeutic interventions in the disruption of stromal osmotic balance.

Abbreviations

AQP	aquaporin
UT	urea transporters

Contributions

L. E. Katkova and G. S. Baturina – equal contribution, obtaining experimental data, discussion of research results, writing the article text; I. A. Iskakov – discussion of research results; E. I. Solenov – concept, obtaining experimental data, writing the article text.

Funding

This work was carried out within the framework of the state assignment of ICG SB RAS “Mechanisms of Genetic Control of Development, Physiological Processes, and Animal Behavior” FWNR-2022-0019.

Ethics approval and consent to participate

All procedures performed in studies involving animals were in accordance with the following documents: “Rules of Good Laboratory Practice in the Russian Federation,” approved by Order no. 199n of the Ministry of Health of the Russian Federation dated 01.04.2016; interstate standards GOST 33215-2014 “Guidelines for the Care and Use of Laboratory Animals” (Rules for equipping premises and organizing procedures, effective date 01.07.2015). The conditions for keeping animals and the experimental procedures performed were approved by the Bioethics Commission of ICG SB RAS (protocol no. 115 dated 20.12.2021).

Conflict of interest

The authors of this work declare that they have no conflicts of interest.

REFERENCES

1. Riley, M. V., Winkler, B. S., Peters, M. I., and Czajkowski, C. A. (1994) Relationship between fluid transport and in situ inhibition of Na^+-K^+ adenosine triphosphatase in corneal endothelium, *Invest. Ophthalmol. Vis. Sci.*, **35**, 560-567.
2. Riley, M. V., Winkler, B. S., Starnes, C. A., Peters, M. I., and Dang, L. (1998) Regulation of corneal endothelial barrier function by adenosine, cyclic AMP, and protein kinases, *Invest. Ophthalmol. Vis. Sci.*, **39**, 2076-2084.
3. Maurice, D. M. (1972) The location of the fluid pump in the cornea, *J. Physiol.*, **221**, 43-54, https://doi.org/10.1113/jphysiol.1972.sp009737.
4. Verkman, A. S., Ruiz-Ederra, J., and Levin, M. H. (2008) Functions of aquaporins in the eye, *Prog. Retin. Eye Res.*, **27**, 420-433, https://doi.org/10.1016/j.preteyeres.2008.04.001.
5. Hampel, U., Chinnery, H. R., Garreis, F., Paulsen, F., de Iongh, R., Bui, B. V., Nguyen, C., Parry, L., and

Huei Leo, C. (2020) Ocular phenotype of relaxin gene knockout mice, *Curr. Eye Res.*, **45**, 1211-1221, https://doi.org/10.1080/02713683.2020.1737714.

6. Echevarría, M., Windhager, E. E., and Frindt, G. (1996) Selectivity of the renal collecting duct water channel aquaporin-3, *J. Biol. Chem.*, **271**, 25079-25082, https://doi.org/10.1074/jbc.271.41.25079.
7. Miller, E. W., Dickinson, B. C., and Chang, C. J. (2010) Aquaporin-3 mediates hydrogen peroxide uptake to regulate downstream intracellular signaling, *Proc. Natl. Acad. Sci. USA*, **107**, 15681-15686, https://doi.org/10.1073/pnas.1005776107.
8. Bonanno, J. A., Guan, Y., Jelamskii, S., and Kang, X. J. (1999) Apical and basolateral CO_2-HCO^{3-} permeability in cultured bovine corneal endothelial cells, *Am. J. Physiol.*, **277**, C545-C553, https://doi.org/10.1152/ajpcell.1999.277.3.C545.
9. Velamakanni, G. S., Sharma, A., Batra, H. S., Velamakanni, S. M., Khan, M., and Gupta, S. (2021) Evaluation of tear film urea level as a diagnostic marker of dry eye disease: a pilot study, *J. Lab. Physicians*, **14**, 43-46, https://doi.org/10.1055/s-0041-1739537.
10. Shayakul, C., and Hediger, M. A. (2004) The SLC14 gene family of urea transporters, *Pflugers Arch.*, **447**, 603-609, https://doi.org/10.1007/s00424-003-1124-x.
11. Shei, W., Liu, J., Htoon, H. M., Aung, T., and Vithana, E. N. (2013) Differential expression of the *Slc4* bicarbonate transporter family in murine corneal endothelium and cell culture, *Mol. Vis.*, **19**, 1096-1106.
12. Alvarez, B. V., Piché, M., Aizouki, C., Rahman, F., Derry, J. M. J., Brunette, I., and Casey, J. R. (2021) Altered gene expression in *slc4a11*$^{-/-}$ mouse cornea highlights SLC4A11 roles, *Sci. Rep.*, **11**, 20885, https://doi.org/10.1038/s41598-021-98921-w.
13. Habtemariam, S. (2023) The molecular pharmacology of phloretin: anti-inflammatory mechanisms of action, *Biomedicines*, **11**, 143, https://doi.org/10.3390/biomedicines11010143.
14. Hytti, M., Ruuth, J., Kanerva, I., Bhattarai, N., Pedersen, M. L., Nielsen, C. U., and Kauppinen, A. (2023) Phloretin inhibits glucose transport and reduces inflammation in human retinal pigment epithelial cells, *Mol. Cell. Biochem.*, **478**, 215-227, https://doi.org/10.1007/s11010-022-04504-2.
15. Bagnasco, S. M., Peng, T., Janech, M. G., Karakashian, A., and Sands, J. M. (2001) Cloning and characterization of the human urea transporter UT-A1 and mapping of the human Slc14a2 gene, *Am. J. Physiol. Renal Physiol.*, **281**, F400-F406, https://doi.org/10.1152/ajprenal.2001.281.3.F400.
16. Brahm, J., Dziegiel, M. H., and Leifelt, J. (2023) Urea and water are transported through different pathways in the red blood cell membrane, *J. Gen. Physiol.*, **155**, e202213322, https://doi.org/10.1085/jgp.202213322.
17. Tsukaguchi, H., Shayakul, C., Berger, U. V., Mackenzie, B., Devidas, S., Guggino, W. B., van Hoek, A. N., and Hediger, M. A. (1998) Molecular characterization of a broad selectivity neutral solute channel, *J. Biol. Chem.*, **273**, 24737-24743, https://doi.org/10.1074/jbc.273.38.24737.
18. Tsukaguchi, H., Weremowicz, S., Morton, C. C., and Hediger, M. A. (1999) Functional and molecular characterization of the human neutral solute channel aquaporin-9, *Am. J. Physiol.*, **277**, F685-F696, https://doi.org/10.1152/ajprenal.1999.277.5.F685.
19. Haddoub, R., Rützler, M., Robin, A., and Flitsch, S. L. (2009) Design, synthesis and assaying of potential aquaporin inhibitors, *Handb. Exp. Pharmacol.*, **190**, 385-402, https://doi.org/10.1007/978-3-540-79885-9_19.
20. Kuseina, I. M., Katkova, L. E., Baturina, G. S., Palchikova, I. G., Iskakov, I. A., and Solenov, E. I. (2024) Corneal endothelial cell volume regulation disorders in keratoconus, *Biochem. Moscow Suppl. Ser. A*, **18**, 44-50, https://doi.org/10.1134/S1990747824700065.
21. Solenov, E. I., Nesterov, V. V., Baturina, G. S., Khodus, G. R., and Ivanova, L. N. (2003) Effect of dDAVP on basolateral cell surface water permeability in the outer medullary collecting duct, *Eur. Biophys. J.*, **32**, 614-619, https://doi.org/10.1007/s00249-003-0308-9.
22. Solenov, E., Watanabe, H., Manley, G. T., and Verkman, A. S. (2004) Sevenfold-reduced osmotic water permeability in primary astrocyte cultures from AQP-4-deficient mice, measured by a fluorescence quenching method, *Am. J. Physiol. Cell Physiol.*, **286**, C426-C432, https://doi.org/10.1152/ajpcell.00298.2003.
23. Zarogiannis, S. G., Ilyaskin, A. V., Baturina, G. S., Katkova, L. E., Medvedev, D. A., Karpov, D. I., Ershov, A. P., and Solenov, E. I. (2013) Regulatory volume decrease of rat kidney principal cells after successive hypo-osmotic shocks, *Math. Biosci.*, **244**, 176-187, https://doi.org/10.1016/j.mbs.2013.05.007.
24. Price, M. O., Mehta, J. S., Jurkunas, U. V., and Price, F. W., Jr. (2021) Corneal endothelial dysfunction: evolving understanding and treatment options, *Prog. Retin. Eye Res.*, **82**, 100904, https://doi.org/10.1016/j.preteyeres.2020.100904.
25. Ilyaskin, A. V., Baturina, G. S., Medvedev, D. A., Ershov, A. P., and Solenov, E. I. (2011) Study of the reaction of kidney collecting duct principal cells to hypotonic shock. Experiment and mathematical model, *Biophysics*, **56**, 516-524, https://doi.org/10.1134/S0006350911030092.
26. Fischbarg, J., and Lim, J. (1974) Role of cations, anions, and carbonic anhydrase in fluid transport across rabbit corneal endothelium, *J. Physiol.*, **241**, 647-675, https://doi.org/10.1113/jphysiol.1974.sp010676.
27. Riley, M. V., Winkler, B. S., Czajkowski, C. A., and Peters, M. I. (1995) The roles of bicarbonate and CO2 in transendothelial fluid movement and control of corneal thickness, *Invest. Ophthalmol. Vis. Sci.*, **36**, 103-112.

28. Winkler, B., Riley, M., Peters, M., and Williams, F. (1992) Chloride is required for fluid transport by the rabbit corneal endothelium, *Am. J. Physiol. Cell Physiol.*, **262**, C1167-C1174, https://doi.org/10.1152/ajpcell.1992.262.5.C1167.
29. Bogner, B., Schroedl, F., Trost, A., Kaser-Eichberger, A., Runge, C., Strohmaier, C., Motloch, K. A., Bruckner, D., Hauser-Kronberger, C., Bauer, H. C., and Reitsamer, H. A. (2016) Aquaporin expression and localization in the rabbit eye, *Exp. Eye Res.*, **147**, 20-30, https://doi.org/10.1016/j.exer.2016.04.013.
30. Nautscher, N., Bauer, A., Steffl, M., and Amselgruber, W. M. (2016) Comparative morphological evaluation of domestic animal cornea, *Vet. Ophthalmol.*, **19**, 297-304, https://doi.org/10.1111/vop.12298.
31. Yu, D., Thelin, W. R., Randell, S. H., and Boucher, R. C. (2012) Expression profiles of aquaporins in rat conjunctiva, cornea, lacrimal gland and Meibomian gland, *Exp. Eye Res.*, **103**, 22-32, https://doi.org/10.1016/j.exer.2012.07.005.
32. Thiagarajah, J. R., and Verkman, A. S. (2002) Aquaporin deletion in mice reduces corneal water permeability and delays restoration of transparency after swelling, *J. Biol. Chem.*, **277**, 19139-19144, https://doi.org/10.1074/jbc.M202071200.
33. Melnyk, S., and Bollag, W. B. (2024) Aquaporins in the cornea, *Int. J. Mol. Sci.*, **25**, 3748, https://doi.org/10.3390/ijms25073748.
34. Kuang, K., Yiming, M., Wen, Q., Li, Y., Ma, L., Iserovich, P., Verkman, A. S., and Fischbarg, J. (2004) Fluid transport across cultured layers of corneal endothelium from aquaporin-1 null mice, *Exp. Eye Res.*, **78**, 791-798, https://doi.org/10.1016/j.exer.2003.11.017.
35. Allnoch, L., Beythien, G., Leitzen, E., Becker, K., Kaup, F. J., Stanelle-Bertram, S., Schaumburg, B., Mounogou Kouassi, N., Beck, S., Zickler, M., Herder, V., Gabriel, G., and Baumgärtner, W. (2021) Vascular inflammation is associated with loss of aquaporin 1 expression on endothelial cells and increased fluid leakage in SARS-CoV-2 infected golden Syrian hamsters, *Viruses*, **13**, 639, https://doi.org/10.3390/v13040639.
36. Dikstein, S., and Maurice, D. M. (1972) The metabolic basis to the fluid pump in the cornea, *J. Physiol.*, **221**, 29-41, https://doi.org/10.1113/jphysiol.1972.sp009736.
37. Sun, X. C., and Bonanno, J. A. (2002) Expression, localization, and functional evaluation of CFTR in bovine corneal endothelial cells, *Am. J. Physiol. Cell Physiol.*, **282**, C673-C683, https://doi.org/10.1152/ajpcell.00384.2001.
38. Sun, X. C., Bonanno, J. A., Jelamskii, S., and Xie, Q. (2000) Expression and localization of $NaHCO_3$ cotransporter in bovine corneal endothelium, *Am. J. Physiol. Cell Physiol.*, **279**, C1648-C1655, https://doi.org/10.1152/ajpcell.2000.279.5.c1648.
39. Kuang, K., Li, Y., Wen, Q., Wang, Z., Li, J., Yang, Y., Iserovich, P., Reinach, P. S., Sparrow, J., Diecke, F. P., and Fischbarg, J. (2001) Corneal endothelial NKCC: molecular identification, location, and contribution to fluid transport, *Am. J. Physiol. Cell Physiol.*, **280**, C491-C499, https://doi.org/10.1152/ajpcell.2001.280.3.c491.
40. Vilas, G. L., Loganathan, S. K., Liu, J., Riau, A. K., Young, J. D., Mehta, J. S., and Vithana, E. N. (2013) Transmembrane water-flux through SLC4A11: a route defective in genetic corneal diseases, *Hum. Mol. Genet.*, **22**, 4579-4590, https://doi.org/10.1093/hmg/ddt307.
41. Myers, E. J., Marshall, A., Jennings, M. L., and Parker, M. D. (2016) Mouse Slc4a11 expressed in *Xenopus* oocytes is an ideally selective H^+/OH^- conductance pathway that is stimulated by rises in intracellular and extracellular pH, *Am. J. Physiol. Cell Physiol.*, **311**, C945-C959, https://doi.org/10.1152/ajpcell.00259.2016.
42. Kao, L., Azimov, R., Abuladze, N., Newman, D., Kurtz, I., and Pushkin, A. (2020) SLC4A11 function: evidence for $H^+(OH^-)$ and NH_3-H^+ transport, *Am. J. Physiol. Cell Physiol.*, **318**, C392-C405, https://doi.org/10.1152/ajpcell.00425.2019.
43. Vithana, E. N., Morgan, P., Sundaresan, P., Ebenezer, N. D., Tan, D. T., Mohamed, M. D., Anand, S., Khine, K. O., Venkataraman, D., Yong, V. H., Salto-Tellez, M., Venkatraman, A., Guo, K., Hemadevi, B., Srinivasan, M., Prajna, V., Khine, M., and Casey, J. R. (2008) *SLC4A11* mutations in Fuchs endothelial corneal dystrophy, *Hum. Mol. Genet.*, **17**, 656-666, https://doi.org/10.1093/hmg/ddm337.
44. Roy, S., Praneetha, D. C., and Vendra, V. P. (2015) Mutations in the corneal endothelial dystrophy-associated gene *SLC4A11* render the cells more vulnerable to oxidative insults, *Cornea*, **34**, 668-674, https://doi.org/10.1097/ICO.0000000000000421.
45. Alka, K., and Casey, J. R. (2018) Molecular phenotype of SLC4A11 missense mutants: setting the stage for personalized medicine in corneal dystrophies, *Hum. Mutat.*, **39**, 676-690, https://doi.org/10.1002/humu.23401.
46. Li, S., Kim, E., Bonanno, J. A., and Vithana, E. N. (2019) R125H, W240S, C386R, and V507I SLC4A11 mutations associated with corneal endothelial dystrophy affect the transporter function but not trafficking in PS120 cells, *Exp. Eye Res.*, **180**, 86-91, https://doi.org/10.1016/j.exer.2018.12.003.
47. Malhotra, D., Loganathan, S. K., Chiu, A. M., Lukowski, C. M., Casey, J. R., and Vithana, E. N. (2020) Defective cell adhesion function of solute transporter, SLC4A11, in endothelial corneal dystrophies, *Hum. Mol. Genet.*, **29**, 97-116, https://doi.org/10.1093/hmg/ddz259.
48. Wang, Y., Klein, J. D., and Sands, J. M. (2023) Phosphatases decrease water and urea permeability in rat inner medullary collecting ducts, *Int. J. Mol. Sci.*, **24**, 6537, https://doi.org/10.3390/ijms24076537.

49. Tamma, G., Procino, G., Svelto, M., and Valenti, G. (2012) Cell culture models and animal models for studying the patho-physiological role of renal aquaporins, *Cell. Mol. Life Sci.*, **69**, 1931-1946, https://doi.org/10.1007/s00018-011-0903-3.
50. Huber, V. J., Tsujita, M., Yamazaki, M., Sakimura, K., and Nakada, T. (2007) Identification of arylsulfonamides as Aquaporin 4 inhibitors, *Bioorg. Med. Chem. Lett.*, **17**, 1270-1273, https://doi.org/10.1016/j.bmcl.2006.12.010.
51. Kabutomori, J., Pina-Lopes, N., and Musa-Aziz, R. (2020) Water transport mediated by murine urea transporters: implications for urine concentration mechanisms, *Biol. Open*, **9**, bio051805, https://doi.org/10.1242/bio.051805.
52. Fenton, R. A., Chou, C. L., Stewart, G. S., Smith, C. P., and Knepper, M. A. (2004) Urinary concentrating defect in mice with selective deletion of phloretin-sensitive urea transporters in the renal collecting duct, *Proc. Natl. Acad. Sci. USA*, **101**, 7469-7474, https://doi.org/10.1073/pnas.0401704101.