Uroplakins Do Not Restrict CO₂ Transport through Urothelium*

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Florian Zocher†, Mark L. Zeidel§, Andreas Missner¶, Tung-Tien Sun*, Ge Zhou*, Yi Liao*, Maximilian von Bodungen†, Warren G. Hill‡, Susan Meyers‖, Peter Pohl‡, and John C. Mathai‡

From the †Institut für Biophysik, Johannes Kepler Universität, 4020 Linz, Austria, the §Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts 02215, the ¶Department of Cell Biology, New York University, New York, New York 10016, and the ‡Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

Lipid bilayers and biological membranes are freely permeable to CO₂, and yet partial CO₂ pressure in the urine is 3–4-fold higher than in blood. We hypothesized that the responsible permeability barrier to CO₂ resides in the umbrella cell apical membrane of the bladder with its dense array of uroplakin complexes. We found that disrupting the uroplakin layer of the urothelium resulted in water and urea permeabilities (P) that were 7- to 8-fold higher than in wild type mice with intact urothelium. However, these interventions had no impact on bladder PCO₂ (~1.6 × 10⁻⁴ cm/s). To test whether the observed permeability barrier to CO₂ was due to an unstirred layer effect or due to kinetics of CO₂ hydration, we first measured the carbonic anhydrase (CA) activity of the bladder epithelium. Finding none, we reduced the experimental system to an epithelial monolayer, Madin-Darby canine kidney cells. With CA present inside and outside the cells, we showed that PCO₂ was unstirred layer limited (~7 × 10⁻³ cm/s). However, in the total absence of CA activity PCO₂ decreased 14-fold (~5.1 × 10⁻⁴ cm/s), indicating that now CO₂ transport is limited by the kinetics of CO₂ hydration. Expression of aquaporin-1 did not alter PCO₂ (and thus the limiting transport step), which confirmed the conclusion that in the urinary bladder, low PCO₂ is due to the lack of CA. The observed dependence of PCO₂ on CA activity suggests that the tightness of biological membranes to CO₂ may uniquely be regulated via CA expression.

The urinary bladder, which stores urine of a composition vastly different from that of blood, exhibits extremely low permeability to water and solutes such as urea and other metabolites in the urine (1, 2). The urine osmolality varies widely between 50 and 1000 mosmol/kg compared with near constant osmolality of blood of ~290 mosmol/kg. The primary permeability barrier of the bladder is formed by the umbrella cells whose apical side faces the lumen along with the tight junctions (2–4). The apical surface of these cells contains urothelial plaques, which cover ~90% of the membrane surface of the bladder. These plaques are made of a paracrystalline array of proteins consisting of a family of integral membrane proteins known as uroplakins (UPs), which include UPIa, UPIb, UPII, UPIIIa and UPIIIb proteins (5, 6). The urinary bladders of UPIIIa-deficient mice showed a marked diminution of plaque surface area and significantly enhanced water and urea permeability, which suggested that uroplakins represent a significant component of the apical membrane barrier to water and solute flux across the bladder (3, 5–7).

When the kidney excretes acidic urine, PCO₂ of urine reaches 80–100 mm Hg, 3–4-fold higher than the blood PCO₂ (8–10). The accumulation of CO₂ in the bladder and its failure to diffuse down its concentration gradient suggest that the permeability of the bladder to CO₂ might be very low. We have shown previously that gases such as CO₂ and H₂S are freely permeable across the lipid membrane and are limited only by the unstirred layer adjacent to the membrane (11, 12). Because the umbrella cell apical membrane forms a barrier to water and solute flux (1, 3, 13), we hypothesized that this specialized membrane may also act as a barrier to CO₂ flux.

We measured the CO₂ flux across a normal urothelium and urothelium in which apical barrier function was disrupted. We found that disrupting apical membrane barrier function by protamine sulfate treatment or by genetic ablation of uroplakins (II/IIIA) resulted in water and urea permeabilities that were 7- to 8-fold higher than in wild type mice with intact urothelium. However, these interventions had no impact on CO₂ permeability across the bladder. To test whether the observed permeability was due to an unstirred layer effect or due to kinetics of CO₂ hydration, we measured the CO₂ perme-
ability in Madin-Darby canine kidney (MDCK)² cells in the presence and absence of carbonic anhydrase inhibitor. Our studies show that the lowered permeability of CO₂ across the bladder is mainly due to a lack of carbonic anhydrase (CA) in the urothelium.

**EXPERIMENTAL PROCEDURES**

*Generation of Uroplakin II/III Knock-out Mice*—The generation and characterization of the UPIIIa and UPII knock-out mice were described previously (23, 24). UPII/UPIIIa double knock-out mice were generated by cross-breeding UPII and UPIIIa knock-out mice. To identify the UPII/IIIa homozygote, the following primer pairs were used for genotyping. For deletion of UPIII gene, one forward (5’-TCCACTCCGAGACAAATC-3’) and two reverse (5’-TATCGCTTCTTGGAGACGGATTTGCTTTGA-3’) primers were used; for detecting a 600-bp product of the neomycin sequence, forward (5’-CACCTCCTCTTATCGCCTTCTTGACGAGTTC-3’) and reverse (5’-TCCACACCAATAAGAGTATGCAAAT-3’) primers were used; for detecting a 198-bp product of the native UPII gene. For deletion of the UPIIIa gene, forward (5’-GGAGCCGGACTCTTGATGCAAAT-3’) and reverse (5’-TATCGCTTCTTGGAGACGGATTTGCTTTGA-3’) primers were used; for detecting a 700-bp product of the neomycin sequence, forward (5’-CACCTCCTCTTATCGCCTTCTTGACGAGTTC-3’) and reverse (5’-GCCACACTACATCCCCAGTGT-3’) for a 250-bp product of the native UPIIIa gene were used.

*Measurement of Urothelial Barrier Function*—Animal experiments were performed in accordance with the animal use and care committees of New York University, University of Pittsburgh, Beth Israel Deaconess Medical Center, and Johannes Kepler University. Bladders were excised after lethal anesthesia, washed in placed in NaCl-Ringer buffer (110 mM NaCl, 5.8 mM KCl, 25 mM NaHCO₃, 1.2 mM KH₂PO₄, 2.0 mM CaCl₂, 1.2 mM MgSO₄, and 11.1 mM glucose (pH 7.4) at 37 °C, bubbled with 95% O₂, 5% CO₂ gas) and carefully stretched and mounted on a small ring in the same solution at 37 °C. The bladder was then placed in a modified Ussing chamber (11). Both compartments of the chamber were under constant stirring and temperature control and allowed electrical measurements and sampling.

In all other transepithelial resistance measurements both voltage-sensing and current-passing electrodes were connected to an automatic voltage clamp (EC-825, Warner Instruments, Hamden, CT), which was, in turn, connected to a microcomputer with a MacLab interface (3, 11). All permeability measurements were performed at 37 °C after stabilization of the transepithelial resistance. Diffusive water and urea permeability coefficients were determined by using isotopic fluxes as described (13, 27). Briefly, tritiated water (1 μCi/ml) or [¹⁴C]urea (0.25 μCi/ml) were added to the apical chamber and 100-μl samples of both apical and basolateral chambers were taken every 15 min. Sample volumes were replaced quantitatively with warmed NaCl-Ringer. Sample radioactivities were counted with a liquid scintillation counter (model 1500, Packard Tri-Carb), and flux rates and permeabilities were calculated as described previously (13, 27).

²The abbreviations used are: MDCK, Madin-Darby canine kidney; CA, carbonic anhydrase; PS, protamine sulfate; USL, unstirred layers; AQP1, aquaporin-1.

**Carbonic Anhydrase Activity Measurement**—Carbonic anhydrase activity of bladder tissue was measured by pH electrophometric method (28). Briefly, we added 2 ml of CO₂ saturated water to 3 ml of a Tris-sulfate solution (pH 8.3) and monitored the time that elapsed until pH dropped to 6.3. CA activity of bladder tissue was measured similarly by adding bladder tissue homogenate (100 μg of protein). The bladder musculature was dissected away before homogenization. All CA activities were measured on ice.

*Protamine Sulfate Treatment and Confocal Imaging*—Protamine sulfate was instilled into mouse bladder transuretherally using a lubricated catheter under light halothane anesthesia. Protamine sulfate (PS) was instilled as a bolus (100 μl of 15 mg/ml PS in PBS) for 15 min before emptying, and the animals were euthanized. Mouse bladders were removed according to animal protocols, and its muscle layers were dissected away. These bladders were mounted in modified Ussing chamber and washed in PBS, and its permeability to water and urea were measured as described above. For CO₂ studies, the bladders were mounted in the Ussing chambers, and protamine sulfate (15 mg/ml) was then added to the apical or luminal surface and incubated for 15 min. The tissue was washed with PBS at the end of incubation and fixed for antibody staining and confocal imaging as described previously (29). Monoclonal antibody for uroplakin II was a kind gift from Dr. Apodaca, University of Pittsburgh. F-actin and nuclei was visualized by using Alexa Fluor® 546 phalloidin and Topro-3 probes respectively. Both these probes and secondary antibodies were purchased from Invitrogen.

*Cell Culture*—Stably aquaporin-1-overexpressing Madin-Darby canine kidney (MDCK-AQP1) cells (30), and MDCK cells were cultured in DMEM supplemented with 110 mg/liter sodium pyruvate, 584 mg/liter l-glutamine, non-essential amino acids, 5% FCS, 20 mM HEPES, 0.1% NaHCO₃, and penicillin/streptomycin at 37° in 8.5% CO₂. For MDCK-AQP1, 75 μg/ml hygromycin B was added. For microelectrode measurements, the cells were seeded 1:1 onto semipermeable supports (Transwell, Costar) with a surface area of 0.33 cm² and cultured again until the electrical resistance reached >3 Kilo ohm, indicating a tight monolayer (usually after 3–4 days). All cell experiments were carried out in HBBS buffer at 37°.

**CO₂ Flux Measurements**—The flux of the weak acid CO₂ from the apical to the basolateral compartment of the Ussing chamber caused acidification of the external unstirred layers (USL) adjacent to the basolateral membrane as shown previously (11). The pH shift is a result of the following chemical reactions,

\[
\begin{align*}
H^+ + OH^- & \iff H_2O \\
H^+ + HEPES & \iff HEPES H^+ \\
CO_2 + H_2O & \iff HCO_3^- + H^+ + CO_2^- \\
CO_2 + OH^- & \iff HCO_3^- \\
\end{align*}
\]

REATIONS 1–4

Because both the CO₂ flux \( j_{CO2}^{USL} \) and the HCO₃⁻ flux \( j_{CO2}^{USL} \) through the USL contribute to the membrane CO₂ flux \( j_{CO2}^{USL} \), the latter is determined by the former.

[2] The abbreviations used are: MDCK, Madin-Darby canine kidney; CA, carbonic anhydrase; PS, protamine sulfate; USL, unstirred layers; AQP1, aquaporin-1.
the simplified diffusion model (15) is valid,

\[ J_{CO_2} = J_{HCO_3}^{USL} + J_{CO_2}^{USL} \]

(Eq. 1)

where the bicarbonate concentration in the apical compartment \([HCO_3^-]\) is much larger than the bicarbonate concentration in the basolateral compartment. In the presence of CA, we assume the hydration and dehydration reactions of \(CO_2\) to be in equilibrium because diffusion through the membrane or through adjacent unstirred layers is rate-limiting.

Equation 2 transforms the problem of determining \(P_{CO_2}\) into the task of determining \(J_{HCO_3}^{USL} = P_{USL}[HCO_3^-]\). Because the only bicarbonate sink is the formation of \(CO_2\), \(J_{HCO_3}^{USL}\) must be equal to the proton flux \(J_P\) through the USL. Due to the high buffer concentration the proton is carried by buffer molecules. The flux is calculated according to Ref. 31,

\[ J_{HCO_3}^{USL} = J_P = \frac{\beta \cdot \Delta pH}{\delta} \]

(Eq. 3)

where \(\beta\), \(\delta\), and \(D_P\) are the buffering capacity of the solution, the USL thickness, and the buffer diffusion coefficient, respectively. We measured local pH as a function of the distance \(x\) to the epithelium. \(\Delta pH/\delta\) was the first derivative of the experimental pH profiles at \(x = 0\).

The proton-sensitive microelectrode had a tip size of 2–4 \(\mu m\). Their 90% rise time was below 0.8 s. Addition of CA to the calibrating solution did not affect the sensitivity of the electrodes. Voltage recording was performed every second by an electrometer (model 6514; Keithley Instruments) connected via an IEEE-488 interface to a personal computer. A hydraulic microdrive manipulator (Narishige, Tokyo, Japan) moved the microelectrodes with a velocity of 2 \(\mu m\) s\(^{-1}\).

We performed the experiments in HBBS buffer (118 mM NaCl, 4.6 mM KCl, 10 mM glucose, and 20 mM HEPES, pH 7.4) at 37 °C and applied the \(CO_2\) flux acidified the USL adjacent to the basal membrane. Increasing concentrations of bicarbonate in the apical compartment caused increased USL acidification on the basal side of the bladder. Bladder \(P_{CO_2}\) was equal to 1.5 \(\times\) \(10^{-4}\) cm/s and 1.5 \(\times\) \(10^{-4}\) cm/s, respectively.

**RESULTS**

First, we measured the flux of \(CO_2\) across MDCK cell monolayers grown on filter supports and mounted in an Ussing type chamber (11). The \(CO_2\) flux acidified the USL adjacent to the basal membrane. Increasing concentrations of bicarbonate in the apical medium led to increased acidification of the basolateral USL (Fig. 1, top). According to Equation 2, we plotted \(1/J_{CO_2}\) versus \(1/[CO_2]\) and calculated the \(P_{CO_2}\) to be \(7 \times 10^{-3}\) cm/s (Fig. 1, top, inset). Comparison of this value with the unstirred layer permeability, \(P_{USL} = D_{CO_2}/\delta = 2.9 \times 10^{-5}/1 \times 10^{-2} cm/s = 2.9 \times 10^{-3}\) cm/s suggest that transport is limited by diffusion through the USL.

We measured \(P_{CO_2}\) of the urinary bladder similarly by mounting the bladder on a circular holder with pins, which in turn separated the two halves of a modified Ussing type chamber. Both the apical and basal sides were bathed in modified Ringer’s solution. As shown in Fig. 1B, increased apical \(CO_2\) concentrations caused increased USL acidification on the basal side of the bladder. Bladder \(P_{CO_2}\) was equal to 1.5 \(\times\) \(10^{-4}\) cm/s (Fig. 1), which is \(\sim 45\)-fold lower than that of MDCK monolayers. These results confirmed that urothelium acts as a barrier to \(CO_2\) transport.

We had shown previously that the urothelium has a very low permeability to water and urea and that the permeability barrier resides mainly in the uroplakin layer of the urothelium (4, 7). To investigate whether the uroplakins are also responsible for reduced permeability of \(CO_2\), we selectively damaged the uroplakin layer by exposing the apical side of the bladder to PS. In rats, instillation of proamine sulfate (10 mg/ml) into bladders in vivo was shown to selectively damage the uppermost umbrella cell layer and caused enhanced leakage of urea and water from the bladder (4, 7). Fig. 2A shows that in mice, proamine sulfate treatment leads to a loss of umbrella cells and uroplakins. Staining and confocal imaging of bladder tissue sections using antibodies to uroplakin shows uroplakin II staining (Fig. 2, green) in control (Fig. 2A) and a loss of uroplakin II staining in proamine sulfate-treated bladder (Fig. 2B). The localization of cell layers in the bladder was visualized by staining for F-actin (red) and nuclei (blue). Fig. 2B clearly shows the loss of umbrella cells in the PS-treated bladder. Furthermore, the loss of umbrella cell disrupts apical membrane barrier function, as evidenced by a decrease in transepithelial resist-
tance and ~3-fold increase in water and urea permeability (Fig. 3). PS treatment in rats was shown to selectively remove umbrella cells from the urothelium without causing inflammation (4, 7).

Although our confocal studies indicate that protamine sulfate treatment selectively damaged the upper urothelial layers, we used a second method to enhance the apical membrane leakiness by genetic ablation of uroplakins II and IIIa. The bladders from UPII knock-out mice were shown to be devoid of uroplakin plaques (14). Fig. 4 shows that in contrast to PS-treated bladders, in knock-out mice, UPII^{-}/UPIIIa^{-} bladders lacking uroplakins the transepithelial resistance did not change. However, there was ~9-fold increase in water permeability and an ~8-fold increase in urea permeability, which shows that uroplakins indeed form a permeability barrier to these molecules.

To determine whether uroplakin removal increases PCO2, we measured USL acidification in the presence of a CO2 gradient. Fig. 5 shows that the pH profiles generated by CO2 flux across wild type, uroplakin knock-out, and protamine sulfate-treated mice bladders are similar. These results show that ablation of uroplakins by protamine sulfate treatment and genetic knock-out does not increase the membrane leakiness to CO2, although it does so for water and urea. Furthermore, these results also suggest that in contrast to the physical nature of the barrier for water and urea flux, the barrier to CO2 transport may not be physical in nature. Rather, it may be (i) the thickness of the multi-layered cell layers causing diffusion limitations or (ii) low CA concentrations leading to slow down of the hydration-dehydration of CO2. Because at physiological pH the equilibrium between bicarbonate and CO2 is shifted to 95% in favor of the anionic form and as bicarbonate is membrane impermeable, this chemical reaction accompanies every CO2 passage across a membrane. If not facilitated by CA, the reaction becomes the rate-limiting transport step, a scenario that recurs every time CO2 crosses one of the multiple membrane barriers of the urothelium.

To identify the nature of the CO2 permeability barrier, we studied the kinetics of CO2 hydration and dehydration. It is known that hydration-dehydration reactions of CO2 are very slow at neutral pH with a rate constant of 14 s^{-1} (for dehydration, $K_{-2}$) in absence of CA enzyme (15). To test whether the CO2 hydration-dehydration reaction is the rate-limiting step for CO2 permeation, we measured the CA activity of the bladder. A homogenate of bladder tissue devoid of musculature did not show any measurable CA activity and was similar to that of the control containing only the buffer (Fig. 6). As a positive control, we measured the CA activity of mouse blood and the activity of purified carbonic anhydrase at various enzyme concentrations. This result suggests that the lack of CA activity leads to the reduced CO2 permeability of the urothelium.

To unambiguously show that absence of CA lowers the apparent CO2 permeability, we recapitulated the bladder permeability results in MDCK monolayers. These cells have the advantage of an intrinsically active CA. Adding CA also to the bathing solution produced rather large pH shifts in the USL in response to a transepithelial CO2 gradient (Fig. 7). P_{CO2} was equal to 7 \times 10^{-3} cm/s, indicating that the system was USL-limited (Fig. 8). Omitting CA from the bathing solution

![Figure 2: Protamine sulfate treatment removes uroplakins and umbrella cells. A (upper panel) shows control bladder showing uroplakin staining, green (left). Middle panel shows actin cytoskeleton and nuclear staining of the umbrella cells. Right most panel shows overlay of all three staining. B (lower panel), Protamine sulfate-treated cells show very little uroplakin staining (left) and loss of umbrella cells causing exposure of intermediate cells (right). UC, umbrella cell; IC, intermediate cell.](image)

![Figure 3: PS treatment and bladder permeability. PS exposure leads to decrease in transepithelial resistance (TER; A) and increased permeability to water, (P_{d, water}) (B) and to urea (P_{d, urea}) (C). Standard error bars are shown. Control (n of 6) and PS-treated mice (n of 10) are shown.](image)
decreased the pH shift, which mimicked a drop in $P_{\text{CO}_2}$. Inhibition of intracellular CA by acetazolamide further reduced the pH shift in the USL. The apparent $P_{\text{CO}_2}$ value of $5 \times 10^{-4}$ cm/s decreased (a 14-fold drop) indicates that the system may be reaction-limited (Fig. 8) rather than membrane-limited.

The implication of this result is that CA is perfectly suited to regulate $CO_2$ transport. This result contrasts with a large body of literature (for an overview, see Ref. 16), which ascribes a role for the regulation of $CO_2$ permeability to aquaporins. To test whether aquaporins may reverse the inhibitory effect of missing CA activity...
on CO2 transport, we repeated the experiments in aquaporin-1-overexpressing cells (11). The measured pH profiles in presence and absence of AQP1 (Fig. 7) show no difference. These results suggest that AQP1 does not facilitate CO2 transport and that the flux is just limited by CO2 hydration/dehydration kinetics. Furthermore, these results also confirm our earlier studies showing that AQP1 does not transport CO2 (11).

**DISCUSSION**

When the kidney generates acidic urine, partial CO2 pressure \( P_{CO2} \) of urine reaches 80–100 mm Hg, 3–4-fold higher than blood \( P_{CO2} \) (8) (9, 10). This indicates that CO2 equilibrates across the bladder very slowly and suggests that the bladder is a barrier to CO2 transport. Such a conclusion contrasts with our earlier observation that CO2 transport across membranes is not limited by the membrane itself but by adjacent USLs (11). \( P_{CO2} \) of lipid membranes is so high that it exceeds the permeabilities to ammonia or water by at least 2 orders of magnitude (17). In contrast, the bladder shows a ~45-fold reduced \( P_{CO2} \) compared with monolayer of MDCK cells. Such an unusual low \( P_{CO2} \) across the bladder could be due to: (a) the uroplakin plaques that cover the urothelium, (b) a large unstirred layer created by the four to five layers of cells, or (c) slow kinetics of CO2 hydration and/or dehydration.

Our results show that, in contrast to water and solute flux, removal of uroplakins by proteamine sulfate treatment or by genetic ablation does not influence the flux of CO2. A USL created by the four to five cell layers of the urothelium would be ~50 μm in size. A simple calculation shows that its permeability \( P_{USL} = D_{CO2}/\delta = 2.9 \times 10^{-7}/5 \times 10^{-3} \text{ cm/s} = 6 \times 10^{-7} \text{ cm/s} \) is much too large to explain the ~45-fold decrease in \( P_{CO2} \) of the bladder compared with a monolayer of MDCK cells.

Such a large reduction must be due to slow CO2 hydration-dehydration kinetics caused by lack of CA in the urothelium. Inhibition of the CA in MDCK cells decreased apparent \( P_{CO2} \) by a factor of 10 and thus recapitulated the reaction limited transport of the bladder, validating our conclusion. A series connection of four or five monolayers, as represented by the bladder epithelium, would have a 40–50-fold lower apparent \( P_{CO2} \) because CO2 must undergo the same chemical reaction every time it crosses a membrane on its way. These observations suggest that CA activity can regulate \( P_{CO2} \).

Sometimes regulation of \( P_{CO2} \) is attributed to aquaporins. But even if aquaporin-1 could facilitate CO2 transport in red blood cells and in *Xenopus* oocytes (11, 18–20), it would still transport only the neutral species. This means that CO2 transport must be limited in the absence of CA, even if AQP1 is expressed because there is simply no CO2 available for transport. These considerations are in perfect agreement with our experiment (Fig. 7). That is, the identification of membrane barriers to CO2 transport does not necessarily imply that proteinaceous transport machineries are required. It may simply mean that CA activity is low as we have now shown for the bladder epithelium.

With a partition coefficient of 1.3 (hexadecane), CO2 partitions into lipids as easily as it does into an aqueous pore. Thus, the inability of AQPs to facilitate CO2 transport is already implied by the comparison of the total membrane surface area \( A \) of \( 3.2 \times 10^{-13} \text{ cm}^2 \) of the aquaporin tetramer, and the maximal pore area of \( 3 \times 10^{-15} \text{ cm}^2 \) provided by the tetramer for CO2 diffusion. A more quantitative analysis must also account for mobility differences in the two environments. Thus, the ratio \( J_{lip}/J_{AQP} \) of the fluxes through a lipid patch the size of an aquaporin tetramer and through four aqueous pores is calculated as follows,

\[
\frac{J_{lip}}{J_{AQP}} = \frac{P_{M}[CO_2]}{4p[CO_2]} = \frac{3.2 \text{ cm/s} \times \frac{3.2 \times 10^{-13} \text{ cm}^2}{4 \times 1.2 \times 10^{-14} \text{ cm}^3/\text{s}} = 21
\]

(Eq. 4)

where \( P_{M} \) and \( p \) are the lower limit of membrane permeability to CO2 (11) and the upper limit of single channel CO2 permeability, respectively. The factor four in the denominator indicates the four pores of the tetramer. Given that CO2 is highly diluted in the aqueous solution (i.e. that the ratio of H2O to CO2 concentrations is roughly equal to \( 10^8 \)), it is safe to assume that there is no more than one CO2 molecule in the pore at one time. Because molecules cannot pass each other in single file transport, this leads to \( p = p_f/10 \), where 10 indicates the total number of molecules in the pore and \( p_f = 11.7 \times 10^{-14} \text{ cm}^3/\text{s} \) (32) denotes single pore permeability to H2O. This calculation does not account for the central pore. Molecular dynamics simulations, however, predict that the central pore is either entirely non-conductive (33) or its permeability to CO2 is smaller than \( p_f \) (34). We conclude that \( J_{lip}/J_{AQP} > 1 \), indicating that aquaporin insertion into a membrane tends to lower its permeability. This result is in agreement with previous MD simulations of CO2 passages through lipid membranes and aquaporins (21, 34). Ammonia is the only gas, which according to the solubility diffusion model, may be facilitated by presence of channel such as an aquaporin (22).

We have shown that expression of aquaporin-1 in MDCK cells leads to a 3-fold increase in water permeability (11) but with no detectable change in CO2 permeability, which is consistent with our earlier results (11). Presence or absence of aquaporin-1 in cells lacking CA activity did not alter the CO2
flux. The urothelium functions as a low permeability barrier to CO$_2$ due to lack of detectable CA in the urothelium. We suggest that P$_{CO2}$ of biological barriers is generally regulated by CA activity.

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