Biol. Chem., Vol. 385, pp. 921–926, October 2004 • Copyright © by Walter de Gruyter • Berlin • New York. DOI 10.1515/BC.2004.120

Minireview

Combined transport of water and ions through membrane channels

Peter Pohl

Forschungsinstitut für Molekulare Pharmakologie, Robert-Rössle-Str. 10, D-13125 Berlin, Germany
e-mail: pohl@fmp-berlin.de

Abstract

The coupling of ion and water flow through membrane channels is under dispute. Among all human aquaporins only aquaporin-6 exhibits ion channel activity. Whether aquaporin-6 functions also as a water channel cannot yet be determined with confidence. Similarly, a comparison of single-channel water permeabilities from ion channels and aquaporins suggests that ion channels may play a secondary role as water channels. However, the fraction of absorbed fluid that crosses epithelial ion channels still remains to be determined.

Keywords: cholesterol; cyclic nucleotides; planar membranes; potassium channel; scanning electrochemical microscopy; single file transport.

Introduction

Water is essential to all forms of life. Its transport into cells and organelles through epithelia and endothelia has been studied for over a century. Nevertheless, the pathways taken by water across a membrane barrier and the mechanism of solute-solvent coupling are still not entirely resolved (Spring, 1999). This review aims to give a short overview of the coupling of ion and water fluxes through membrane channels. Excellent recent reviews about water channel proteins (Nielsen et al., 2002; Fujiyoshi et al., 2002) and epithelial water transport (Matthay et al., 2002; Schafer, 2002) are available elsewhere.

The water selectivity of aquaporins

Commonly it is considered that water may pass the membrane lipid bilayer by diffusion. The extent to which the lipid part of membranes contributes to water transport varies considerably between different cells. Epithelial cells, for example, have to maintain large chemical and osmotic gradients. Consequently, the membrane matrix has to be effectively impermeable to ions and most other small molecules. Tightening of the membrane barrier is achieved by high concentrations of glycosphingolipids and sphingomyelin in the outer leaflet of the epithelial plasma membrane. Formation of complexes of these lipids with cholesterol result in a more than ten-fold lower water permeability for the exofacial leaflet than the cytoplasmic leaflet which lacks these complexes (Hill and Zeidel, 2000; Krylov et al., 2001). Experimental evidence for the independent and additive resistances of both leaflets to permeation has been obtained with asymmetric planar bilayers which mimic the apical membrane of renal epithelial cells. They were folded from two monolayers of different composition in the aperture of a teflon film (Krylov et al., 2001). Water flow was measured by imposing an osmotic gradient across these bilayers and detecting the resulting small changes in solute concentration close to the membrane surface (Pohl et al., 1997). The change in the interfacial Na^+ concentration measured by scanning microelectrodes (Figure 1A) is related to the velocity of the osmotic volume flow, v, as defined by equation (1):

$$c(x)=c_s e^{\frac{-vx}{D}+\frac{ax^3}{D}} \quad (1)$$

where a, x, c_s, D are the stirring parameter, the distance to the membrane, the solute concentration in the immediate membrane vicinity and the solute diffusion coefficient (Pohl et al., 1997). v allows calculation of membrane water permeability P_f:

$$P_f=\frac{v}{c_{osm}V_w} \quad (2)$$

where c_{osm} and V_w are, respectively, the effective transmembrane osmolyte concentration gradient and the molar volume of water (Finkelstein, 1987). Equations (1) and (2) yield a P_f of 6.7 ± 0.7 μm/s for planar bilayers mimicking the leaflet asymmetry of epithelial cells (Figure 1B). The result indicates that the permeability P_{AB} of a bilayer composed of leaflets A and B is equal to:

$$\frac{1}{P_{AB}}=\frac{1}{P_A}+\frac{1}{P_B} \quad (3)$$

where P_A and P_B are the permeabilities of leaflet A and B, respectively (Krylov et al., 2001). Thus, most of the resistance presented by the apical membrane to water permeation is contributed by the outer leaflet which is enriched in cholesterol and sphingolipids. This lipid composition provides cells specialized in water transport with the low background conductivity required for water flux adaptation by regulation of water channel protein abundance in the plasma membrane.

The latter may reach 1300 molecules/μm^2 in aquaporin-rich tissues (Nielsen et al., 1993). If reconstituted at a comparable density (300 aquaporin-1 molecules/μm^2)

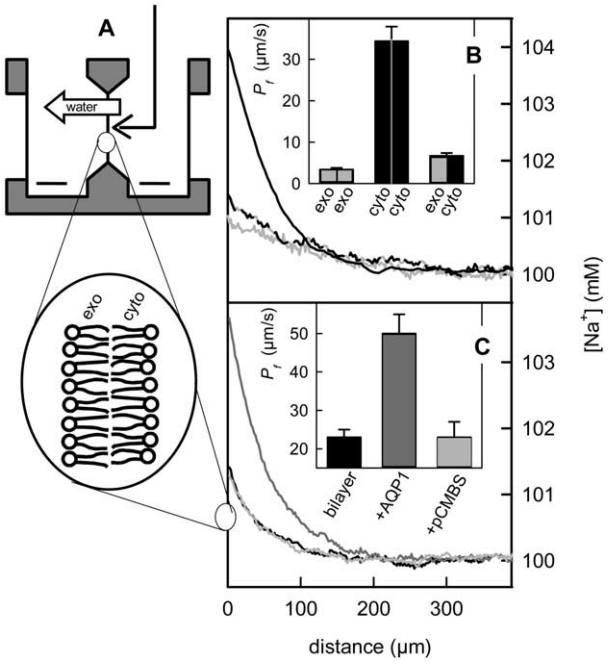

Figure 1 The extremely low water permeability of the lipid matrix enables cells specialized in water transport to regulate water flux by adaptation of water channel protein abundance in the plasma membrane.
(A) Scheme of the experimental setup used for water flux measurements. Planar bilayers are formed in the aperture of a Teflon chamber by folding of two monolayers. Osmotic water flow passing through this membrane dilutes the solution in the hypertonic compartment and concentrates the solution in the hypotonic compartment. The resulting ion concentration polarization measured in the immediate vicinity of the membrane allows to calculate the membrane water permeability (compare eq. 1–2). (B) Representative sodium concentrations measured at the indicated distances to the bilayer in the hypotonic compartment (temperature: 36°C). The hypertonic compartment contained 1 M urea. Symmetric bilayers were formed from exofacial (exo) and cytoplasmic (cyto) lipids shown in grey and black, respectively. Asymmetric membranes were folded from one cytoplasmic and one exofacial leaflet (black and grey). The panel was adapted from Krylov et al. (2001). (C) Water permeability of planar bilayers reconstituted with purified human red blood cell aquaporin-1. Plain lipid bilayers (black) were formed from the *E. coli* lipid extract. The hypertonic compartment contained 1 M urea (temperature: 24°C). Reconstitution of about 10^7 copies of the aquaporin-1 molecule leads to a marked increase of water permeability. Chloromethylbenzenesulfonate (pCMBS), a known inhibitor of water channel proteins, reduces the membrane water permeability to baseline levels, thereby confirming that the increase in water permeability occurred through aquaporin-1. The panel was adapted from Saparov et al. (2001).

into bare bilayers, P_f is about ten-fold higher than in bilayers mimicking the apical membranes of epithelial cells (Figure 1B, C). Similar P_f values of cell membranes permit water absorption, release or redistribution with remarkable speed and precision.

At the same time, water transport across cell membranes must be sufficiently selective to prevent the movement of other solutes, ions, and even protons (Finkelstein, 1987). Aquaporins accomplish this task with an unparalleled perfection as has been shown by reconstitution into planar bilayers. The model system allows the simultaneous measurement of the electrical and hydraulic conductivities of the bacterial aquaporin-Z (Pohl et al., 2001). The P_f of aquaporin-Z-containing membranes is about 50 µm/s, i.e. similar to the P_f value observed after aquaporin-1 reconstitution (Figure 1C). It translates into an aquaporin-Z-mediated water flux density of 4.3×10^{-6} mol s^{-1} cm^{-2} (Pohl et al., 2001). In contrast, the aquaporin-Z-induced ion conductance is astonishingly small. It does not differ significantly from the conductivity of bare bilayers. The corresponding ion flux is equal to 7×10^{-16} mol s^{-1} cm^{-2}. A slightly enhanced membrane conductivity at acidic pH does not indicate aquaporin-mediated facilitation of proton transport since it is entirely brought about by the lipid bilayer (Figure 2A). The water-to-ion flux ratio indicates that less than one ion is transported per 10^9 water molecules!

Several studies have suggested that water-ion cotransport may occur in some aquaporins after cyclic nucleotide binding (Yool et al., 1996; Anthony et al., 2000), raising the question of whether aquaporins may participate in transmembrane signaling. Whereas the cAMP channel activity could not be reproduced by several research groups (Agre et al., 1997; Saparov et al., 2001), cGMP-mediated ion channel activity has been observed in reconstituted planar bilayers (Saparov et al., 2001). However, only a tiny fraction of functionally active

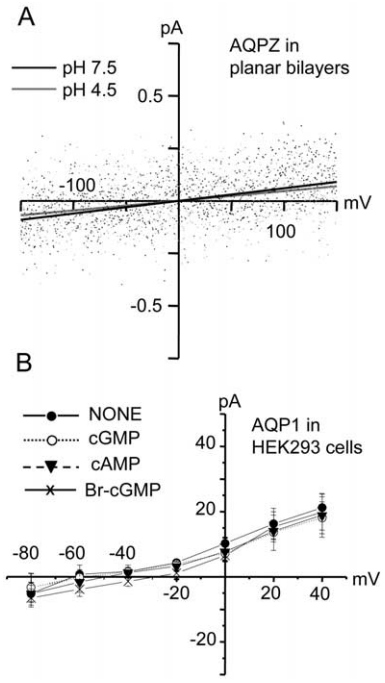

Figure 2 Functional reconstitution of aquaporins into planar bilayers, but not overexpression in HEK293 cells, provides the experimental system with the low background conductivity required to detect openings of single ion channels.
(A) Aquaporin-Z was reconstituted at a protein-to-lipid mass ratio of 1:100. The aquaporin-Z-mediated water flux density of 4.3×10^{-6} mol s^{-1} cm^{-2} contrasts with an ion flux density of only 7×10^{-16} mol s^{-1} cm^{-2} (compare Pohl et al., 2001). The ratio of water-to-ion fluxes of 10^9 is not significantly altered by acidification of the milieu, indicating that protons are excluded from permeating aquaporin-Z. (B) Whole-cell patch clamp experiments. Mean current-voltage (*I*/*V*) relationships of peak current recorded in the absence or presence of cyclic nucleotides indicate that neither cGMP nor Br-cGMP nor cAMP induces ion channel activity in aquaporin-1 transfected HEK293 cells (modified from Tsunoda et al., 2004).

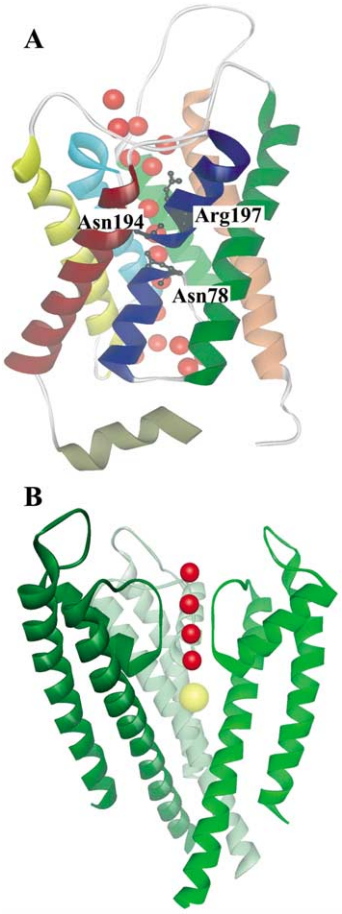

Figure 3 The pore structures of the water channel AQP1 (protein databank file 1J4N) and the potassium channel KcsA (protein databank file 1R3L) allow single-file transport, i.e. within the narrow part of the channels molecules cannot pass or overtake each other.

(A) The six transmembrane helices and two short membrane helices of the bovine AQP1 are shown (Sui et al., 2001). The two short helices meet with their fingerprint NPA motifs in the middle of the channel where the electrostatic barrier for proton movement is centered (de Groot et al., 2003; Chakrabarti et al., 2004). The asparagines (Asn78 and Asn194) of the NPA motifs are highlighted in black. With Arg197 (also black) a smaller electrostatic barrier is located at the constriction region. (B) The channel is formed by a tetrameric assembly of KcsA molecules, each possessing two transmembrane helices (Zhou and MacKinnon, 2003). One of the four molecules has been removed to show the selectivity filter; it has four ion binding sites. For electrostatic reasons the maximum occupancy is two ions. The minimum occupancy of the selectivity filter observed by X-ray diffraction is one ion, which is in contrast with water flux measurements. Fast osmotic water flow occurs only when all ions are rinsed out of the filter (Saparov and Pohl, 2004). This situation is illustrated in the Figure where all binding sites are occupied by water molecules (red). The potassium ion (yellow) has been placed into the cavity which, in contrast to the selectivity filter, is wide enough to allow different molecules to pass each other.

water channels exhibits ion channel activity in bilayers. Their astonishingly small open probability of less than 10^{-6} transforms into less than one open channel per cell even in aquaporin-1-rich membranes such as those of red blood cells (Nielsen et al., 1993). The open probability of ion channels, which play a role in cell physiology, may range from only 0.004 to 0.014 (Firsov et al., 1996), as has been shown with epitope-tagged epithelial sodium channels. The million-fold discrepancy found for aquaporins is unprecedented and, conceivably, represents an evolutionary relic of doubtful physiological significance. The lack of an ion channel function for aquaporin-1 is suggested also by the low sequence similarity with other cyclic nucleotide channels (Fotiadis et al., 2002; Tsunoda et al., 2004).

The hypothesized roles of aquaporin-1 ion channels in (i) transmembrane signaling (Boassa and Yool, 2002) and (ii) kidney proximal-tubule function (Yool and Weinstein, 2002) are supported by ion channel recordings in *Xenopus* oocytes. However, the experiments were likely to be biased by regulatory processes of the expression system, which may have been misinterpreted as aquaporin-1 ion channel activity. Neither open probability nor channel conductivity found in *Xenopus* oocytes (Anthony et al., 2000) are reproducible in HEK293 cells (Tsunoda et al., 2004). Despite expression levels comparable to aquaporin-rich tissues, cyclic nucleotides were without any effect on whole-cell membrane conductivity (Figure 2). Potential single-channel openings should remain unnoticed in these patch clamp experiments because of the high background conductivity of HEK293 cells. In planar bilayers background conductivity is two orders of magnitude lower (compare panels A and B in Figure 2), providing them with the exquisite sensitivity required to detect the opening of one channel out of a million (Saparov et al., 2001).

The lack of cyclic nucleotide-gated ion channel activity in HEK293 rules out a secondary role of aquaporins in transmembrane signaling. Regulation of water channel function by cyclic nucleotides is restricted to an increase in aquaporin-2 abundance in the plasma membrane by exocytosis (Lorenz et al., 2003). The fast augmentation of aquaporin-2 plasma membrane density is required for the acute regulation of body water balance. The signaling cascade starts with the binding of the antidiuretic hormone arginine vasopressin to the V_2-receptor of principal cells. Stimulation of the latter leads to a rise in cyclic adenosine monophosphate (cAMP) and cytoplasmic Ca^{2+}. The subsequent phosphorylation of aquaporin-2 initiates a long-range aquaporin-2 redistribution from vesicles distributed throughout the collecting duct cell to the apical plasma membrane. The final step is the exocytic insertion of water channels into the plasma membrane resulting in an increase in the osmotic water permeability. This process does not alter the electrical plasma membrane conductivity, indicating that aquaporin-2 excludes ions, too (Lorenz et al., 2003).

Molecular dynamics simulations of water and proton transport through aquaporins (Jensen et al., 2003; de Groot et al., 2003) provide the explanation for the uniquely selective mechanism for free permeation by water and the exclusion of ions. The main barriers to ion and proton permeation are: (i) the electrostatic field centered at the conserved Asn-Pro-Ala (NPA) motif (characteristic of all aquaporins) and (ii) the positive charge (Arg-197) located at the narrowest constriction of the aqueous pathway through aquaporin-1, approximately 8 Å above the middle of the bilayer (Figure 3A). The electrostatic barrier inside the channel is large enough (de Groot et al., 2003) to inhibit not only the movement of proton vehicles

(H_3O^+), but to prevent structural proton diffusion according to the Grotthuss mechanism (Agmon, 1995) as well.

Surprisingly, permeation through aquaporin-6 seems to be different. This is the only member of the water channel family whose ion channel function is completely accepted (Yasui et al., 1999). Similarly to aquaporin-2, it is found in the kidney collecting duct. In contrast to aquaporin-2, aquaporin-6 resides not in principal cells but in intercalated cells and is stacked to its intracellular location, i.e. the vesicles bearing aquaporin-6 do no shuttle to the plasma membrane. Aquaporin-6 allows cation and anion permeation with marked specificity for nitrate, an observations that implies that the primary role of aquaporin-6 may be in cellular regulation rather than fluid transport (Ikeda et al., 2002).

Water and ion cotransport

Transport through aquaporins is a single-file process, i.e. water molecules within the pore cannot pass or overtake each other (Figure 3A). This would certainly occur also in ion channels if their pore radius is less than twice that of a water molecule, i.e. water molecules and ions are transported in a single file (Figure 3B). In this case, the coupling of ion and water fluxes can be determined from an electrokinetic measurement, which can come either from an electroosmotic experiment or from a streaming potential experiment (Finkelstein, 1987). Electrokinetic measurements have revealed that translocation of one ion through a membrane channel obeying single-file kinetics is accompanied by the movement of at least one water molecule. For example, about two water molecules are translocated together with a single Na^+ ion through epithelial sodium channels (Ismailov et al., 1997) or with a single K^+ ion through potassium channels (Miller, 1982; Alcayaga et al., 1989). The number of water molecules increases to five for gramicidin channels (Finkelstein, 1987).

Vice versa, the number of ions accompanying one water molecule cannot be derived from streaming potential measurements or electroosmotic experiments. For example, it is well known that gramicidin channels most of the time do not contain an ion (Finkelstein, 1987), i.e. water molecules may pass the channel without being accompanied by an ion. Such a situation is evident from solvent drag experiments (Pohl and Saparov, 2000), another type of electrokinetic analysis. The only difference to streaming potential measurements is that solute flow (and thus osmotic solvent flow) is allowed (the current is different from zero), while the transmembrane potential is clamped to zero mV. In this case, the osmotic water flux, J_w, is derived from the velocity of volume flow, v_c, observed through the membrane channels:

$$J_w = \frac{v_c}{V_w} \tag{4}$$

By using scanning microelectrodes (Figure 1) v_c is determined as the difference in v (according to eq. 1) of the membrane with reconstituted channels and of the bare lipid bilayer. During solvent drag a solute is pushed through pores by the solvent, i.e. the solute flux is increased in the direction of the osmotic water flow and is retarded in the opposite direction. The incremental solute flux, J_{ion}, can easily be monitored by measurement of membrane current, I:

$$J_{ion} = \frac{I}{zF} \tag{5}$$

If determined in the absence of a transmembrane gradient of the permeant ion, J_{ion} enables calculation of the number of water molecules (N) that are moving in a single-file fashion along with one ion (Pohl and Saparov, 2000):

$$N = \frac{J_{ion} z^2 F^2}{RTGc_{osm}V_w} \tag{6}$$

where G denotes the electrical membrane conductivity. As illustrated by experiments with gramicidin, N must not to be mixed up with the water/ion flux ratio. The latter may be as large as ca. 10^6 for gramicidin, whereas N is limited to 5 (Pohl and Saparov, 2000). The large flux ratio indicates that the channel operates most of the time as a water channel and that it is only occasionally entered by an ion, but if entered, the narrow channel accommodates $N=5$ water molecules at the same time. Accordingly, the single channel water permeability of gramicidin is comparable to the one of water channels, suggesting that ion channels in general may significantly contribute to epithelial water transport.

Such a possibility has been questioned by recent experiments, in which the water permeability of epithelial potassium channels (ROMK1) was tested in the oocyte expression system. Even though potassium channels are water-filled pores, the K^+-selective ion-transporting pathway was found to be virtually water-impermeable (Sabirov et al., 1998). Contrasting evidence has been obtained with KcsA, the *Streptomyces lividans* potassium channel (Figure 3B). Upon reconstitution into planar bilayers, KcsA was found to transport water faster than any other channel obeying single-file kinetics (Saparov and Pohl, 2004). KcsA water permeability exceeds that of of aquaporins by at least one order of magnitude (Figure 4), suggesting that water flux through ion channels may be important at places where their membrane density reaches 10% of the density typically found for aquaporins. Such a situation is encountered in the nodes of Ranvier (Hille, 2001). Because neurons lack channels specialized in water transport, it is intriguing to ask whether potassium channels may contribute to neuronal water homeostasis. In this case, a shift of the resting potential due to osmotic fluid flow is not expected because a substantial water flow occurs only through channels that do not contain ions. Due to the much slower velocity of ion movement, the faster water flux is inhibited as soon as an ion enters the channel (Saparov and Pohl, 2004). The difference in the ion occupancy of ROMK and KcsA in the oocyte (Sabirov et al., 1998) and planar bilayer systems (Saparov and Pohl, 2004), respectively, may be the reason for the measured differences in water permeabilities.

A variety of other channels has been found to conduct substantial amounts of water (Figure 4), including the

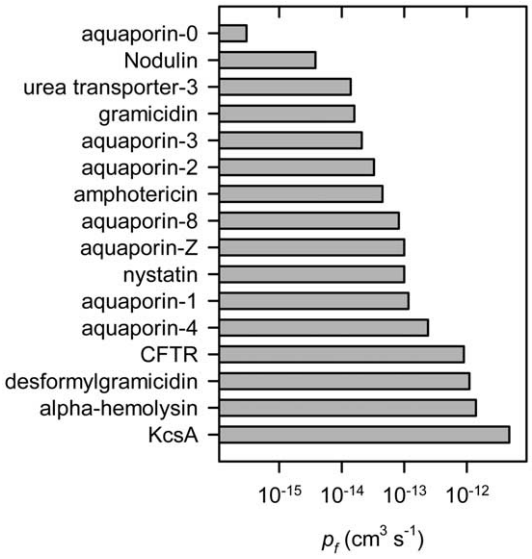

Figure 4 Single-channel water permeabilities, p_f, of aquaporins and ion channels.
Usually the aquaglyceroproteins (members of the water channel family that are permeable to water and small osmolytes) have a lower water permeability than orthodox aquaporins (water-selective members of the protein family). Aquaporin-0 (Chandy et al., 1997), nodulin (Dean et al., 1999), and aquaporin-3 (Yang and Verkman, 1997) belong to the aquaglyceroproteins, whereas aquaporin-1 (Zeidel et al., 1992), aquaporin-2 (Yang and Verkman, 1997), aquaporin-4 (Yang and Verkman, 1997), and aquaporin-Z (Borgnia et al., 1999) function as orthodox aquaporins. Aquaporin-8 is unique among other aquaporins in primary structure and tissue distribution. It is impermeable to glycerol, but mouse aquaporin-8 transports urea as well as water (Ma et al., 1997). *Vice versa*, urea transporters are found to be permeable also for water (Yang and Verkman, 1998). Nystatin and amphotericin are bacterial channels that allow both cation and anion movement (Finkelstein, 1987). The bacterial channels gramicidin (Pohl and Saparov, 2000), its desformylated derivative (Saparov et al., 2000) and KcsA (Saparov and Pohl, 2004) are cation-selective. The bacterial α-hemolysin (Paula et al., 1999) and the human cystic fibrosis transmembrane conductance regulator (CFTR Hasegawa et al., 1992) are anion-selective channels.

cystic fibrosis transmembrane conductance regulator (Hasegawa et al., 1992) and the urea transporter (Yang and Verkman, 1998). So far two cation and two anion channels are known which exceed the aquaporins in water transport efficiency (Figure 4). Recently, a contribution of the volume-regulated anion channel to the exchange of water has been proposed (Nilius, 2004). Closure of the volume-regulated anion channel reduced the permeability of endothelial cells for water, suggesting that the volume-regulated anion channel itself can be permeated by water. Provided that the outward flux of osmolytes through the volume-regulated anion channel causes a local osmotic gradient, the resulting water flux could efficiently contribute to the protection of the osmotic thermodynamic equilibrium across the plasma membrane.

Although ion channels have been shown to represent a potentially efficient water pathway, the physiological importance of water fluxes through ion channels remains to be elucidated. The only mammalian aquaporin known to function as an ion channel is not an exception as the water transporting function of aquaporin-6 has not yet been demonstrated.

Acknowledgments

I am grateful to Sandro Keller, Berlin, Germany, for critically reading the manuscript. The author is a Heisenberg fellow of the Deutsche Forschungsgemeinschaft (Po 533/7-1). The work was also supported by the Deutsche Forschungsgemeinschaft (Po 533/8-1).

References

Agmon, N. (1995). The Grotthuss Mechanism. Chem. Phys. Lett. *244*, 456–462.

Agre, P., Lee, M.D., Devidas, S., Guggino, W.B., Sasaki, S., Uchida, S., Kuwahara, M., Fushimi, K., Marumo, F., Verkman, A.S. et al. (1997). Aquaporins and ion conductance. Science *275*, 1490–1492.

Alcayaga, C., Cecchi, X., Alvarez, O., and Latorre, R. (1989). Streaming potential measurements in Ca^{2+}-activated K^+ channels from skeletal and smooth muscle. Coupling of ion and water fluxes. Biophys. J. *55*, 367–371.

Anthony, T.L., Brooks, H.L., Boassa, D., Leonov, S., Yanochko, G.M., Regan, J.W., and Yool, A.J. (2000). Cloned human aquaporin-1 is a cyclic GMP-gated ion channel. Mol. Pharmacol. *57*, 576–588.

Boassa, D. and Yool, A.J. (2002). A fascinating tail: cGMP activation of aquaporin-1 ion channels. Trends Pharmacol. Sci. *23*, 558–562.

Borgnia, M.J., Kozono, D., Calamita, G., Maloney, P.C., and Agre, P. (1999). Functional reconstitution and characterization of AqpZ, the *E. coli* water channel protein. J. Mol. Biol. *291*, 1169–1179.

Chakrabarti, N., Tajkhorshid, E., Roux, B., and Pomes, R. (2004). Molecular basis of proton blockage in aquaporins. Structure *12*, 65–74.

Chandy, G., Zampighi, G.A., Kreman, M., and Hall, J.E. (1997). Comparison of the water transporting properties of MIP and AQP1. J. Membr. Biol. *159*, 29–39.

de Groot, B.L., Frigato, T., Helms, V., and Grubmüller, H. (2003). The mechanism of proton exclusion in the aquaporin-1 water channel. J. Mol. Biol. *333*, 279–293.

Dean, R.M., Rivers, R.L., Zeidel, M.L., and Roberts, D.M. (1999). Purification and functional reconstitution of soybean nodulin 26. An aquaporin with water and glycerol transport properties. Biochemistry *38*, 347–353.

Finkelstein, A. 1987. Water movement through lipid bilayers, pores, and plasma membranes (New York, USA: J. Wiley & Sons).

Firsov, D., Schild, L., Gautschi, I., Merillat, A.M., Schneeberger, E., and Rossier, B.C. (1996). Cell surface expression of the epithelial Na channel and a mutant causing Liddle syndrome: a quantitative approach. Proc. Natl. Acad. Sci. USA *93*, 15370–15375.

Fotiadis, D., Suda, K., Tittmann, P., Jenö, P., Philippsen, A., Müller, D.J., Gross, H., and Engel, A. (2002). Identification and structure of a putative Ca^{2+}-binding domain at the C-terminus of AQP1. J. Mol. Biol. *318*, 1381–1394.

Fujiyoshi, Y., Mitsuoka, K., de Groot, B.L., Philippsen, A., Grubmüller, H., Agre, P., and Engel, A. (2002). Structure and function of water channels. Curr. Opin. Struct. Biol. *12*, 509–515.

Hasegawa, H., Skach, W., Baker, O., Calayag, M.C., Lingappa, V., and Verkman, A.S. (1992). A multifunctional aqueous channel formed by CFTR. Science *258*, 1477–1479.

Hill, W.G. and Zeidel, M.L. (2000). Reconstituting the barrier properties of a water-tight epithelial membrane by design of leaflet-specific liposomes. J. Biol. Chem. *275*, 30176–30185.

Hille, B. 2001. Ion Channels of Excitable Membranes (Sunderland, MA, USA: Sinauer Associates, Inc.).

Ikeda, M., Beitz, E., Kozono, D., Guggino, W.B., Agre, P., and Yasui, M. (2002). Characterization of aquaporin-6 as a nitrate channel in mammalian cells: requirement of pore-lining residue threonine-63. J. Biol. Chem. *277*, 39873–39879.

Ismailov, I.I., Shlyonsky, V.G., and Benos, D.J. (1997). Streaming potential measurements in abg-rat epithelial Na^+-channel in planar lipid bilayers. Proc. Natl. Acad. Sci. USA *94*, 7651–7654.

Jensen, M.O., Tajkhorshid, E., and Schulten, K. (2003). Electrostatic tuning of permeation and selectivity in aquaporin water channels. Biophys. J. *85*, 2884–2899.

Krylov, A.V., Pohl, P., Zeidel, M.L., and Hill, W.G. (2001). Water permeability of asymmetric planar lipid bilayers: leaflets of different composition offer independent and additive resistances to permeation. J. Gen. Physiol. *118*, 333–340.

Lorenz, D., Krylov, A., Hahm, D., Hagen, V., Rosenthal, W., Pohl, P., and Maric, K. (2003). Cyclic AMP is sufficient for triggering the exocytic recruitment of aquaporin-2 in renal epithelial cells. EMBO Rep. *4*, 88–93.

Ma, T., Yang, B., and Verkman, A.S. (1997). Cloning of a novel water and urea-permeable aquaporin from mouse expressed strongly in colon, placenta, liver, and heart. Biochem. Biophys. Res. Commun. *240*, 324–328.

Matthay, M.A., Folkesson, H.G., and Clerici, C. (2002). Lung epithelial fluid transport and the resolution of pulmonary edema. Physiol. Rev. *82*, 569–600.

Miller, C. (1982). Coupling of water and ion fluxes in a K^+-selective channel of sarcoplasmic reticulum. Biophys. J. *38*, 227–230.

Nielsen, S., Smith, B.L., Christensen, E.I., Knepper, M.A., and Agre, P. (1993). CHIP28 water channels are localized in constitutively water-permeable segments of the nephron. J. Cell Biol. *120*, 371–383.

Nielsen, S., Frokiaer, J., Marples, D., Kwon, T.H., Agre, P., and Knepper, M.A. (2002). Aquaporins in the kidney: from molecules to medicine. Physiol. Rev. *82*, 205–244.

Nilius, B. (2004). Is the volume-regulated anion channel VRAC a 'water-permeable' channel? Neurochem. Res. *29*, 3–8.

Paula, S., Akeson, M., and Deamer, D. (1999). Water transport by the bacterial channel α-hemolysin. Biochim. Biophys. Acta *1418*, 117–126.

Pohl, P. and Saparov, S.M. (2000). Solvent drag across gramicidin channels demonstrated by microelectrodes. Biophys. J. *78*, 2426–2434.

Pohl, P., Saparov, S.M., and Antonenko, Y.N. (1997). The effect of a transmembrane osmotic flux on the ion concentration distribution in the immediate membrane vicinity measured by microelectrodes. Biophys. J. *72*, 1711–1718.

Pohl, P., Saparov, S.M., Borgnia, M.J., and Agre, P. (2001). High selectivity of water channel activity measured by voltage clamp: analysis of planar lipid bilayers reconstituted with purified AqpZ. Proc. Natl. Acad. Sci. USA *98*, 9624–9629.

Sabirov, R.Z., Morishima, S., and Okada, Y. (1998). Probing the water permeability of ROMK1 and amphotericin B channels using *Xenopus* oocytes. Biochim. Biophys. Acta *1368*, 19–26.

Saparov, S.M. and Pohl, P. (2004). Beyond the diffusion limit: water flow through the empty bacterial potassium channel. Proc. Natl. Acad. Sci. USA *101*, 4805–4809.

Saparov, S.M., Antonenko, Y.N., Koeppe, R.E., and Pohl, P. (2000). Desformylgramicidin: a model channel with an extremely high water permeability. Biophys. J. *79*, 2526–2534.

Saparov, S.M., Kozono, D., Rothe, U., Agre, P., and Pohl, P. (2001). Water and ion permeation of aquaporin-1 in planar lipid bilayers: major differences in structural determinants and stoichiometry. J. Biol. Chem. *276*, 31515–31520.

Schafer, J.A. (2002). Abnormal regulation of ENaC: syndromes of salt retention and salt wasting by the collecting duct. Am. J. Physiol. Renal Physiol. *283*, F221–F235.

Spring, K.R. (1999). Epithelial fluid transport-a century of investigation. News Physiol. Sci. *14*, 92–98.

Sui, H., Han, B.G., Lee, J.K., Walian, P., and Jap, B.K. (2001). Structural basis of water-specific transport through the AQP1 water channel. Nature *414*, 872–878.

Tsunoda, S.P., Wiesner, B., Lorenz, D., Rosenthal, W., and Pohl, P. (2004). Aquaporin-1, nothing but a water channel. J. Biol. Chem. *279*, 11364–11367.

Yang, B. and Verkman, A.S. (1997). Water and glycerol permeabilities of aquaporins 1-5 and MIP determined quantitatively by expression of epitope-tagged constructs in *Xenopus* oocytes. J. Biol. Chem. *272*, 16140–16146.

Yang, B. and Verkman, A.S. (1998). Urea transporter UT3 functions as an efficient water channel. Direct evidence for a common water/urea pathway. J. Biol. Chem. *273*, 9369–9372.

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

Yool, A.J. and Weinstein, A.M. (2002). New roles for old holes: ion channel function in aquaporin-1. News Physiol. Sci. *17*, 68–72.

Yool, A.J., Stamer, W.D., and Regan, J.W. (1996). Forskolin stimulation of water and cation permeability in aquaporin-1 water channels. Science *273*, 1216–1218.

Zeidel, M.L., Ambudkar, S.V., Smith, B.L., and Agre, P. (1992). Reconstituion of functional water channels in liposomes containing purified red cell chip28 protein. Biochemistry *31*, 7436–7440.

Zhou, Y. and MacKinnon, R. (2003). The occupancy of ions in the K^+ selectivity filter: charge balance and coupling of ion binding to a protein conformational change underlie high conduction rates. J. Mol. Biol. *333*, 965–975.