Pflugers Arch - Eur J Physiol (2011) 462:623–630
DOI 10.1007/s00424-011-1002-x

TRANSPORT PHYSIOLOGY

Aquaporins as gas channels

Marcela Herrera · Jeffrey L. Garvin

Received: 5 July 2011 / Revised: 18 July 2011 / Accepted: 20 July 2011 / Published online: 2 August 2011

Abstract Gas molecules play important roles in human physiology. Volatile substances produced by one cell often regulate neighboring cells in a paracrine fashion. While gaseous molecules have traditionally been thought to travel from cell to cell by free diffusion through the bilayer portion of the membrane, this does not explain their rapid physiological actions. The recent observations that: (1) water channels can transport other molecules besides water, and (2) aquaporins are often expressed in tissues where gas (but not water) transport is essential suggest that these channels conduct physiologically important gases in addition to water. This review summarizes recent findings on the role of aquaporins as gas transporters as well as their physiological significance.

Keywords Nitric oxide · Transport · Membrane · Gas exchange · Aquaporins

Aquaporins are a family of integral membrane proteins found in many organisms from bacteria to mammals. Thirteen mammalian aquaporins (0–12) have been identified to date, and although they all share structural similarities, their expression is tissue specific [1, 26, 39, 62]. The first one to be described was aquaporin-1 (AQP1), discovered by chance in 1991 by Preston and Agre who were trying to identify Rh blood group polypeptides found in the erythrocyte plasma membrane [12, 45, 46]. Human AQP1 is a 269-amino acid chain (28 kDa) containing six bilayer-spanning domains and five connecting loops, of which three (A, C, and E) are located outside the cell and two (B and D) within the cytoplasm. Two identical asparagine–proline–alanine (NPA) motifs at residues 76–78 (in cytoplasmic loop B) and 192–194 (in extracellular loop E) are connected to each other within the membrane forming a single narrow aqueous pathway ("aquapore") of 2.8 Å in diameter at the narrowest point (the constriction site) as calculated by electron crystallography [9, 27, 36, 47, 52] (Fig. 1 a–b). The size of the pore varies among AQPs and determines whether the channel passes larger molecules in addition to water. Unlike AQP1, the narrowest pore in AQP3 is reportedly 3.4 Å and this AQP also transports glycerol, which is a larger molecule compared to water [20, 25]. Based on their selectivity, the AQP family has been divided into "aquaporins" (AQP1, 2, 4, and 5), which pass water through the single pore, and "aquaglyceroporins" (AQP3, 6, 7, 9, and 10), which also transport glycerol. In addition to constriction size, AQP selectivity is further determined by the nature of the amino acids lining the pore. All AQPs have a positively charged arginine residue positioned near the constriction site (R195) which serve to exclude protons, as replacing it with valine results in proton leakage [2, 8]. However, the amino acids that line the pores of glycerol-transporting AQPs are more hydrophobic [53]. Only AQP8 has been shown to pass H_2O_2 [3], a molecule very similar to water in terms of both size and dielectric properties; however, the molecular

M. Herrera · J. L. Garvin
Hypertension and Vascular Research Division,
Department of Internal Medicine, Henry Ford Hospital,
Detroit, MI, USA

J. L. Garvin
Department of Physiology, Wayne State University,
Detroit, MI, USA

M. Herrera (✉)
Division of Nephrology, Department of Medicine,
Duke University,
2 Genome Court, MSRB2 2018, Box 103015, Durham,
NC 27710, USA
e-mail: marcela.herrera@duke.edu

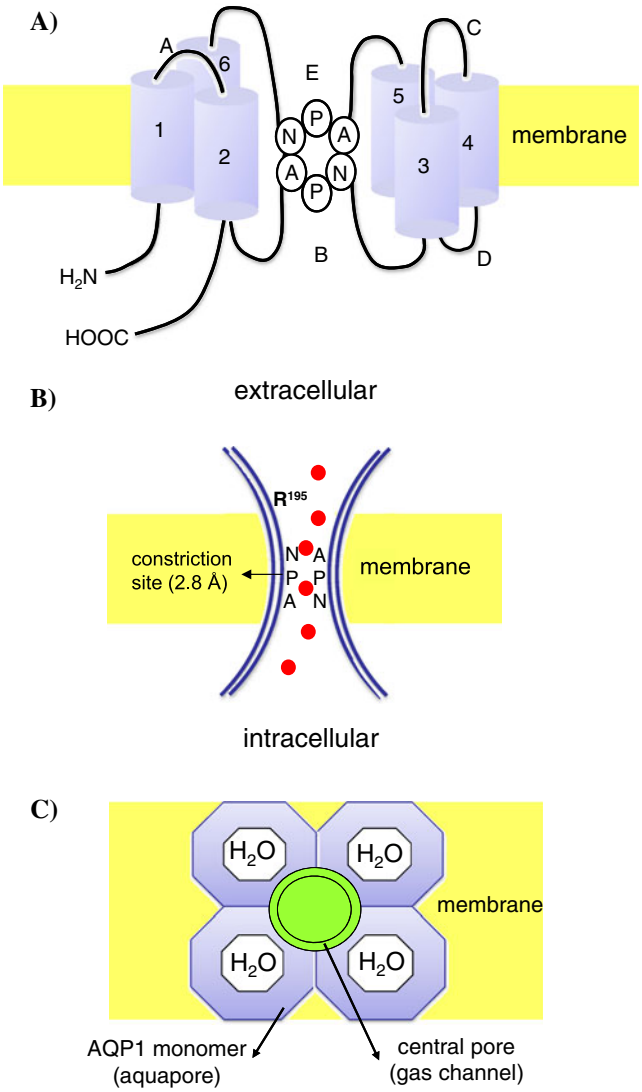

Fig. 1 **a** Topology of the aquaporin molecule. Each AQP1 monomer consist of six transmembrane domains (*1–6*) connected by five loops (*A–E*). The channel is formed by connecting loops B and E with two NPA boxes shaping the water pore, and the amino and carboxy termini oriented toward the cytoplasm. **b** The channel consists of an extracellular and intracellular vestibule joined in the center where water molecules (*red dots*) pass in single file. The arginine in position 195 (*R195*) provides fixed positive charges which prevent proton passage. The constriction site is bounded by two asparagine residues from the highly conserved NPA motif. **c** Schematic representation of the AQP1 tetramer (*top view*). Each AQP1 monomer transports water. AQP1 is thought to conduct CO_2 and NO via the central pore

determinants of H_2O_2 selectivity in AQP8 as well as its physiological significance in mammals have not been established to our knowledge.

In the plasma membrane, AQP1 exists as a homotetramer with each subunit containing an individually functional water pore [52, 58]. At the center of the four monomers lies a fifth pore composed mainly of hydrophobic amino acids which might provide a path for non-polar molecules [59] (Fig. 1c). Up to now, scientists have presumed that gas molecules enter and exit the cell by freely diffusing through the plasma membrane, however, we have seen no direct measurements that would support this assumption and in fact this dogma has recently been challenged by several observations. First, some cell membranes are impermeable to gases (NH_3 and CO_2), among them the apical membranes of colonic crypts, gastric glands, and renal thick ascending limbs [7, 19, 29, 57]. Second, the thermodynamics of diffusion through the bilayer would suggest that the hydrophobic nature of many gases makes penetration of the polar phospholipid head groups unfavorable due to the large amount of energy that would be required during such process and, by the same token, hydrophobic gases would be trapped in the lipid phase of the cell membrane and unable to reenter the aqueous phase of the cytoplasm or intercellular space; thus, free diffusion seems like a relatively inefficient means of crossing the cell membrane. Third, gaseous molecules [such as nitric oxide (NO)] produced in one cell often quickly exert paracrine actions on neighboring cells [16]. We believe facilitated diffusion of gaseous molecules offers a more realistic explanation, since free diffusion is most likely a slower process. Many gases have high partition coefficients, ranging from 2 for CO_2 [50] to 5 for NO [34]. These coefficients are measured at equilibrium, and it could take hours or even days before equilibrium between phases is reached; thus, partition coefficients do not provide information about rates of gaseous molecules traversing the lipid bilayer. Accordingly, during free diffusion, any gas would have to partition between the extra- and the intramembrane space before it could be extruded into the cytoplasm (a process driven by the concentration gradient), making the movement of such molecules a rather slower process. In addition to the potential toxic effects of gases when trapped in the lipid phase of the membrane, transport of gases across cell membranes at a certain time would depend exclusively on the temporal concentration of each gas within the lipid phase. Therefore, facilitated transport would seem to be a more likely means for gaseous molecules to traverse lipid bilayers.

CO_2

AQP1 transports CO_2

AQP1 is often highly expressed by cells necessary for transport of gases (such as CO_2, O_2, and NO) but not water, like the pulmonary capillaries, epithelial, vascular smooth muscle, and red blood cells [13, 45, 49, 56], supporting the hypothesis that AQP1 could function as a gas channel. The first experimental evidence suggesting that AQP1 might be

a gas channel was published by the Boron's group in 1998. These investigators used *Xenopus* oocytes and intracellular electrodes to measure the rate of acidification caused by CO_2 influx across the cell membrane [38]. They found that injecting oocytes with AQP1 mRNA tripled the rate of CO_2 influx which in turn correlated with that of water. They also showed that mercurial agents known to block water transport by AQP1 also blocked CO_2, whereas substituting Ser for Cys189 (the amino acid that confers mercurial inhibition of the channel) failed to enhance CO_2 transport [11]. At the same time, Zeidel's group tested whether differences in lipid composition or membrane fluidity affects CO_2 permeability and found that whereas artificial liposomes with varying degrees of membrane fluidity, all exhibited similar CO_2 transport; in proteoliposomes containing purified AQP1, transport increased fourfold [44]. Like Boron's group, they found that mercuric chloride blocked AQP1-dependent CO_2 transport. Since mercurial agents inhibit AQP1 by binding to Cys189, which is located in the water pore itself, these data suggest that either (1) CO_2 also uses the single water channel to traverse the channel or (2) blocking the aquapores causes conformational changes in the central pore, making it less permeable to CO_2.

Studies using mammalian cells have indicated that AQP1-dependent CO_2 transport also occurs in native tissues. Using human red blood cells, Forster et al. reported that CO_2 transport across the erythrocyte membrane is blocked by 4, 4′-diisothiocyanato-stilbene-2,2′-disulfonate (DIDS, a known inhibitor of ion exchangers), most likely by directly suppressing a membrane protein that facilitates diffusion of CO_2 [14]. While we do not know how DIDS inhibits AQP1-dependent CO_2 transport, Boron found that DIDS blocks permeability of CO_2 but not water [5], suggesting that either (1) DIDS directly inhibits the central pore of AQP1, leaving the aquapores open to water, or (2) DIDS inhibits a CO_2 transporter other than AQP1.

The first report suggesting that AQP1-dependent CO_2 transport is significant physiologically came from Uehlein et al. [54] who found that AQP1 derived from tobacco plants (NtAQP1), which shares high sequence and structural homology to human AQP1, plays an important role in photosynthesis and leaf growth [54]. Their experiments showed that NtAQP1 increased transmembrane CO_2 transport by 45% when expressed in *Xenopus* oocytes, indicating that just like human AQP1, NtAQP1 also functions as a CO_2 channel. In addition, altering NtAQP1 expression using either antisense technology or tetracyclin-inducible plant lines correlated with rates of $^{14}CO_2$ incorporation into photosynthetic products of leaf discs and in turn, transport correlated directly with photosynthesis and plant growth. Thus, AQP1 appears to expedite both CO_2 influx and availability, being of great importance given that the atmosphere/plant CO_2 gradient is relatively low compared to lungs/atmosphere. More recently, Otto et al. investigated the ability of monomeric vs. tetrameric arrangement of NtAQP1 to transport CO_2 and found that when expressed in yeast, AQP1 tetramers exhibited higher rates of CO_2 transport than monomers [41], consistent with the concept that CO_2 traverses NtAQP1 via the central pore of the aquaporin tetramer. Whether additional gas paths are created in between tetramers or not remain to be answered.

Boron's group also investigated the physiological significance of CO_2 transport by AQP1 in mammals. AQP1 is abundantly expressed in the proximal tubules of the kidney, where it plays an important role in fluid and HCO_3^- reabsorption, taking up 80% of the filtered HCO_3^- [51]. The transport of CO_2 across the apical membrane of the proximal tubule is the first step in the reabsorption of HCO_3^-. Using isolated perfused proximal tubules, these investigators showed that HCO_3^- reabsorption is inhibited by about 50% when inhibitors of AQP1 are added to the preparation. In addition, proximal tubules isolated from AQP1 −/− mice exhibited 50% less HCO_3^- reabsorption [65]. Thus, in the proximal tubule, AQP1 may play an important role in acid/base balance by facilitating CO_2 influx and therefore HCO_3^- reabsorption. More recently, Xu et al. studied the response to metabolic acidosis in mice lacking AQP1 and found that ammonium chloride feeding, an established model of metabolic acidosis, lowered arterial pH and HCO_3^- even more [61], suggesting that AQP1 plays an important role in regulating arterial pH during metabolic acidosis, possibly by acting as a CO_2 transporter in the proximal tubule (Fig. 2).

In contrast to the above reports, some investigators showed that AQP1-dependent CO_2 transport has no physiological relevance [55, 63]. Several technical limitations could explain the negative findings, among them (1) the existence of as yet unidentified CO_2 channels could mask the contribution of AQP1 to CO_2 permeability across different cell membranes, including AQP1 −/− mice, (2) the existence of unstirred layers or perfusion-limited conditions in different experimental settings could mask the contribution of AQP1 to CO_2 permeability, and (3) the lack of methodological tools allowing direct measurement of CO_2 traversing cell membranes could confound interpretation of the data [6, 33].

CO_2 transport by other proteins

In addition to AQP1, other membrane proteins have been shown to function as gas channels. Recently Musa-Aziz et al. [37] monitored CO_2 transport across the oocyte membrane using pH electrodes placed near the outer surface, thereby detecting transient changes in surface pH

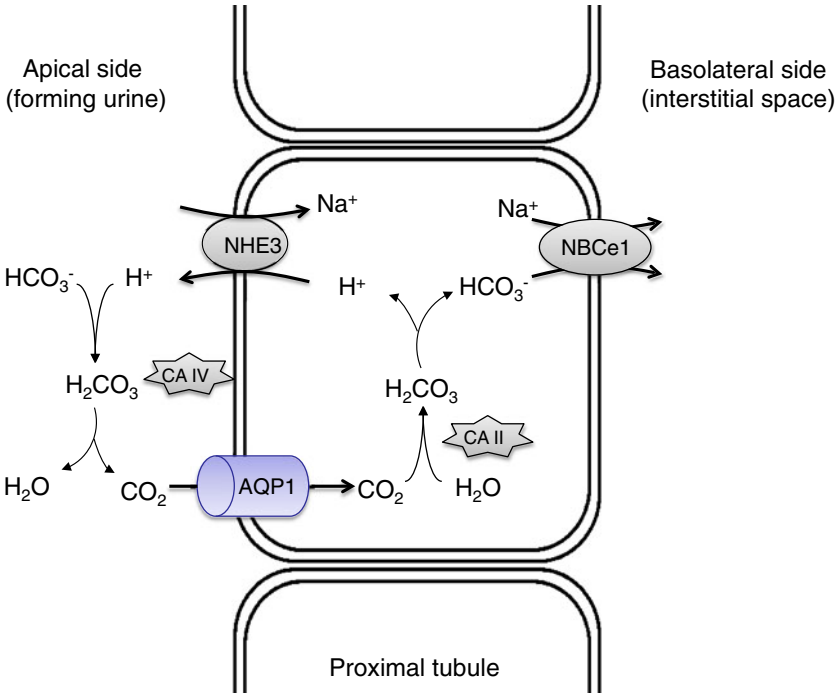

Fig. 2 Physiological role of aquaporin 1 (*AQP1*) in the renal proximal tubule. In the forming urine, HCO_3^- is converted to CO_2 by luminal carbonic anhydrase IV (*CA IV*). AQP1 transports CO_2 into the epithelial cells, where it is converted back to HCO_3^- by carbonic anhydrase II (*CA II*). In turn, HCO_3^- is extruded across the basolateral membrane into the interstitium via the electrogenic Na^+/HCO_3^- cotransporter (*NBCe1*). In the absence of AQP1, free diffusion of CO_2 is not sufficient to enhance HCO_3^- reabsorption and restore acid–base balance during metabolic acidosis. *NHE3* Na^+–H^+ exchanger type 3

due to the disruption of the HCO_3^-/CO_2 equilibrium upon the addition of CO_2 to the bulk solution which can be used to estimate rates of CO_2 influx. When they injected oocytes with cRNA for different membrane proteins including the Na^+–glucose cotransporter SGLT1, the Na^+–K^+–2Cl cotransporter $NKCC_2$, and the H^+–oligopeptide cotransporter PepT1 to see if this would stimulate CO_2 flux, they found that none of them had any effect on CO_2 permeability compared to water-injected oocytes. However, when they injected their oocytes with cRNA for AQP1, 4, and 5 they measured higher rates of CO_2 transport. By normalizing the rates of CO_2 transport by AQP expression (water permeability), they found that AQP5 exhibited the highest permeability to CO_2, followed by AQP1 and AQP4 [37]; thus, other aquaporins can also transport CO_2 although affinities vary. While we still do not know why different aquaporins have different permeabilities to CO_2, based on molecular dynamic simulations, one can predict that central pore size and chemical interactions are important determinants of gas permeability through aquaporins [59].

NO

AQP1 transports NO

Like CO_2, NO is a gas that plays important roles in human physiology. It was originally called "endothelium-derived relaxing factor", since the first studies by Furchgott and Zawadski in 1980 and later on Palmer and Moncada in 1987 showed that NO produced by the endothelial cells of the vasculature relaxes adjacent vascular smooth muscle cells [15, 16, 35, 43]. In this process, NO must traverse two lipid bilayers to exit the endothelial cells where it is produced and enter the vascular smooth muscle cells where it controls contractility.

AQP1 is abundantly expressed in endothelial and vascular smooth muscle cells [17, 18, 22, 32, 40, 49], where transport of NO across the membrane—unlike water —is extremely important for vascular physiology. For this reason, we wanted to know whether AQP1 also transports NO across cell membranes. Using fluorescence microscopy to monitor intracellular NO in real time and Chinese hamster ovary cells transfected to express AQP1, we found that AQP1 increased NO influx across the cell membrane. When we used reconstituted liposomes containing AQP1, NO influx across the lipid bilayer occurred significantly faster when purified human AQP1 was inserted into the membrane, suggesting that the accelerated flux was due to AQP1 and not some other protein. Lipid bilayers containing no protein still showed NO flux but at a significantly slower rate (75% lower compared to AQP1-containing liposomes) [23]. These data showed that: (a) AQP1 facilitates diffusion of NO across cell membranes and (b) the lipid bilayer is a significant barrier to NO diffusion. We concluded that free diffusion still occurs in the absence of AQP1, albeit more slowly.

In additional experiments, we studied the rate of NO influx in response to varying NO gradients and found that it saturated at 3 μM NO, while the half-maximum transport rate ($K_{1/2}$) occurred at a concentration of 0.54 μM,

indicating that NO transport is saturable and further supporting the conclusion that NO is transported by AQP1 across cell membranes rather than diffusing freely which would result in a linear relationship between NO concentration and rate of influx [23].

Finally, we examined whether transport of NO by AQP1 has any physiological significance. NO exits the endothelial cells of the vasculature where it is produced, entering vascular smooth muscle cells and causing relaxation. Since AQP1 is expressed in both types of cells [17, 18, 22, 32, 40, 49], we investigated its role in endothelium-dependent relaxation. Measurements of NO efflux across the plasma membrane showed that endothelial cells from AQP1 −/− mice exhibited significantly reduced NO efflux while vascular smooth muscle cells showed significantly less NO influx, confirming that AQP1 transports NO out of endothelial cells and into vascular smooth muscle cells. In addition, when we treated intact vessels with acetylcholine to stimulate the production of NO by endothelial cells and measured smooth muscle relaxation, we found that NO-induced relaxation at any given concentration of acetylcholine was decreased in vessels isolated from AQP1 −/− mice, indicating that endothelium-dependent relaxation was impaired in the absence of AQP1 [22]. These studies confirmed that endothelium-dependent relaxation requires AQP1-dependent transport of NO across cell membranes (Fig. 3). These findings raised the question of whether AQP1 −/− mice are hypertensive. Measurements done in our laboratory indicated that they are not hypertensive; if anything, they have lower blood pressure than controls (unpublished data). This discrepancy is likely explained by the fact that these mice cannot concentrate urine and as a result they are polyuric [10, 31, 42]; thus, the hypotension caused by the renal defect is likely masking the hypertensive phenotype dictated by defective NO-dependent relaxation in the systemic vasculature. In addition, unpublished data from our laboratory suggest that other humoral factors compensate for impaired NO-induced dilatation in AQP1 −/− mice, and we are currently exploring these pathways. Unfortunately, the lack of specific inhibitors for AQP1 has limited our ability to advance research in this field. The development of new pharmacological and molecular tools would greatly expand our understanding of the role of AQP1 in cardiovascular physiology and pathology.

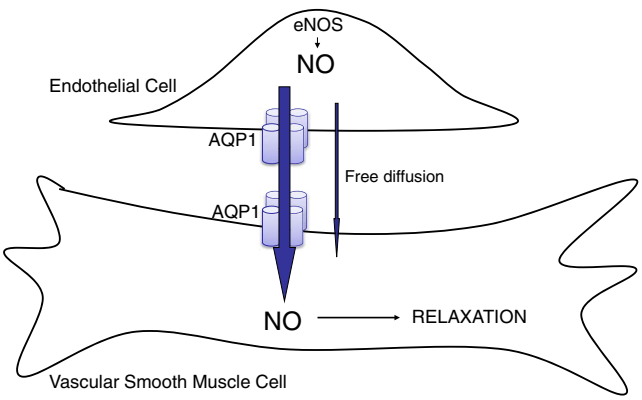

Fig. 3 Physiological role of aquaporin-1 (*AQP1*) within the vasculature. Endothelial NO synthase (*eNOS*) generates NO. AQP1 transports NO out of the endothelial cells and into the vascular smooth muscle cells where it induces relaxation. In the absence of AQP1, free diffusion of NO is not sufficient to support full expression of endothelium-dependent relaxation

To our knowledge, the molecular mechanism of NO transport by AQP1 is still unknown. Molecular simulation analyses suggested that gas molecules traverse the highly hydrophobic central pore formed by the AQP1 tetramer [59]. In addition, it has been proposed that cGMP interacts with cytoplasmic loops to confine the central pore, and that the loop functions as a "lid" that opens and closes the channel to regulate NO flux across cell membranes [64]. The molecular dynamics of NO flux through the central pore of the AQP1 tetramer are still unknown.

In summary, AQP1 carries NO across cell membranes by facilitated transport, which is three times faster than free diffusion. The transport of NO by AQP1 has physiological significance because it mediates NO-dependent vasorelaxation. The ability of aquaporins to transport NO may permit tight control of intracellular NO concentrations in target cells and directional release from cells where it is produced. Free diffusion would rule out control over intracellular NO concentration or exit at a specific site; large amounts of NO could become trapped between the inner and outer leaflet of the membrane, leading to oxidative damage and cell death. Because we know the intracellular environment is tightly controlled with regard to ions and signaling molecules, it seems unlikely that there would be no means of regulating entry and exit of NO; thus, facilitated transport of NO offers a better explanation for this gaseous molecule's rapid paracrine actions within the vasculature.

Transport of NO by other AQPs

Recent molecular simulation studies suggest that aquaporin-4 may be a more likely NO channel [60]. However, experiments addressing whether or not other aquaporins also serve as NO channels have not being yet reported. Future studies are needed before we can fully understand the dynamics of gas transport. Whether or not other aquaporins transport NO, which one transports NO the best, what molecular mechanisms regulate channel activity, whether other tissues rely on gas transport under physiological conditions, whether disease alters gas transport, these are all open questions that remain to be answered.

NH_3

Transport of NH_3 by AQPs

Unlike CO_2 and NO, NH_3 transport by AQP has not been extensively studied. The first observations suggesting a role for aquaporins as NH_3 channels were reported by Holm et al. [24] who used *Xenopus* oocytes under open-circuit and voltage-clamped conditions (to exclude NH_4^+ and H^+ transport) to study the effect of several aquaporins on NH_3 transport by monitoring the rate of acidification of a low-buffer bath. Although APQ1 had no effect on NH_3 permeability, AQP3, AQP8, and AQP9 all increased acidification, suggesting enhanced NH_3 influx across the cell membrane [24]. Using planar lipid bilayers expressing AQP8 and monitoring surface pH changes in response to bath NH_3, Saparov et al. confirmed Holm's observation that AQP8 serves as a channel for NH_3 [48]. Using a similar technique, Musa-Aziz et al. recently reported that in *Xenopus* oocytes AQP1 enhanced NH_3 influx significantly more than AQP4 and AQP5 [37], pointing to facilitated transport of NH_3 by AQP1 and contradicting Holm's data showing that AQP1 does not significantly affect NH_3 transport. While the reason for this discrepancy remains unknown, varying sensitivities of the methods used could be the explanation.

The physiological significance of any aquaporin as a NH_3 transporter remains speculative. In the basolateral membrane of the renal thick ascending limb, aquaporins could explain the relatively high basolateral/luminal permeability to NH_3 measured in isolated perfused tubules [19]. More research is needed in order to understand the molecular mechanisms of NH_3 homeostasis.

Transport of NH_3 by other proteins

Unlike CO_2 and NO, other transmembrane proteins appear to be more efficient NH_3 channels, among them AmtB and RhCG (members of the Rh family). Monitoring the rate of surface pH changes in response to adding extracellular NH_3, Boron's group has shown that these transporters, when expressed in *Xenopus* oocytes, preferentially transport NH_3 over other gases and at higher rates than any AQP tested under the same experimental conditions [37], suggesting that gas channels also exhibit selectivity. Similar to these observations, measurements of vesicular pH change revealed higher rates of NH_3 influx in proteoliposomes containing purified RhCG [21], suggesting that RhCG transports NH_3 lipid bilayers.

To investigate the physiological significance of RhCG-mediated NH_3 transport, investigators focused their attention on NH_3 secretion by the distal nephron, an important mechanism regulating acid–base homeostasis. Biver et al. showed that in isolated perfused cortical-collecting ducts, the transepithelial NH_3 permeability was reduced by 60% in tubules from RhCH −/− mice. The reduced bath to lumen permeability was due to lower basolateral as well as apical NH_3 permeability across the plasma membrane in the absence of RhCG [4]. In line with the in vitro studies, mice with collecting duct-specific deletion of RhCG exhibited reduced ammonium excretion and severe metabolic acidosis after an acid load [30]. Thus, RhCG plays an important role in NH_3 excretion by facilitating the movement of NH_3 across the basolateral and apical plasma membrane of the collecting duct during NH_3 secretion. The molecular mechanisms of NH_3 permeation through RhCG are not fully understood, however, the crystal structure of human RhCG at high resolution indicates that the pore is lined by histidine residues that exclude charged molecules and allow the passage of neutral NH_3 [21, 28].

In summary, AQP1 transports CO_2, NO, and NH_3 across plasma membranes, thereby expediting their traversal of lipid bilayers. AQP1-dependent CO_2 and NO transport plays an important role in mammalian physiology, but transport of gases by other AQPs is less clear and needs further research. Channel-dependent transport of gaseous molecules fits better with the tightly controlled intracellular environment and the rapid paracrine actions of gaseous molecules (which would have to be shorter than their half-life) and offers a means of controlling directional release. The molecular mechanisms that confer selectivity to gases remain to be explored. Regulation of AQPs by intracellular trafficking and/or channel gating may represent a tight regulatory mechanism for gas cell homeostasis and opens a new and promising area of research.

Acknowledgments This work was supported by NIH grants HL 28982, HL 07985, and HL 09550 (JLG) and NIH F32 National Service Award grant DK081333-01 (MH).

References

1. Agre P (2006) The aquaporin water channels. Proc Am Thorac Soc 3(1):5–13
2. Beitz E, Wu B, Holm LM, Schultz JE, Zeuthen T (2006) Point mutations in the aromatic/arginine region in aquaporin 1 allow passage of urea, glycerol, ammonia, and protons. Proc Natl Acad Sci U S A 103(2):269–274
3. Bienert GP, Moller AL, Kristiansen KA, Schulz A, Moller IM, Schjoerring JK, Jahn TP (2007) Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. J Biol Chem 282(2):1183–1192
4. Biver S, Belge H, Bourgeois S, Van Vooren P, Nowik M, Scohy S, Houillier P, Szpirer J, Szpirer C, Wagner CA, Devuyst O, Marini AM (2008) A role for Rhesus factor Rhcg in renal ammonium excretion and male fertility. Nature 456 (7220):339–343
5. Boron WF, Cooper GJ (1998) Effect of DIDS on the CO2 permeability of the water channel AQP1. FASEB J 12

6. Boron WF, Waisbren SJ, Modlin IM, Geibel JP (1994) Unique permeability barrier of the apical surface of parietal and chief cells in isolated perfused gastric glands. J Exp Biol 196:347–360
7. Boron WF, Endeward V, Gros G, Musa-Aziz R, Pohl P (2011) Intrinsic CO2 permeability of cell membranes and potential biological relevance of CO2 channels. Chemphyschem 12 (5):1017–1019. doi:10.1002/cphc.201100034
8. Chen H, Wu Y, Voth GA (2006) Origins of proton transport behavior from selectivity domain mutations of the aquaporin-1 channel. Biophys J 90(10):L73–L75
9. Cheng A, van Hoek AN, Yeager M, Verkman AS, Mitra AK (1997) Three-dimensional organization of a human water channel. Nature 387(6633):627–630. doi:10.1038/42517
10. Chou CL, Knepper MA, Hoek AN, Brown D, Yang B, Ma T, Verkman AS (1999) Reduced water permeability and altered ultrastructure in thin descending limb of Henle in aquaporin-1 null mice. J Clin Invest 103(4):491–496. doi:10.1172/JCI5704
11. Cooper GJ, Boron WF (1998) Effect of PCMBS on CO2 permeability of Xenopus oocytes expressing aquaporin 1 or its C189S mutant. Am J Physiol 275(6 Pt 1):C1481–C1486
12. Denker BM, Smith BL, Kuhajda FP, Agre P (1988) Identification, purification, and partial characterization of a novel Mr 28,000 integral membrane protein from erythrocytes and renal tubules. J Biol Chem 263(30):15634–15642
13. Effros RM, Darin C, Jacobs ER, Rogers RA, Krenz G, Schneeberger EE (1997) Water transport and the distribution of aquaporin-1 in pulmonary air spaces. J Appl Physiol 83(3):1002–1016
14. Forster RE, Gros G, Lin L, Ono Y, Wunder M (1998) The effect of 4,4′-diisothiocyanato-stilbene-2,2′-disulfonate on CO2 permeability of the red blood cell membrane. Proc Natl Acad Sci U S A 95 (26):15815–15820
15. Furchgott RF (1990) The 1989 Ulf von Euler lecture. Studies on endothelium-dependent vasodilation and the endothelium-derived relaxing factor. Acta Physiol Scand 139(2):257–270
16. Furchgott RF, Zawadzki JV (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288(5789):373–376
17. Gannon BJ, Carati CJ (2003) Endothelial distribution of the membrane water channel molecule aquaporin-1: implications for tissue and lymph fluid physiology? Lymphat Res Biol 1(1):55–66
18. Gannon BJ, Warnes GM, Carati CJ, Verco CJ (2000) Aquaporin-1 expression in visceral smooth muscle cells of female rat reproductive tract. J Smooth Muscle Res 36(5):155–167
19. Garvin JL, Burg MB, Knepper MA (1988) Active NH4+ absorption by the thick ascending limb. Am J Physiol 255(1 Pt 2):F57–F65
20. Gonen T, Walz T (2006) The structure of aquaporins. Q Rev Biophys 39(4):361–396
21. Gruswitz F, Chaudhary S, Ho JD, Schlessinger A, Pezeshki B, Ho CM, Sali A, Westhoff CM, Stroud RM (2010) Function of human Rh based on structure of RhCG at 2.1 A. Proc Natl Acad Sci U S A 107(21):9638–9643. doi:1003587107
22. Herrera M, Garvin JL (2007) Novel role of AQP-1 in NO-dependent vasorelaxation. Am J Physiol Renal Physiol 292(5): F1443–F1451
23. Herrera M, Hong NJ, Garvin JL (2006) Aquaporin-1 transports NO across cell membranes. Hypertension 48(1):157–164
24. Holm LM, Jahn TP, Moller AL, Schjoerring JK, Ferri D, Klaerke DA, Zeuthen T (2005) NH3 and NH4+ permeability in aquaporin-expressing Xenopus oocytes. Pflugers Arch 450(6):415–428. doi:10.1007/s00424-005-1399-1
25. Hub JS, de Groot BL (2008) Mechanism of selectivity in aquaporins and aquaglyceroporins. Proc Natl Acad Sci U S A 105(4):1198–1203
26. Ishibashi K (2009) New members of mammalian aquaporins: AQP10-AQP12. Handb Exp Pharmacol 190:251–262. doi:10.1007/978-3-540-79885-9_13
27. Jung JS, Preston GM, Smith BL, Guggino WB, Agre P (1994) Molecular structure of the water channel through aquaporin CHIP. The hourglass model. J Biol Chem 269(20):14648–14654
28. Khademi S, III O'Connell J, Remis J, Robles-Colmenares Y, Miercke LJ, Stroud RM (2004) Mechanism of ammonia transport by Amt/MEP/Rh: structure of AmtB at 1.35 Å. Science 305 (5690):1587–1594. doi:10.1126/science.1101952
29. Kikeri D, Sun A, Zeidel ML, Hebert SC (1989) Cell membranes impermeable to NH3. Nature 339(6224):478–480. doi:10.1038/339478a0
30. Lee HW, Verlander JW, Bishop JM, Igarashi P, Handlogten ME, Weiner ID (2009) Collecting duct-specific Rh C glycoprotein deletion alters basal and acidosis-stimulated renal ammonia excretion. Am J Physiol Renal Physiol 296(6):F1364–F1375
31. Ma T, Yang B, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS (1998) Severely impaired urinary concentrating ability in transgenic mice lacking aquaporin-1 water channels. J Biol Chem 273 (8):4296–4299
32. Matsuzaki T, Tajika Y, Ablimit A, Aoki T, Hagiwara H, Takata K (2004) Aquaporins in the digestive system. Med Electron Microsc 37(2):71–80. doi:10.1007/s00795-004-0246-3
33. Missner A, Kugler P, Saparov SM, Sommer K, Mathai JC, Zeidel ML, Pohl P (2008) Carbon dioxide transport through membranes. J Biol Chem 283(37):25340–25347
34. Moller M, Botti H, Batthyany C, Rubbo H, Radi R, Denicola A (2005) Direct measurement of nitric oxide and oxygen partitioning into liposomes and low density lipoprotein. J Biol Chem 280 (10):8850–8854
35. Moncada S, Palmer RM, Higgs EA (1988) The discovery of nitric oxide as the endogenous nitrovasodilator. Hypertension 12 (4):365–372
36. Murata K, Mitsuoka K, Hirai T, Walz T, Agre P, Heymann JB, Engel A, Fujiyoshi Y (2000) Structural determinants of water permeation through aquaporin-1. Nature 407(6804):599–605. doi:10.1038/35036519
37. Musa-Aziz R, Chen LM, Pelletier MF, Boron WF (2009) Relative CO2/NH3 selectivities of AQP1, AQP4, AQP5, AmtB, and RhAG. Proc Natl Acad Sci U S A 106(13):5406–5411
38. Nakhoul NL, Davis BA, Romero MF, Boron WF (1998) Effect of expressing the water channel aquaporin-1 on the CO2 permeability of Xenopus oocytes. Am J Physiol 274(2 Pt 1):C543–C548
39. Nielsen S, Smith BL, Christensen EI, Agre P (1993) Distribution of the aquaporin CHIP in secretory and resorptive epithelia and capillary endothelia. Proc Natl Acad Sci U S A 90(15):7275–7279
40. Nielsen S, Frokiaer J, Marples D, Kwon TH, Agre P, Knepper MA (2002) Aquaporins in the kidney: from molecules to medicine. Physiol Rev 82(1):205–244. doi:10.1152/physrev.00024.2001
41. Otto B, Uehlein N, Sdorra S, Fischer M, Ayaz M, Belastegui-Macadam X, Heckwolf M, Lachnit M, Pede N, Priem N, Reinhard A, Siegfart S, Urban M, Kaldenhoff R (2010) Aquaporin tetramer composition modifies the function of tobacco aquaporins. J Biol Chem 285(41):31253–31260
42. Pallone TL, Edwards A, Ma T, Silldorff EP, Verkman AS (2000) Requirement of aquaporin-1 for NaCl-driven water transport across descending vasa recta. J Clin Invest 105(2):215–222. doi:10.1172/JCI8214
43. Palmer RM, Ferrige AG, Moncada S (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327(6122):524–526. doi:10.1038/327524a0
44. Prasad GV, Coury LA, Finn F, Zeidel ML (1998) Reconstituted aquaporin 1 water channels transport CO2 across membranes. J Biol Chem 273(50):33123–33126

45. Preston GM, Agre P (1991) Isolation of the cDNA for erythrocyte integral membrane protein of 28 kilodaltons: member of an ancient channel family. Proc Natl Acad Sci U S A 88(24):11110–11114
46. Preston GM, Carroll TP, Guggino WB, Agre P (1992) Appearance of water channels in Xenopus oocytes expressing red cell CHIP28 protein. Science 256(5055):385–387
47. Preston GM, Jung JS, Guggino WB, Agre P (1994) Membrane topology of aquaporin CHIP. Analysis of functional epitope-scanning mutants by vectorial proteolysis. J Biol Chem 269 (3):1668–1673
48. Saparov SM, Liu K, Agre P, Pohl P (2007) Fast and selective ammonia transport by aquaporin-8. J Biol Chem 282(8):5296–5301
49. Shanahan CM, Connolly DL, Tyson KL, Cary NR, Osbourn JK, Agre P, Weissberg PL (1999) Aquaporin-1 is expressed by vascular smooth muscle cells and mediates rapid water transport across vascular cell membranes. J Vasc Res 36(5):353–362
50. Simon SA, Gutknecht J (1980) Solubility of carbon dioxide in lipid bilayer membranes and organic solvents. Biochim Biophys Acta 596(3):352–358
51. Skelton LA, Boron WF, Zhou Y (2010) Acid-base transport by the renal proximal tubule. J Nephrol 23(Suppl 16):S4–S18
52. Smith BL, Agre P (1991) Erythrocyte Mr 28,000 transmembrane protein exists as a multisubunit oligomer similar to channel proteins. J Biol Chem 266(10):6407–6415
53. Stroud RM, Miercke LJ, O'Connell J, Khademi S, Lee JK, Remis J, Harries W, Robles Y, Akhavan D (2003) Glycerol facilitator GlpF and the associated aquaporin family of channels. Curr Opin Struct Biol 13(4):424–431
54. Uehlein N, Lovisolo C, Siefritz F, Kaldenhoff R (2003) The tobacco aquaporin NtAQP1 is a membrane CO2 pore with physiological functions. Nature 425(6959):734–737. doi:10.1038/nature02027
55. Verkman AS (2002) Does aquaporin-1 pass gas? An opposing view. J Physiol 542(Pt 1):31
56. Verkman AS (2006) Aquaporins in endothelia. Kidney Int 69 (7):1120–1123
57. Waisbren SJ, Geibel JP, Modlin IM, Boron WF (1994) Unusual permeability properties of gastric gland cells. Nature 368 (6469):332–335. doi:10.1038/368332a0
58. Walz T, Smith BL, Zeidel ML, Engel A, Agre P (1994) Biologically active two-dimensional crystals of aquaporin CHIP. J Biol Chem 269(3):1583–1586
59. Wang Y, Tajkhorshid E (2010) Nitric oxide conduction by the brain aquaporin AQP4. Proteins 78(3):661–670. doi:10.1002/prot.22595
60. Wang Y, Cohen J, Boron WF, Schulten K, Tajkhorshid E (2007) Exploring gas permeability of cellular membranes and membrane channels with molecular dynamics. J Struct Biol 157(3):534–544
61. Xu L, Zhou, Y, Boron WF (2011) Role of aquaporin 1 (AQP1) as a CO2 channel in chronic metabolic acidosis. FASEB J 25 (834.1)
62. Yakata K, Tani K, Fujiyoshi Y (2011) Water permeability and characterization of aquaporin-11. J Struct Biol 174(2):315–320
63. Yang B, Fukuda N, van Hoek A, Matthay MA, Ma T, Verkman AS (2000) Carbon dioxide permeability of aquaporin-1 measured in erythrocytes and lung of aquaporin-1 null mice and in reconstituted proteoliposomes. J Biol Chem 275(4):2686–2692
64. Yu J, Yool AJ, Schulten K, Tajkhorshid E (2006) Mechanism of gating and ion conductivity of a possible tetrameric pore in aquaporin-1. Structure 14(9):1411–1423
65. Zhou Y, Bouyer P, Virkki L, Davis BA, Boron WF (2000) Effect of Zn or DIDS on HCO3-reabsorption by S2 proximal tubule. FASEB J 14