Aquaporin water channels and lung physiology

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Verkman, A. S., Michael A. Matthay, and Yuanlin Song. Aquaporin water channels and lung physiology. Am J Physiol Lung Cell Mol Physiol 278: L867–L879, 2000.—Fluid transport across epithelial and endothelial barriers occurs in the neonatal and adult lungs. Biophysical measurements in the intact lung and cell isolates have indicated that osmotic water permeability is exceptionally high across alveolar epithelia and endothelia and moderately high across airway epithelia. This review is focused on the role of membrane water-transporting proteins, the aquaporins (AQPs), in high lung water permeability and lung physiology. The lung expresses several AQPs: AQP1 in microvascular endothelia, AQP3 in large airways, AQP4 in large- and small-airway epithelia, and AQP5 in type I alveolar epithelial cells. Lung phenotype analysis of transgenic mice lacking each of these AQPs has been informative. Osmotically driven water permeability between the air space and capillary compartments is reduced 10-fold by deletion of AQP1 or AQP5 and reduced even more by deletion of AQP1 and AQP4 or AQP1 and AQP5 together. AQP1 deletion greatly reduces osmotically driven water transport across alveolar capillaries but has only a minor effect on hydrostatic lung filtration, which primarily involves paracellular water movement. However, despite the major role of AQPs in lung osmotic water permeabilities, AQP deletion has little or no effect on physiologically important lung functions, such as alveolar fluid clearance in adult and neonatal lung, and edema accumulation after lung injury. Although AQPs play a major role in renal and central nervous system physiology, the data to date on AQP knockout mice do not support an important role of high lung water permeabilities or AQPs in lung physiology. However, there remain unresolved questions about possible non-water-transporting roles of AQPs and about the role of AQPs in airway physiology, pleural fluid dynamics, and edema after lung infection.

water transport; water permeability; alveolus; fluid clearance; transgenic mice

FLUID MOVEMENT BETWEEN THE AIR SPACE and vascular compartments in lung plays an important physiological role in many processes such as regulation of airway hydration, reabsorption of alveolar fluid in the neonatal period in preparation for alveolar respiration, and the resolution of pulmonary edema (5, 46, 56). Fluid movement across epithelial and endothelial barriers occurs in interstitial and alveolar pulmonary edema resulting from numerous etiologies such as congestive heart failure, acute respiratory distress syndrome, infection, acute lung injury after acid aspiration, and subacute hypoxic lung injury. The ability to control fluid transport in the lung by pharmacological means, gene therapy, or other approaches could have profound consequences in cardiovascular and lung medicine.

Biological fluids consist of salts, macromolecules, and water, with water as the major component on a molar basis. Water transport can be driven across membrane barriers by hydrostatic and oncotic or osmotic driving forces. The general paradigm for the transport of fluid across tight cell layers such as epithelia in the lung is that active salt transport drives water transport by creating osmotic gradients. Hydrostatic fluid movement can occur when a permeability barrier becomes leaky to both solutes and water. The reader is referred elsewhere for additional details about the biophysics of
fluid transport across membrane and complex tissue barriers (17, 75). From the above considerations, it follows that active and secondary active ion and solute transport processes play a major role in fluid transport in the lung and extrapulmonary tissues. There is a considerable body of information about ion pumps (e.g., Na\(^+\)-K\(^+\)-ATPase), channels (e.g., epithelial Na channel, cystic fibrosis transmembrane conductance regulator, Cl\(^{-}\)2), and transporters (e.g., Cl\(^{-}\)/HCO\(_3\)\(^{-}\) and Na\(^{+}\)/H\(^{+}\) exchangers) involved in maintaining cellular homeostasis and vectorial fluid transport in airways and lung. This review addresses the issue of how water moves across barriers in the lung.

The story begins with the definition of permeability barriers in lung and measurement of their water permeability properties. The general observation is that lung water permeabilities are high and in the case of type I alveolar epithelial cells, higher than in other mammalian cell membranes studied to date. The aquaporin (AQP) family of membrane water channels is reviewed, and indirect evidence is described supporting a role for AQPs in lung physiology, such as the specific AQP expression pattern in the lung and developmental expression-function correlates. The generation and phenotype analysis of transgenic knockout mice lacking each of the lung AQPs is then described. The knockout mice have been very informative in defining the role of AQPs in lung physiology. The review then points out important unresolved questions in the field and directions for future research.

BARRIERS TO WATER TRANSPORT IN LUNG

There are several barriers to the transport of water between the air space, interstitial, and vascular compartments in the lung (Fig. 1). The trachea and large airways contain an epithelial cell layer but represent a uniform barriers is an oversimplification. The trachea and large airways contain an epithelial cell layer but still represent a relatively small surface area. In human lung, the total airway surface area is \(\sim 1.4\) m\(^2\), only \(\sim 1\)% of the alveolar surface area of 143 m\(^2\) (80). The alveolar epithelium thus provides the major surface lining the air space. The alveolar epithelium contains type I cells, which are flat cells comprising the majority of the alveolar epithelial surface, and type II cells, which transport salt actively and produce surfactant. Movement of water between the air space and capillary compartments through the alveolar epithelium also encounters potential permeability barriers in the interstitium and capillary endothelia. As shown in Fig. 1, the pulmonary artery runs with the airways and divides as the airways divide (48, 65). The capillary bed is then formed in the alveolar acinar region beyond the terminal bronchioli. The bronchial artery forms a capillary plexus that supplies the muscle and submucosa of the airways. This plexus communicates with branches of the pulmonary artery and empties into the pulmonary veins. Figure 1 also shows the complex cluster organization of alveolar acini arising from distal airways. Heterogeneity in vascular geometry, blood flow, and gas exchange throughout the lung provide yet an additional level of complexity. Thus although the basic concept of vascular, interstitial, and air space compartments is valid, the description of lung fluid movement in terms of passage across well-demarcated uniform barriers is an oversimplification.

WATER PERMEABILITIES IN LUNG

Water permeability across a simple barrier separating two compartments is characterized by an osmotic water permeability coefficient (\(P_f\)), defined as the volume flux (\(J_v\)) induced by an osmotic gradient: 
\[
P_f = \frac{J_v}{S(V_w \Delta C)}
\]
where \(S\) is barrier surface area, \(V_w\) is the partial molar volume of water (18 cm\(^3\)/mol), and \(\Delta C\) is the osmotic gradient. High \(P_f\) (generally >0.005–0.01 cm/s) suggests a facilitated water pathway involving molecular water channels (17). The Arrhenius activation energy (\(E_a\)), which is deduced from the temperature dependence of \(P_f\), also provides potentially useful information. \(E_a\) is generally <4–6 kcal/mol for a pore-mediated water pathway (or unstirred layer-limited water transport) and >8–10 kcal/mol for lipid-mediated water transport. Many biological membranes contain parallel pore and lipid pathways that warrant consideration in the interpretation of \(P_f\) and \(E_a\) values. Other biophysical properties describing a water-transporting barrier include the diffusional water permeability (\(P_d\)) and solute reflection coefficient. However, as discussed by Verkman (75), \(P_d\) and solute reflection coefficient probably have little utility in describing complex structures like the lung because of unstirred layer effects and uncertainties in barrier geometry. Characterization of a water-transporting pathway also generally involves the testing of mercury compounds (e.g., HgCl\(_2\)) known to inhibit many of the AQP water channels, with the caveat that mercury compounds are highly toxic to living cells. The reader is
referred to Refs. 16, 17, and 75 for a more detailed description of the biophysics of water permeability. An initial estimate of osmotic water permeability ($P_f$) between the air space and the capillary compartments utilized an air space “instillation and sample” approach in the in situ perfused sheep lung (19). The vascular compartment was perfused continuously with isosmolar saline, and the air space was filled rapidly with hypertonic saline. Serial samples of air space fluid showed that osmolality equilibrated rapidly, with a half-time of $\sim 45$ s. Osmotic equilibration was reversibly slowed approximately threefold on addition of HgCl$_2$ to the perfusate. $P_f$ was estimated to be 0.02 cm/s, a very high value that was similar to that of erythrocytes and epithelial cell membranes from kidney tubules. It was concluded that water movement between the air space and the capillary compartments in the intact lung was transcellular and facilitated by mercurial-sensitive water channels. Another approach to study air space-capillary water permeability involves perfusing isolated lungs with an isosmolar and then an anisosmolar solution and measuring serial osmolalities of fluid exiting the pulmonary vein (14); however, this approach does not provide quantitative permeability values and is subject to concerns about the effects of heterogeneity in vascular flow. Newer quantitative methods are described below to measure water permeability across specific barriers in the lung.

Pleural surface fluorescence recordings. For studies in small animals, where rapid air space fluid instillation and sampling are not practical, a pleural surface fluorescence method was developed in which air space osmolality is deduced from the fluorescence of an aqueous-phase indicator added to the air space fluid (7). The principle of the method is shown in Fig. 2A. The air space is filled with fluid containing a membrane-impermeant fluorophore, and the pulmonary artery is perfused with solutions of specified osmolalities. Because of the finite penetration depth of the excitation light, only lung tissue within 100–200 µm of the pleural surface is illuminated. Under these conditions, the surface fluorescence signal is directly proportional to the air space fluorophore concentration. In response to an osmotic gradient, water flows between the air space and the perfusate compartments, resulting in a change in fluorophore concentration that is detected continuously by measurement of pleural surface fluorescence. This approach has excellent time resolution without the need for invasive fluid sampling. The representative pleural surface recording shown in Fig. 2B indicates a stable signal with remarkably little experimental noise. In response to doubling of perfusate osmolality from 300 to 600 mosM, the pleural surface fluorescence signal approximately doubles as predicted theoretically. $P_f$ in the mouse lung was 0.017 cm/s at 23°C, independent of the solute used to induce osmosis, independent of osmotic gradient size and direction, weakly temperature dependent, and inhibited by HgCl$_2$. The pleural surface fluorescence method was used in a developmental study to show increased air space-capillary water permeability in the first 24 h after birth (9) and, as discussed below, in lungs from various AQP knockout mice. The pleural surface fluorescence method has also been used to measure $P_d$ using an H$_2$O/H$_2$O mixture.
exchange method and a $^2\text{H}_2\text{O}$-sensitive fluorophore (7) and can be used to study $\text{H}, \text{Na}^{+}, \text{Cl}^{-}$ transport with suitable membrane-impermeant fluorescent indicators added to the air space fluid.

The air space-capillary osmotic water permeability measured by the approach above includes serial contributions from epithelial and endothelial barriers. A modified pleural surface fluorescence strategy was developed to directly measure microvascular endothelial water permeability in intact lung (8). The air space is filled with an inert, water-insoluble perfluorocarbon to restrict lung water to two compartments, the interstitium and capillaries, and thus establish a single rate-limiting permeability barrier, the capillary endothelium. The pulmonary artery is perfused with solutions of specified osmolalities containing identical concentrations of high-molecular-weight fluorescein-dextran. In response to a change in perfusate osmolality, water is osmotically driven into or out of the capillaries, resulting in fluorophore dilution or concentration, respectively. The change in fluorophore concentration is recorded as a prompt change (decrease for fluorophore dilution; Fig. 2C) in pleural surface fluorescence. The magnitude of the prompt deflection in fluorescence signal increases with increased water permeability or decreased pulmonary arterial flow. The prompt deflection is followed by a slower return of fluorescence signal to the original level as interstitial and capillary osmotically driven water movement into or out of the capillaries, resulting in fluorophore dilution or concentration, respectively. The change in fluorophore concentration is recorded as a prompt change (decrease for fluorophore dilution; Fig. 2C) in pleural surface fluorescence. The magnitude of the prompt deflection in fluorescence signal increases with increased water permeability or decreased pulmonary arterial flow. The prompt deflection is followed by a slower return of fluorescence signal to the original level as interstitial and capillary osmotically driven water movement into or out of the capillaries, resulting in fluorophore dilution or concentration, respectively.

Gravimetry has been used to measure hydrostatic capillary filtration in dog lungs (15, 23, 59). Lung weight is the sum of solids and lung water in all compartments. For measurement of lung filtration in large animals, the pulmonary artery is generally perfused with a constant-flow infusion pump, and lung weight is measured in response to an increase in venous outflow pressure. The kinetics of lung weight increase consist of a relatively rapid phase corresponding to vascular engorgement, followed by a slower phase corresponding to extravascular fluid accumulation.

Song et al. (67) recently modified the classic gravimetric method to measure both osmotically induced water transport and hydrostatic filtration in mouse lungs. In contrast to the pleural surface fluorescence approach, gravimetry measures water transport throughout the lung. Figure 3A shows the apparatus in which lung weight is measured during perfusion. When the air space compartment is filled with isosmolar saline, changes in perfusate osmolality drive water movement across endothelial and epithelial barriers, producing changes in the water content of the interstitial and air space compartments observed as changes in lung weight. Figure 3B shows representative gravimetric data in which increasing the perfusate osmolality from 300 to 400, 500, and 600 mosM produced reversible decreases in lung weight as was drawn out of the air spaces of the lung. Figure 3C shows a filtration study in which the pulmonary artery was continuously perfused with saline and the pulmonary arterial pressure was increased from 8 to 18 cmH$_2$O at a constant left atrial pressure of 5 cmH$_2$O. The increased pressure produced a prompt increase in lung weight due to vascular engorgement, followed by a further approximately linear increase in lung weight due to fluid filtration. In a variation of this approach, the air spaces were filled with an inert perfluorocarbon to selectively probe microvascular permeability properties. As de-
scribed below, the gravimetry method has been used to analyze lung water permeability in various AQP knockout mice.

Water transport across isolated microperfused distal airways. It is not possible to measure water permeability in small airways in the intact lung because the alveolar epithelium comprises the majority of the surface area. To measure the water transport properties of small airways directly, a microperfusion technique was developed based on methods developed originally for perfusion of isolated kidney tubules (35). Small airways from the guinea pig were used because relatively long segments of viable distal airway (up to 2 mm length, 0.1–0.2 mm diameter) without bifurcations could be microdissected (18). The airway lumen was perfused at constant nanoliter per minute flow rates with isosmolar saline containing a membrane-impermeant fluorophore. The bath solution surrounding the airway was changed from isosmolar saline to a solution of different osmolality. The anisosmolar bath drives water transport across the airway epithelium, resulting in a steady-state profile of increasing fluorphore concentration (for a hyperosmolar bath) along the axis of the airway.

The fluorescence signal at any point along the airway, proportional to lumen osmolality, is then related to transepithelial $P_{f}$, airway size, and lumen flow rate. The $P_{f}$ in guinea pig distal airways was $4.5 \times 10^{-3}$ cm/s at $23^\circ$C, independent of the lumen flow rate and the magnitude and direction of the osmotic gradient, and weakly temperature dependent ($E_{a}$ 4.4 kcal/mol). Unlike alveolar water permeability, airway $P_{f}$ was not inhibited by HgCl$_{2}$, consistent with a role for the mercurial-insensitive water channel AQP4. The in vitro microperfusion method with fluorescence detection was also used to measure diffusional water transport by $H_2O/ 2H_2O$ exchange (18); however, unstirred layers preclude interpretation of $P_{f}$ values in terms of intrinsic membrane barrier properties.

Water permeability across plasma membranes of isolated lung and airway cells. The measurement of water permeability in isolated cells or plasma membrane vesicles can provide information about the contributions of specific cell types and individual plasma membranes in the overall water permeability. The stop-flow light-scattering technique has been used to measure water permeability in vesicles or cells in suspension. Cell or vesicle suspensions are subjected to an osmotic gradient by rapid mixture (generally <1 ms) with a solution of different osmolality in a stop-flow apparatus. Osmotic water movement across the cell plasma membrane produces a change in cell volume and an instantaneous change in the scattered light intensity. Osmotic water permeability is computed from the time course of the light-scattering signal and cell geometry. This approach was used to measure $P_{f}$ in isolated type I and type II alveolar epithelial cells from the rat lung (13). In response to an increase in osmolality from 300 to 600 mosM, light scattering increased, with an apparent half-time of ~50 ms for freshly immunisolated type I cells. The deduced $P_{f}$ was exceptionally high (~0.07 cm/s), weakly temperature dependent, and inhibited by HgCl$_{2}$. This high plasma membrane $P_{f}$ in type I cells quantitatively accounts for the high air space-capillary $P_{f}$ in intact lung.

Water permeability measurements in intact epithelial cells in suspension provide information about the composite water permeabilities of apical and basolateral membranes. However, measurements with suspended cells cannot resolve the individual permeabilities of the two membranes that form serial barriers in the intact epithelium. To measure $P_{f}$ in individual cell membranes, either purified membrane vesicles are studied or water permeability is measured in polarized epithelial layers. To date, methods have not been reported to isolate purified apical or basolateral membranes from lung epithelial cells. However, several methods are suitable for the measurement of cell membrane water permeability in epithelial cell layers that are cultured on rigid supports or mounted in an Ussing-type chamber (reviewed in Ref. 75). A simple approach is spatial filtering microscopy in which cell volume changes are recorded with transmitted light as a probe of intracellular refractive index (16). Cell swelling causes dilution of cytoplasmic contents and decreased intracellular refractive index. Figure 4 shows the time course of cell volume in primary cultures of human tracheal epithelial cells grown on porous supports. The osmolalities of solutions perfusing the apical and basolateral membranes were individually changed to give information about the apical and basolateral membrane water permeabilities. With appropriate modeling and assumptions about cell surface geometry (16), the $P_{f}$ in tracheal cell membranes was in the low-to-moderate range (0.004–0.005 cm/s). Similar permeabilities have been measured in cultured human nasal epithelial cells (82), where water permeability of the apical membrane appeared to be greater than that of the basolateral membrane.

Remaining questions. The functional studies indicate very high water permeabilities in cell membranes of
the alveolar epithelium and microvascular endothelium, with lower water permeabilities in airway cells. The relative apical versus basolateral membrane permeabilities of alveolar and small-airway epithelial cells, which may be important in cell volume regulatory mechanisms, are not known. Such measurements in type I alveolar epithelial cells will be particularly challenging because of their high water permeabilities. As described below, water permeability measurements in lung tissues from mice lacking specific AQPs have provided clear-cut information about the role of transcellular water movement through molecular water channels. Transgenic mice also permit evaluation of the more important issue of whether high lung water permeabilities are required for the functioning of normal and diseased lungs.

AQP WATER CHANNELS IN THE LUNG

Family of AQP water channels. There are at least 10 AQP-type water-transporting proteins identified in mammalian tissues, at least 4 of which are expressed in lung. The AQPs are small (28–31 kDa) hydrophilic proteins that are homologous to the major intrinsic protein of lens fiber. The AQPs contain several conserved amino acid sequences, including two asparagine-proline-alanine (NPA) motifs, with overall amino acid identities of 22–60% after sequence alignment. In addition, there is considerable homology in the amino acid sequence between the first and second halves of each protein, suggesting an early gene duplication event (60). Mutagenesis and topological and crystallographic analyses of AQP1, an erythrocyte water channel that is easily purified, indicated that AQP1 monomers contain water pores but associate in the membrane as tetramers. Each AQP1 monomer consists of six membrane-spanning helical domains forming a tilted helical barrier surrounding a putative aqueous pore. The reader is referred to recent reviews for details about AQP structure and topology (29, 76). Although the only crystallographic information about mammalian AQPs is available for AQP1, it is likely that the general features such as tetrameric association and the tilted helical barrel motif apply to the other AQPs as well.

Descriptions of the genetics, functional analysis, and expression patterns of the mammalian AQPs are provided in numerous recent reviews (12, 54, 76). Briefly, major intrinsic protein (or AQP0) is expressed only in lens fibers and may function as a weak and possibly pH-regulated water transporter. AQP1 is widely expressed in the epithelium and endothelia of the kidney (proximal tubule, thin descending limb of Henle, vasa recta), lung (microvascular endothelia), choroid plexus, ciliary body, and other sites. AQP2 is the vasopressin-regulated water channel expressed primarily in epithelial cells of the kidney collecting duct. AQP3 is expressed most strongly in the kidney collecting duct but also in the gastrointestinal tract, large airways, skin, and urinary bladder. AQP4 (the mercurial-insensitive water channel) was originally cloned from the lung (26) but is most strongly expressed in astroglia of the central nervous system and, to a lesser extent, in the kidney collecting duct, skeletal muscle, and colon. AQP5 is expressed in alveolar epithelial cells as well as in salivary and lacrimal glandular epithelia. AQP6 is expressed only in the kidney. AQP7 is expressed primarily in the testis but is found as well in fat cells and, to a lesser extent, in a subsegment of the kidney proximal tubule. AQP8 is localized primarily to sites in the gastrointestinal tract including liver, pancreas, colon, and salivary gland. AQP9 is also strongly expressed in the liver as well as in peripheral leukocytes and the testis. In general, the AQPs are expressed at sites where rapid vectorial fluid transport is thought to occur. However, there are a number of exceptions such as AQP1 expression in erythrocytes, AQP3 in the skin and urinary bladder, AQP4 in skeletal muscle, AQP7 in fat cells, and AQP9 in leukocytes.

The AQPs function primarily as water-transporting pores in which intrinsic water permeability appears not to be subject to regulation by posttranslational modulation. AQP1, AQP2, AQP4, and AQP8 appear to be selective for the passage of water. AQP1 has also been proposed to transport CO₂ (52), glycerol (1), and cations (88) under some conditions, but these results have been questioned (3, 77, 84, 86). Interestingly, despite its efficient water-transporting ability, AQP1 does not transport protons or small monovalent anions (73, 89). AQP3 and AQP7 also transport glycerol and possibly urea as well as water; however, it is not clear whether glycerol and water share a common aqueous pore. AQP9 appears to be a unique, relatively nonselective solute transporter with the ability to carry a variety of small polar solutes including some monosaccharides (70).

AQP expression in the lung. Four members of the AQP protein family are expressed in the airways and lung. The first localization of an AQP in the lung was reported by in situ hybridization showing AQP1 transcript expression in alveoli (28). Subsequently, AQP1 was immunolocalized on the plasma membrane of microvascular endothelial cells and, to a lesser extent, on some pneumocytes (19, 25, 55, 63). AQP3 is expressed in basal epithelial cells in large airways and throughout the nasopharynx (20, 33, 53). AQP4 is expressed at the basolateral plasma membrane surface in epithelial cells throughout the nasopharynx (20, 33, 53). AQP5 is expressed in the apical membrane of type I alveolar epithelial cells (22, 53). Type I alveolar epithelial cells also express the T1α protein, which has been proposed to be involved in water transport (81); however, an expression study (43) has indicated that T1α does not appear to transport water itself or to regulate AQP-type water channels (43). Of note, water channels have not yet been identified at the basolateral surface of alveolar epithelial cells nor at the apical surface of airway epithelial cells. Small quantities of transcripts encoding AQP8, AQP9, and AQP9L appear to be expressed in the lung, but these proteins have not yet been immunolocalized.

AQP expression and water transport in lung development. The lung at birth undergoes a dramatic transition from a fluid-secreting to a fluid-reabsorbing organ...
in preparation for alveolar respiration (5, 56). Several studies (32, 61, 72, 87) have addressed the issue of AQP developmental expression in lung. AQP1 transcript expression increases slowly throughout fetal life and then strongly just after birth. Quantitative immunoblot analysis showed a progressive increase in AQP1 protein expression over the first week of life. Steroid treatment was associated with increased AQP1 transcript expression. AQP4 transcript expression sharply increases near the time of birth. In contrast, AQP5 transcript expression increases slowly during the first week of life. To address the functional consequences of these observations, the pleural surface fluorescence method was used to measure air space-capillary osmotic water permeability in the developing lung (9). A significant increase in osmotic water permeability in the first 24 h after birth was found, consistent with a role for AQPs in the maintenance of a dry air space during the first few days of postnatal life.

Indirect evidence of a role for AQPs in lung physiology. The specific expression pattern of AQPs in the lung suggests that they play a role in water movement between the air space, interstitial, and capillary compartments. The increased AQP expression and air space-capillary water permeability near the time of birth supports a role for AQPs in neonatal lung fluid clearance. In addition, there is evidence that the expression of various lung AQPs can be regulated by steroids, keratinocyte growth factor, and possibly other hormonal factors (6, 32, 78). Together, these results provide indirect evidence of a role for AQPs in lung physiology. Direct investigation of AQPs in lung physiology requires the development of specific AQP blockers or phenotype analysis of animal models with defined AQP deficiencies. To date, the only AQP inhibitors are mercury compounds that are too toxic for in vivo use. Our laboratory has studied in vivo AQP function by the generation and phenotype analysis of transgenic knockout mice deficient in specific AQPs. Each of the four major airway or lung AQPs has been deleted by targeted gene disruption procedures. In addition, several double-knockout mice have been generated in which pairs of AQPs (AQP1 and AQP3, AQP1 and AQP4, AQP1 and AQP5, AQP3 and AQP4) have been deleted by cross-breeding of single-knockout mice.

FUNCTIONAL ANALYSIS OF AQP KNOCKOUT MICE

Role of AQPs in extrapulmonary fluid secretion and absorption. Phenotype studies in AQP-deficient mice have indicated multiple physiological abnormalities. In the kidney, deletion of AQP1 produces a severe defect in urinary-concentrating ability, resulting in profound fluid loss when mice are deprived of water (42). Mechanistic analysis revealed that the urinary-concentrating defect results from a combination of defective proximal tubule fluid absorption (62) and defective countercurrent exchange because of low water permeability in the thin descending limb of Henle (10) and outer medullary descending vasa recta (57). The decreased active fluid absorption in the proximal tubule after AQP1 deletion supports the general paradigm that high epithelial cell water permeability is required for active, near-isosmotic fluid transport. Mice lacking AQP3, a collecting duct water channel, manifest nephrogenic diabetes insipidus with polyuria, polydipsia, and urinary hypoosmolality (38). The decreased basolateral membrane water permeability impairs the osmotic extraction of water from the collecting duct lumen. Mice lacking AQP4, a basolateral membrane water channel expressed in the inner medullary collecting duct, have a mildly impaired maximal urinary-concentrating ability (41) despite a fourfold decrease in transepithelial water permeability in the inner medullary collecting duct (11). Phenotype studies in AQP knockout mice have thus been informative in elucidating basic mechanisms of renal physiology (74).

The salivary gland provides another example where deletion of an AQP is associated with defective active transepithelial fluid transport. Saliva is produced by active salt pumping into the acinar lumen driving osmotic water transport, followed by expulsion through the watertight salivary duct. AQP5 is expressed at the apical membrane of acinar epithelial cells. Whereas wild-type mice produce large amounts of clear, nonviscous saliva after pilocarpine injection, AQP5 knockout mice produce relatively little highly viscous fluid (37). Fluid analysis indicated that the AQP5 knockout mice produced a hyperosmolar hypernatremic saliva, suggesting that AQP5 null mice are able to pump salt actively into the acinar lumen but that water permeability is too low to permit osmotic equilibration. These observations support the paradigm that high epithelial water permeability facilitates active fluid transport.

A recent study of brain edema provides an example of the importance of an AQP in tissue swelling (44). AQP4 is most strongly expressed in the brain at the blood-brain and brain-cerebrospinal fluid barriers. AQP4 protein is found in glial cells lining the ependyma and pial surfaces in contact with the cerebrospinal fluid. Initial evaluation of AQP4 null mice showed no overt neurological abnormalities or defects in osmoregulation (41). However, based on the specific AQP4 expression pattern, the hypothesis that AQP4 plays a role in producing brain edema in response to two established neurological insults, acute water intoxication, producing serum hyponatremia and cellular brain edema, and ischemic stroke, producing a combination of cellular and vasogenic edema, was tested (44). AQP4 deletion conferred remarkable protection from brain edema in these models, with improved mouse survival and clinical outcome as well as reduced brain swelling. In other recent studies, significant phenotype differences were found in peritoneal fluid transport (83) and dietary fat processing (39) in AQP1 null mice and colonic water transport in AQP4 null mice (39).

Organ-specific AQP expression does not indicate physiological significance. The phenotype studies described above provide clear-cut examples of the importance of AQPs in organ function. However, does the tissue-specific expression of an AQP imply a role in normal organ physiology or an adaptation to pathophysiological stress? Recent studies suggest that this is not the case. For
example, AQP4 is expressed at the plasmalemma of fast-twitch skeletal muscle fibers and has been suggested to play a role in muscle physiology and in the pathophysiology of hereditary muscular dystrophies (21). However, analysis of skeletal muscle water permeability and contractile function, including treadmill performance, and muscle force generation and swelling showed no difference between wild-type and AQP4 knockout mice (85). AQP4 is also expressed at the basolateral membrane of gastric parietal cells (21) and has been proposed to play a role in gastric acid secretion (49). However, measurements of basal and agonist-stimulated gastric acidification and serum gastrin levels showed no differences between wild-type and AQP4 knockout mice (78a). As mentioned above, AQP5 deletion caused defective salivary gland fluid secretion; however, deletions of AQP1 and AQP4, which are also expressed in the salivary gland, did not affect saliva production (37). Deletions of the lacrimal gland AQPs (AQP1, AQP3, AQP4, and AQP5) did not affect basal or agonist-stimulated tear production (50). From these and additional examples, we conclude that tissue-specific AQP expression does not imply physiological significance. Therefore, the evaluation of AQP function in organ physiology needs to be done on a case-by-case basis. Lung phenotype studies on aquaporin knockout mice are described in the next section.

Role of AQPs in lung water permeability. Pleural surface fluorescence and gravimetric methods were used to quantify the role of AQPs in lung water permeability (4, 36, 67). Figure 5A shows pleural surface recordings of air space-capillary osmotic water permeability in perfused lungs. Deletion of AQP1 or AQP5 separately produced a remarkable decrease in water permeability. Therefore, AQP1 provides the major route for osmotically driven water movement across lung microvascular endothelia and AQP5 provides the major route for water movement across the alveolar epithelium. Because AQPs are expressed in cell plasma membranes, osmotically driven water transport occurs mainly by a transcellular rather than a paracellular pathway. These findings support the notion that the primary route for water movement between the capillary and air space compartments is by serial passage across alveolar epithelial and microvascular endothelial barriers. The minimal residual water permeability across the alveolar apical surface after AQP5 deletion may involve type II alveolar epithelial cells, the apical membrane lipid of type I cells, and possibly as yet unidentified water transporters. The residual osmotic water permeability across the microvessels probably represents a combination of lipid-mediated and paracellular transport.

Figure 5B summarizes air space-capillary $P_f$ values for AQP single- and double-knockout mice. $P_f$ was greatly reduced when AQP1 and AQP5 were deleted together. AQP4 deletion by itself had little effect on $P_f$, but deletion of AQP4 together with AQP1 resulted in a decreased $P_f$ compared with deletion of AQP1 alone. It was reasoned that the relatively low lung water permeability in AQP1 null mice might permit the detection of a small incremental effect of AQP4 deletion. The 1.4- to 1.6-fold effect of AQP4 deletion on water permeability in the AQP1 null mice suggests an overall contribution of AQP4 to lung water permeability of ~4% (taking into account the 10-fold effect of AQP1 deletion alone). Although this represents a relatively small contribution, it is greater than that predicted based on estimates that the airways comprise only 1.4% of the total air space epithelial surface area (30, 80) and that transepithelial water permeability in the airways is 5- to 10-fold lower than across the alveoli (18). Thus the airways may have a greater role in net lung fluid transport than previously anticipated.

The effect of AQP deletion on hydrostatically driven lung fluid accumulation was also studied. AQP1 deletion produced a small but significant 1.4-fold decrease in lung fluid accumulation in response to a hydrostatic stress despite a 10-fold reduction in osmotic driven air space-capillary water permeability (67). There-

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**Fig. 5.** Osmotic water permeability across air space-capillary barrier in lungs of AQP knockout mice. A: representative time-course data for lungs of mice of indicated genotypes. Pleural surface fluorescence measurements were made as in Fig. 2. Osmalalities were switched between 300 and 500 mosM (up and down arrows). Left arrows, early phase of rapid signal change resulting from rapid transcapillary water transport in AQP5 knockout mice (see Ref. 37). B: averaged air space-capillary water permeability coefficients ($P_f$) determined from surface fluorescence measurements. Values are means ± SE. (Data taken from Refs. 4, 37, and 67.)
fore AQP1-independent pathways, probably involving paracellular water transport, play the major role in hydrostatically driven transcapillary water movement. Paracellular water permeability also dominates over transcellular water permeability in the renal microvasculature (57), where water movement driven by hydrostatic pressure differences and osmotic gradients of large solutes is substantially greater than that driven by small solutes like NaCl. In lung, renal, and other microvascular beds, transvascular water movement is governed by classic Starling forces. Because AQP1-independent pathways account for the vast majority of total water permeability in lung microvessels, it is unlikely that AQP1 would play an important role in hydrostatically driven lung edema in conditions such as congestive heart failure.

AQP5 deletion did not impair the accumulation of lung edema driven by increased hydrostatic pressure (36). Mechanistically, it is understandable why AQP5 should not facilitate hydrostatically driven fluid movement across the relatively tight alveolar epithelial barrier. Because AQP5 is a water-selective transporter with a unity reflection coefficient for solutes, solute-free water driven into the air space compartment by hydrostatic forces, unless accompanied by solute entry, would be opposed by strong osmotic driving forces. The accumulation of alveolar edema probably involves transient breakdown of the integrity of the alveolar barrier, permitting the entry of solutes and protein (47). Such bulk movement of fluid through rifts in the alveolar barrier would not be expected to involve AQP5 as was found experimentally.

Role of AQPs in alveolar epithelial fluid transport. An important function of the alveolar epithelium is the active clearance of fluid from the air spaces, which is responsible for the resolution of pulmonary edema (45). Fluid absorption across the alveolar epithelium involves active salt transport, which is energized by basolateral membrane Na⁺-K⁺-ATPase and requires apical membrane (epithelial) Na⁺ channels (ENaC and non-ENaC proteins) and possibly Cl⁻ transporters. Water transport is driven by osmotic gradients created by salt transport by a presumed near-isomolar transport mechanism in which small osmotic gradients drive water movement across the highly water-permeable alveolar epithelium. As described above, other near-isomolar transport mechanisms require AQPs, including AQP1 in the kidney proximal tubule (62) and AQP5 in salivary gland (37). It was thus postulated that high alveolar epithelial water permeability would facilitate active fluid absorption from the air spaces across the alveolar epithelium.

Alveolar fluid clearance was studied in wild-type mice and mice lacking various AQPs (4, 36). Several experimental strategies were used, including the isolated perfused lung, the in situ perfused and nonperfused lungs, and the in situ ventilated lung (models reviewed in Refs. 24, 46). Interestingly, results from all models indicated that AQP deletion did not affect alveolar fluid clearance. Figure 6 shows original data from a study in which the air space was filled with an isosmolar solution containing radiolabeled albumin as a volume marker. Alveolar fluid clearance is expressed as percentage fluid absorption at 15 min. Values are means ± SD; n = 6–10 mice. Isoproterenol (0.1 mM) was added to instillate solution, and some measurements were made 72 h after treatment of mice with keratinocyte growth factor (KGF). (Data taken from Refs. 4 and 37.)

![Fig. 6. Alveolar fluid clearance in lungs of AQP knockout mice. Measurements were done at 37°C with an in situ perfused lung preparation in which the air space was instilled with an isosmolar solution containing 131I-albumin as a volume marker. Alveolar fluid clearance was inhibited by the sodium inhibitor amiloride. Clearance was enhanced by β-agonists or by pretreatment of mice with keratinocyte growth factor for 3 days to induce alveolar type II cell proliferation (71, 78). Deletion of AQP1 or AQP5, which decreased air space-capillary water permeability 10-fold, did not effect alveolar fluid clearance even under maximal keratinocyte growth factor induction and β-agonist stimulation.](image-url)

The insensitivity of alveolar fluid clearance to AQP deletion is probably the consequence of the substantially lower rate of active fluid absorption (per unit surface area) in the lung compared with the proximal tubule or salivary gland. In the latter systems, active fluid transport exceeds 0.5 µl·min⁻¹·µm⁻² epithelial surface area. With a mouse lung epithelial surface area of 600 cm², an active fluid absorption rate of 10 µl/min (equivalent to 30% absorption in 15 min) gives a rate of 0.016 µl·min⁻¹·µm⁻², substantially lower than that in the kidney proximal tubule and salivary gland. Slower rates of active fluid transport probably do not require very high cell membrane water permeabilities (68). Another consideration is that in the alveolus, unlike in the kidney proximal tubule and salivary gland, salt and water move primarily through different cells, type II and type I cells, respectively. Thus three-compartment models of solute-solvent coupling to accomplish isosmolar fluid transport may not apply to the alveolar epithelium. Another difference between active fluid transport in the alveolus versus the kidney proximal tubule and salivary gland is that fluid is rapidly cleared on both sides of the epithelium in the kidney (by lumen...
and capillary flow) and salivary gland (by capillary flow and saliva expulsion from the acinus), whereas fluid moves relatively slowly in the alveolar air spaces. Whatever the mechanism, the lack of effect of AQP deletion on alveolar fluid clearance raises doubt about the physiological role of AQPs and high water permeability in lung.

A recent study (66) was done to investigate whether air space fluid clearance in the neonatal period requires AQPs. In lungs from wild-type mice harvested at specified times after spontaneous delivery, lung water content (assessed by wet-to-dry weight ratios) decreased from 7.9 at birth to 5.3 at 24 h after birth, with a 50% reduction at ~25 min. At 45 min after birth, wet-to-dry weight ratios were similar for wild-type mice and mice lacking AQP1, AQP4, or AQP5. It thus appears that lung AQPs are not required for fluid clearance in the neonatal period or for maintenance of a dry air space. This result contrasts sharply with the finding that ENaC-deficient mice die within 40 h after birth because of defective fluid clearance from the air spaces (31).

Role of AQPs in acute lung injury. An initial study was done to test the hypothesis that AQPs play a role in lung edema in response to various forms of acute lung injury. Three established models of lung injury were studied: hyperoxia, acid aspiration, and thiourea administration (66). Experiments were done with wild-type mice and mice lacking AQP1, AQP4, or AQP5. In mice exposed to >95% oxygen, mean survival was not affected by AQP deletion nor was the wet-to-dry ratio after 65 h of hyperoxia. In mice undergoing intratracheal acid instillation to produce epithelial cell injury, AQP deletion did not affect mean wet-to-dry weight ratios at 2 h. In mice receiving intraperitoneal thiourea to produce endothelial lung injury, AQP deletion did not affect mean wet-to-dry weight ratios or pleural fluid volume at 3 h. Together, these data suggest that despite their major role in osmotically driven lung water transport, the known lung AQPs have little importance in the physiological absorption of lung water or in the accumulation of fluid in the injured lung. Although not yet tested, the results so far would suggest that AQPs are not important in the pathophysiology of other clinically relevant causes of acute lung injury such as pneumonia, sepsis, or the resolution of pulmonary edema from cardiac failure.

SUMMARY AND DIRECTIONS

The findings reviewed here indicate that water permeabilities across epithelial and endothelial barriers in the lung are in general high and facilitated by AQP-type water channels. Specific deletions of AQPs in mice resulted in remarkably decreased water permeabilities across air space-capillary barriers. However, physiologically important processes such as alveolar fluid clearance and edema accumulation in response to lung injury do not appear to require high lung water permeabilities or AQPs. The substantially lower rates of fluid movement in the lung compared with those in other organs such as the kidney and salivary gland probably account for the findings. Our results thus suggest that modulation of lung AQP expression and function by pharmacological or genetic means would not have clinical utility.

However, a number of issues remain unresolved. It is not clear whether the high level of specific expression of AQPs in the lung represents a vestigial remnant from ancestral mammals or whether AQPs are advantageous in some situations. High basolateral membrane water permeability in lung epithelia might facilitate cell volume regulation during physiological variations in airway fluid osmolarity resulting from changes in hydration state or aspiration of anisosmolar fluids. Based on its expression in pleural membranes, AQP1 may play a role in pleural fluid accumulation as was found for water transport in the peritoneal cavity (83). Phenotype studies of airway physiology in AQP knockout mice are needed to define the role of AQPs in air space humidification and airway surface liquid properties in normal and diseased lungs. The possible role of airway AQPs in the pathophysiology of cystic fibrosis warrants examination. There is evidence that the water permeability of airway AQP3 is regulated by pH (90) and interactions with the cystic fibrosis conductance transmembrane regulator protein (64). The cystic fibrosis conductance transmembrane regulator protein itself has been shown to transport water when expressed at a high density in a heterologous expression system (27). Studies in AQP3 and AQP4 null mice and in recently generated AQP3 and AQP4 double-knockout mice (38) may provide evidence of an important role for AQPs in airway physiology. The identification and physiological analysis of novel lung water transporters is needed, particularly the water transporter responsible for high basolateral membrane water permeability in type I alveolar epithelial cells. Last, possible non-water-transporting roles of AQPs in the lung merit consideration. Although AQP1-mediated carbon dioxide transport in the lung does not appear to be physiologically important (84), other lung AQPs might facilitate gas transport under some conditions. The lung AQPs might also be involved in functions unrelated to membrane transport, such as lung growth and angiogenesis.

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L878 INVITED REVIEW


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