Aquaporins and disease: lessons from mice to humans

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Recent discovery of a family of water-specific membrane channel proteins, the aquaporins, has provided new insights into the molecular basis of membrane water permeability. Eleven mammalian aquaporins have been identified to date, with homolog present across the spectrum of life, including bacteria, yeast and plants. The distribution of the mammalian aquaporins predicts their participation in a range of pathophysiological events. Empirical evidence of a physiological role for aquaporins is emerging from studies in both mice and humans, and suggests that aquaporins are likely to play significant roles in human pathophysiology.

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The existence of water-specific membrane channel proteins in selected tissues was postulated for several decades. The identity of such a channel remained unknown, however, until the isolation of aquaporin-1 (AQP1) from red blood cell membranes and demonstration of its permeability properties by Agre and colleagues [1,2]. Eleven mammalian aquaporins have now been identified, which can roughly be divided by sequence homology and functional characteristics into channels that are permeated only by water and those that are permeated by water and small solutes [3,4]. Recent descriptions of the molecular structure of AQP1 [5,6] have confirmed the previously predicted hourglass model [7], and could provide insight into the unique permeability characteristics of this family of proteins.

The function of aquaporins in different organs is of interest to a wide range of investigators and, with time, is likely to be also directly relevant to clinicians. Although clear examples of aquaporin-limited membrane permeability have been identified, definition of roles at all sites, and for all homologs, is far from trivial. We describe the studies of aquaporin distribution and function in the kidney, lung, eye and secretory glands. For these examples and beyond, however, the story is far from over.

Aquaporins and the kidney

The mammalian kidney has a major role in regulating total body water balance by concentrating or diluting urine, thereby contributing to expansion or reduction in extracellular fluid volume. It is now clear that well-defined segmental permeabilities in the nephron correlate with expression of different members of the AQP family.

Distribution

Seven renal aquaporins (AQP1–4, 6–8) have been identified [8]. In general, the distribution of each of these in the kidney is similar across species. AQP1 (Fig. 1) is abundant in both the apical and basolateral membranes of renal proximal tubular epithelium, thin descending limb epithelium of the loop of Henle, and the endothelium of the descending vasa recta. No aquaporins have been identified in the water-impermeable thin and thick ascending loop of Henle. Several water channel proteins are expressed in the renal collecting duct. AQP2 is present in vesicles of collecting duct principal cells that translocate to the apical membrane in response to vasopressin. AQP3 and AQP4 are expressed in the basolateral membrane of principal cells, AQP3 diffusely along the collecting duct, and AQP4 primarily in the inner medullary collecting duct. AQP6 is an intracellular water channel, in intracellular vesicles of the acid-secreting type-A intercalated cells of the collecting duct. Limited data are available on the distribution of AQP7 and AQP8 in the kidney. In rat, AQP7 appears to be in the brush border of the proximal tubule, whereas AQP8 is present in the cytosol of proximal tubule and collecting duct epithelium.

Physiology

The roles of aquaporins in organ physiology have been best defined – and least controversial – in the kidney. Aquaporin-limited membrane water permeability has been demonstrated at several sites in the nephron. Demonstration of vasopressin-dependent expression of AQP2 in the apical membrane of collecting duct principal cells confirmed the shuttle hypothesis of collecting duct permeability, and predicted a role for AQP2 in urinary concentration [9]. Deen first identified AQP2 mutations in individuals with hereditary nephrogenic diabetes insipidus (NDI) [10] and, with others, has uncovered additional mutations with both autosomal dominant and autosomal recessive inheritance patterns. In general, autosomal dominant mutations in AQP2 produce trafficking defects, whereas autosomal recessive mutations lead to misfolding of the protein [8]. Mice homozygous for a Thr-Met mutation at position 126 of AQP2 (T126M), an autosomal recessive mutation in humans, appeared normal at 2–3 days after birth, but failed to thrive and generally died by day six from severe polyuria, suggesting that the AQP-null phenotype is more severe in mice than in humans [11]. For further details of AQP2, see Refs [12,13].
Acquired NDI occurs in a variety of clinical settings. Animal studies demonstrated the central role of AQP2 dysregulation in acquired NDI. Decreased AQP2 expression was observed in several models of acquired NDI, including treatment with lithium [14], following relief of urinary obstruction [15] and with chronic hypokalemia [16]. At the other end of the water imbalance spectrum, AQP2 overexpression has been described in several conditions associated with fluid retention, including congestive heart failure, cirrhosis and pregnancy [17,18]. These observations confirm a fundamental role for AQP2 in a variety of pathological disorders of water homeostasis.

Recent investigations in both mice and humans have confirmed a physiological role for AQP1 in the proximal nephron. Mice with AQP1 deletion had baseline polyuria, and decreased proximal tubule and descending vasa recta water permeability, consistent with predictions based on the distribution of the protein [19–21]. AQP1-null mice had reduced glomerular filtration rates, thought to result from increased fluid and solute delivery out of the proximal tubule to the macula densa. When water-deprived, AQP1-null mice became profoundly dehydrated, with serum osmolality near 500 mOsm kg$^{-1}$ after 36 h of water deprivation.

Rare AQP1-null humans have also been identified (seven kindreds worldwide), based on the absence of an erythrocyte blood group antigen (Colton) encoded by an extracellular epitope of AQP1 [22]. Although AQP1 deficiency produced no obvious clinical consequences [23], we hypothesized that these individuals would have subclinical defects in water homeostasis that would become apparent under stress conditions. Recent examination of renal function in two AQP1-null individuals revealed that neither was polyuric, and both had normal kidney size, creatinine clearance and glomerular filtration rate [24]. When the individuals were water-deprived, in spite of normal increases in serum osmolality and vasopressin levels, maximal urine osmolality in both subjects was ∼450 mOsm kg$^{-1}$ after >20 h of water deprivation, a moderate urinary concentrating defect compared to normal individuals [25] (Fig. 2a). Additional studies suggested that proximal tubule fluid reabsorption was preserved. Therefore, we believe that, in contrast to mice, the primary defect in these rare AQP1-null humans was not in the proximal tubule, but rather in the thin descending limb, the descending vasa recta, or in both.

The manifestations of AQP1 deficiency in null mice were more severe than in AQP1-null humans. Although compensation in the AQP1-null humans is strongly suspected, the phenotypic differences between AQP1-null humans and mice might simply reflect species differences in mechanisms regulating proximal tubule water reabsorption.

Mice with deletion of the Aqp3 or Aqp4 genes demonstrated the contribution of those proteins to collecting duct function. AQP4-deficient mice have a mild urinary concentrating defect [26]. AQP3-null mice have severe polyuria; however, the interpretation of the phenotype was complicated by a surprising (and unexplained) decrease in AQP2 expression in these animals [27]. To date, humans with AQP3 or AQP4 mutations have not been identified. As with AQP1, extrarenal manifestations of AQP3 or AQP4 deficiency will also be of great interest, for example in skin (AQP3), brain (AQP4), or lung (AQP3 and AQP4).

AQP6 colocalizes with the H$^+$-ATPase in intracellular vesicles of the intercalated cells of the collecting duct [28]. In contrast to the other known AQP, AQP6 is permeated by anions, and activated by both HgCl$_2$ and low pH [29]. This constellation of findings suggests unique functions for AQP6 among water channel proteins, perhaps in regulation of acid-base balance. Studies of AQP6-null mice are ongoing (Yasu, unpublished).

Aquaporins and the lung

Requirements for fluid homeostasis in the lung are complex. Fluid secreted into the lung during gestation must be removed in the perinatal period. Physiological stresses (e.g. exercise) or pathological stresses (e.g. infection) can alter lung fluid balance,
Aquaporin (AQP) expression in the human respiratory tract. The expression and polarization of different AQPs varies between the upper airway (nasopharyngeal and bronchial epithelium), lower airway (bronchiolar epithelium), alveolus (type I and type II pneumocytes) and airway submucosal glands. AQP1, red; AQP3, green; AQP4, orange; AQP5, blue.

Fig. 3. Aquaporin (AQP) expression in the human respiratory tract. The expression and polarization of different AQPs varies between the upper airway (nasopharyngeal and bronchial epithelium), lower airway (bronchiolar epithelium), alveolus (type I and type II pneumocytes) and airway submucosal glands. AQP1, red; AQP3, green; AQP4, orange; AQP5, blue.

Fig. 2. Urinary concentration in control and aquaporin 1 (AQP1)-null individuals. (a) Urine osmolality after water deprivation in two AQP1-null individuals (22 h water deprivation) and 15 controls (overnight water deprivation). (b) Urine osmolality as a function of blood vasopressin levels after water deprivation in two AQP1-null individuals, compared to the response in control individuals. Adapted, with permission, from Ref. [25].

potentially impacting on gas exchange and lung defense. In the airways, strict regulation of the airway surface liquid is required for effective mucociliary transport, and inspired air must be humidified to prevent drying of the distal airways. The molecular basis for each of these phenomena remains incompletely defined.

Distribution
Four aquaporins, AQP1, AQP3, AQP4 and AQP5, have been identified in the human, rat and mouse respiratory tract (Fig. 3). Although some aspects of aquaporin distribution in the respiratory tract are preserved across species, distinct differences have also been noted. Aquaporin distribution in the rat respiratory tract serves as a point of reference [30–32]. In the rat lung, AQP1 is present in the apical and basolateral membrane of microvascular endothelium and visceral pleura. AQP3 is located in the basolateral membrane of basal cells of tracheal and nasopharyngeal epithelium. AQP4 is present in the basolateral membrane of ciliated columnar cells in bronchial, tracheal and nasopharyngeal epithelium. AQP5 is expressed in the apical membrane of type I pneumocytes, as well as in the apical membrane of acinar cells in submucosal glands of the airways and nasopharynx.

In the human lung, as in rat, AQP1 is abundant in the apical and basolateral membrane of microvascular endothelial cells, particularly in the vascular plexus around the airways [33]. Krada and colleagues noted many similarities between the human and rat respiratory tract in the distribution of AQP3–5. In contrast to the rat, however, AQP5 is present in the superficial epithelium of nasopharyngeal and bronchial epithelium, and AQP3 is located in the apical membrane of bronchiolar epithelium [34]. Krane demonstrated a third pattern of distribution for AQP5 in the mouse respiratory tract, where AQP5 was present in both the apical and basolateral membrane of scattered ciliated and secretory cells in the trachea, the apical membrane of ciliated cells in the bronchi, basal cells of the bronchi and lobar bronchioles, and both type I and type II pneumocytes in the alveoli [35].

Species differences in aquaporin distribution raise many questions. In other organs, AQP3 is exclusively a basolateral protein, whereas AQP5 is almost exclusively apical. What mechanisms dictate the difference in distribution for AQP3 and AQP5 in human, rat and mouse lung? No apical water channel proteins have been identified in rat airway epithelium. Do lung epithelial water transport requirements differ among species, and if so, why? And what are the implications of these differences for using animals to model AQP-relevant questions in human lung?

Physiology
The contribution of aquaporins to water homeostasis in the lung is less well defined than in the kidney. A primary role for aquaporins in osmotically driven fluid transport in the lung is unambiguous: mice with AQP1 or AQP5 gene deletions have osmotic water permeability reduced by >90% across lung endothelium (AQP1 null) [36] or alveolar epithelium (AQP5 null) [37]; AQP1-null animals also had a 50% reduction in hydrostatic permeability. Although large osmotic gradients are generally not thought to exist in the lung, it is unclear whether small or local gradients sufficient to drive AQP-mediated water transport might exist. Acute pulmonary edema formation in mice with AQP1, AQP4 or AQP5 deletions following...
inflammatory stimuli was not different from that in wild-type animals [38]. AQP5-null mice have similar isosmolar alveolar fluid reabsorption to their wild-type counterparts, raising questions about the role of AQP5 in mediating transalveolar water movement [37]. Given the extremely high AQP5-mediated water permeability of isolated type I pneumocytes [39], these findings also reinforce our lack of understanding about the mechanisms and sites of fluid reabsorption out of the alveolus. Potential redundancy in transport mechanisms, and compensation in animals with germ-line mutations, might confound attempts to define aquaporin physiology. Alternative functions, such as cell volume regulation, also need to be considered. Indeed, both AQP1 and AQP5 have been shown to be induced by hypertonic stress, although their precise role in that response remains unclear [40,41].

Recent studies strongly suggest potential roles for AQP5 in water homeostasis in the airways. Airway submucosal glands contribute significantly to generation of the airway surface liquid [42], and express AQP5 on the apical membrane of acinar secretory cells [31,34]. Airway submucosal glands are believed to function similarly to the rat submandibular gland [43], an organ in which AQP5 is both abundant [31] and rate-limiting for saliva production [44]. Song and Verkman demonstrated that AQP5-null mice have decreased pilocarpine-stimulated secretion from submucosal glands of the nasopharynx and trachea compared to wild-type controls [45]. Krane and colleagues demonstrated that, in response to acetylcholine or methacholine, AQP5-null mice have greater bronchoconstriction than wild-type mice [35]. The mechanisms underlying increased bronchial reactivity are as yet unknown; however, the potential association between AQP5, airway water homeostasis and asthma is of great potential clinical importance [46].

The first functional analysis of an aquaporin in the human lung was recently performed [33]. Because AQP1 is abundant in the vascular plexus around the airways, high-resolution CT (HRCT) scans were used to measure changes in airway wall thickness before and after fluid loading, previously shown to correlate with peribronchiolar edema formation in dogs [47]. In response to three liters of intravenous saline, pulmonary vessels distended similarly in two AQP1-null individuals and five controls, suggesting similar volume loading. Airway wall thickness increased by 50% in the control group in response to fluid challenge, representing early peribronchiolar edema. In marked contrast, airway wall thickness did not change in AQP1-null individuals with fluid loading, strongly suggesting a role for AQP1 in determining lung endothelial permeability.

Aquaporins and the eye
To date, five aquaporins have been identified in the eye [48]. Major intrinsic protein (MIP; AQP0) is expressed in lens fiber cells. AQP1 is present in scleral fibroblasts, corneal endothelium and keratinocytes, and endothelium covering the trabecular meshwork and canal of Schlemm. AQP3 is in bulbar conjunctival epithelium. AQP4 is present in retinal glia. AQP5 is expressed in the corneal epithelium and lacrimal glands.

MIP is exclusively expressed in lens fiber cells, where it comprises >50% of the total membrane protein. This abundance suggests a structural role for MIP, in addition to its role as a functional water channel. The gene encoding MIP has been identified as the site of two naturally occurring mouse mutations [49]. Mice homozygous for either the cataract Fraser mutation, Catfr, or the lens opacity mutation, Lop, develop bilateral cataracts and progressive degeneration of lens fiber cells. The Catfr mutation produces a truncated MIP protein. The Lop mutation (ala51 to pro substitution) inhibits targeting of MIP to the cell membrane.

Linkage analysis in humans led to identification of two families with inherited cataracts, each with missense mutations in the MIP gene [50]. Two different amino-acid substitutions were identified, glu to gly at residue 134 (E134G), or thr to arg at residue 138 (T138R); the clinical phenotypes of these mutations were surprisingly distinct (Fig. 4). Individuals with the E134G substitution have a unilamellar cataract that is stable after birth. Families with T138R have multifocal lens opacities that increase throughout life. The biochemical basis for the difference in phenotype is not known. When co-expressed in oocytes with wild-type MIP, both E134G and T138R mutants interfere with water permeability, consistent with the dominantly inherited phenotype [51]. The dominant inheritance also supports the hypothesis that MIP might serve a structural role, because mutations in genes encoding structural proteins, such as crystallins, are usually expressed as dominant traits.

Aquaporins and the secretory glands
Secretion of fluid by salivary, lacrimal and sweat glands results from coupling of active electrolyte...
Aquaporins (AQP) are water channels that allow water to traverse through cell membranes. They are involved in various biological processes, including the transport of water across cell membranes. The discovery of aquaporins has provided insight into the molecular basis of membrane water transport and has prompted a reconsideration of water permeability as a factor that can be specifically regulated independently of solute transport. It appears likely that the degree to which aquaporins determine water permeability in specific tissues varies depending on both the organ and the context. Many questions regarding aquaporin biology remain. What are the structural determinants that impart such unique permeability characteristics to this family of proteins? Under what specific circumstances do aquaporins play physiological roles in different tissues? What are the mechanisms that regulate aquaporin expression and function, and how are these altered in pathophysiological states of disrupted water homeostasis? Are aquaporins reasonable targets for therapy, and in what conditions? As with many biological tales, discovery of the aquaporins has advanced our basic understanding of a fundamental process; however, this insight has increased, rather than decreased, the complexity of the relevant questions.

A tale in evolution

The descriptions above, although providing evidence for physiological roles for aquaporins in different organs, are not comprehensive. Recent work presents roles for aquaporins in bile generation [57], hearing [58] and altered brain water homeostasis [59]. Aquaporins are likely to participate in water and glycerol metabolism in liver, adipocytes and the reproductive tract. The list of organs and potential roles continues to expand.

The discovery of aquaporins has provided insight into the molecular basis of membrane water transport, and has prompted a reconsideration of water permeability as a factor that can be specifically regulated independently of solute transport. It appears likely that the degree to which aquaporins determine water permeability in specific tissues varies depending on both the organ and the context. Many questions regarding aquaporin biology remain. What are the structural determinants that impart such unique permeability characteristics to this family of proteins? Under what specific circumstances do aquaporins play physiological roles in different tissues? What are the mechanisms that regulate aquaporin expression and function, and how are these altered in pathophysiological states of disrupted water homeostasis? Are aquaporins reasonable targets for therapy, and in what conditions? As with many biological tales, discovery of the aquaporins has advanced our basic understanding of a fundamental process; however, this insight has increased, rather than decreased, the complexity of the relevant questions.

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