Roles of Aquaporins in Kidney Revealed by Transgenic Mice

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Transgenic mouse models of aquaporin (AQP) deletion and mutation have been instructive in elucidating the role of AQPs in renal physiology. Mice lacking AQP1 are unable to concentrate their urine because of low water permeability in the proximal tubule, thin descending limb of Henle, and outer medullary descending vasa recta, resulting in defective near-isosmolar fluid absorption in the proximal tubule and defective countercurrent multiplication. Mice lacking functional AQP2, AQP3, or AQP4 manifest various degrees of nephrogenic diabetes insipidus resulting from reduced collecting duct water permeability. Mice lacking AQP7 and AQP8 can concentrate their urine fully, although AQP7 null mice manifest an interesting defect in glycerol reabsorption. Two unexpected renal phenotypes of AQP null mice have been discovered recently, including defective proximal tubule cell migration in AQP1 deficiency, and cystic renal disease in AQP11 deficiency. AQPs thus are important in several aspects of the urinary concentrating mechanism and in functions unrelated to tubular fluid transport. The mouse phenotype data suggest the renal AQPs as targets for the development of aquaretics and potentially for therapy of cystic renal disease and acute renal injury.
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The aquaporins (AQP) are a family of small transmembrane proteins that transport water and, in some cases, water and small solutes. In kidney, at least 6 aquaporins are expressed: AQP1 in plasma membranes of the proximal tubule, thin descending limb of Henle, and descending vasa recta; AQP2 in the apical membrane and intracellular vesicles of collecting duct principal cells; AQP3 and AQP4 in the basolateral membranes of the same cells; AQP6 in intracellular vesicles of collecting duct intercalated cells; and AQP7 in the S3 segment of the proximal tubule (Fig 1A).[1,2] Because the primary role of AQPs is to facilitate osmotically driven water transport, this tissue distribution suggests involvement of the renal AQPs in the urinary concentrating mechanism,

which requires near-isosmolar fluid absorption in the proximal tubule, countercurrent multiplication/exchange to generate a hypertonic medullary interstitium, and regulated transepithelial water permeability in the collecting duct. AQPs also have a wide extrarenal distribution in many water-transporting epithelial, endothelial, and other cell types in the brain, eye, lung, secretory glands, and the gastrointestinal tract, and in cells that do not require high water permeability in skin, fat, and urinary bladder.

Transgenic mouse models of AQP deletion and mutation have been very informative in elucidating the role of AQPs in renal and extrarenal tissues. In many cases, as in the urinary concentrating mechanism and secretory gland function, mouse phenotype data have confirmed the predicted roles of AQPs, and in other cases new biological functions for AQPs have been discovered. Because selective nontoxic AQP inhibitors are not yet available, transgenic mouse models of AQP deletion are, at present, the best way to define the physiologic roles of AQPs, recognizing the general caveats in the use of transgenic mouse models such as the possibility of compensatory changes in expression of other cell proteins,[3,4] and the possibility of a phenotype being a secondary rather than a primary effect of AQP deficiency.[5] In addition, in some cases mouse physiology may differ, at least in quantitative terms,
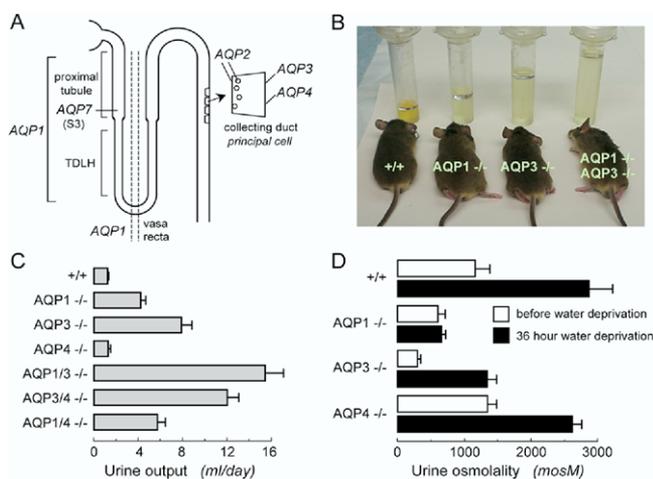
Figure 1 Urinary concentrating function in AQP null mice. (A) Sites of AQP expression in kidney. (B) Twenty-four–hour urine collections showing polyuria in mice lacking AQP1 and AQP3, individually and together. (C) Twenty-four–hour urine output (mean ± SE) from mice of indicated genotype. (D) Urine osmolalities before and after 36-hour water deprivation. Data summarized from Ma et al[6,7,9] and Yang et al.[8] (Color version of figure is available online.)

from that in larger mammals and human beings, as in the ability of the mouse kidney to generate a urine osmolality of 3,500 mOsm. This article focuses on insights into renal physiology that have emerged from phenotype analysis of AQP null and mutant mice. Also, new paradigms about roles of AQPs in mammalian physiology that have emerged from analysis of extrarenal phenotypes of AQP null mice are mentioned.

Nephrogenic Diabetes Insipidus in Aquaporin Null Mice

Deletion of AQP1 and/or AQP3 in mice produces marked polyuria, as seen from 24-hour urine collections in metabolic cages (Fig 1B).[6-8] Figure 1C summarizes urine output data for single- and double-knockout mice. Although AQP4 deletion by itself does not cause polyuria,[9] its codeletion with AQP3 increases urine output to greater than that found in mice lacking AQP3 by itself. Figure 1D summarizes urine osmolalities before and after a 36-hour water deprivation. Urinary osmolality in AQP1 null mice is low and does not increase after water deprivation, whereas AQP3 null mice can generate a partially concentrated urine. The AQP1 null mice became severely dehydrated after water deprivation, manifesting marked serum hyperosmolality and lethargy. AQP4 null mice did not manifest signs of dehydration and had only a mild defect in maximum urinary concentrating ability. These results confirmed the expected importance of AQPs in the urinary concentrating mechanism. The urinary diluting ability in these AQP null mice was intact, as shown by the ability to generate urine of less than 120 mOsm after intraperitoneal infusion of half-normal saline. AQP2 mutant mice do not

survive beyond the neonatal period,[10] although a recently generated model of inducible AQP2 gene deletion in adult mice manifests marked polyuria, with daily urine output exceeding their body weight.[11]

Mechanisms of Defective Urinary Concentrating Ability in AQP1 Deficiency

AQP1 is expressed at the apical and basolateral membranes of the proximal tubule, where most of the fluid filtered by the glomerulus is reabsorbed by an active near-isosmolar transport mechanism, and in the thin descending limb of Henle and descending vasa recta, where countercurrent multiplication and exchange occur. Transepithelial osmotic water permeability (P_f) in isolated microperfused S2 segments of the proximal tubule driven by a raffinose gradient was decreased by nearly 5-fold in AQP1 knockout mice (Fig 2A),[12] indicating that the major pathway for osmotically driven water transport in this segment is transcellular and AQP1 dependent. Free-flow micropuncture with end-proximal tubule fluid sampling showed approximately 50% reduced fluid absorption, with little effect on the single-nephron glomerular filtration rate (Fig 2B). The data support a 3-compartment model in which mild luminal hypotonicity drives osmotic water movement through highly water-permeable cell membranes. As predicted from these findings, a substantial approximately 40 mOsm transepithelial osmotic gradient in end proximal tubule fluid was found in AQP1 null mice, compared with an approximately 10 mOsm gradient in wild-type mice,[13] indicating active pumping of salt out of the tubule lumen without adequate water movement to dissipate the osmotic gradient. Micropuncture collections
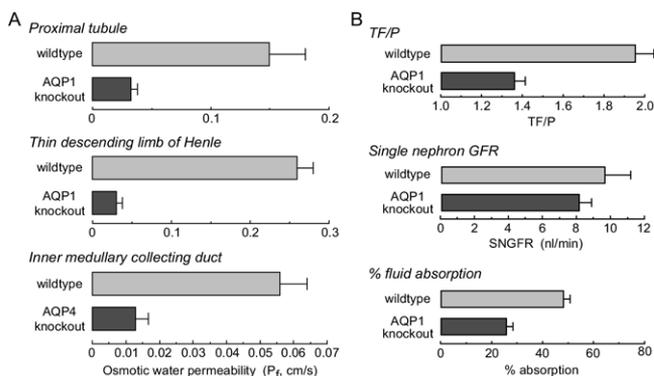

Figure 2 Mechanistic analysis of defective urinary concentrating ability in AQP deficiency. (A) P_f in the microperfused kidney proximal tubule, thin descending limb of Henle, and inner medullary collecting duct from mice of indicated genotype (mean ± SE). Data summarized from Schnermann et al[12] and Chou et al.[15,18] (B) Free-flow micropuncture in wild-type versus AQP1 knockout mice. Data summarized from Schnermann et al[12] for the end proximal tubule fluid collections. TF/P, ratio of marker in collected tubule fluid versus plasma; GFR, glomerular filtration rate.

from superficial distal tubules provided evidence for a tubuloglomerular feedback mechanism in which the single-nephron glomerular filtration rate decreases in AQP1 null mice so as to reduce distal fluid delivery to near-normal values. Recent data from double-knockout mice lacking AQP1 and the adenosine-1 receptor, which lacks tubuloglomerular feedback control of glomerular filtration, suggested that adaptations in distal fluid transport mechanisms in AQP1 deficiency in part accounts for their lack of more profound polyuria.[14] Together these data provide evidence for defective near-isosmolar fluid absorption in the proximal tubule in AQP1 deficiency.

The high expression of AQP1 in the thin descending limb of Henle epithelium and vasa recta endothelium suggests defective countercurrent multiplication and exchange in AQP1 deficiency, preventing the formation of a hypertonic medullary interstitium. This conclusion is supported by the lack of increase in urine osmolality in AQP1 null mice after administration of a vasopressin agonist,[6] and measured low medullary interstitial osmolality. As summarized in Figure 2A, osmotic water permeability P_f across microperfused thin descending limb of Henle (TDLH) was 10-fold reduced by AQP1 deletion,[15] indicating that AQP1 is the principal water channel in TDLH, and supports the view that osmotic equilibration along TDLH by water transport plays a key role in the renal countercurrent concentrating mechanism. The AQP1 null mice also were used to confirm that AQP1 is responsible for the high density of intramembrane particles observed in TDLH membranes (and proximal tubule) by freeze-fracture electron microscopy (Fig 3A).[15] In isolated microperfused outer medullary descending vasa recta subjected to NaCl gradients, P_f was reduced more than 50-fold by AQP1 deletion.[16] When raffinose rather than NaCl was used to drive osmotic water movement, P_f was doubled in wild-type mice and reduced only 2-fold by AQP1 deletion. Together with mercurial inhibition studies, it was concluded that solutes larger than NaCl are able to drive water movement both through AQP1 and an AQP1-independent, mercurial-insensitive pathway that may involve paracellular transport. Mathematic modeling predicted that the decreased medullary hypertonicity in AQP1 null mice results in part from enhanced papillary blood flow. Together, the microperfusion and micropuncture results suggest that AQP1 deletion produces nephrogenic diabetes insipidus in mice primarily by interfering with the normally hypertonic medullary interstitium generated by countercurrent multiplication and exchange. Human beings lacking AQP1 manifest a qualitatively similar urinary concentrating defect to mice, with urinary hypotonicity and insensitivity to water deprivation.[17]

Urinary Concentrating Function in AQP3 and AQP4 Null Mice

AQP3 and AQP4 are expressed at the basolateral membrane of collecting duct epithelium, with relatively greater expression of AQP3 in the cortical and outer medullary collecting
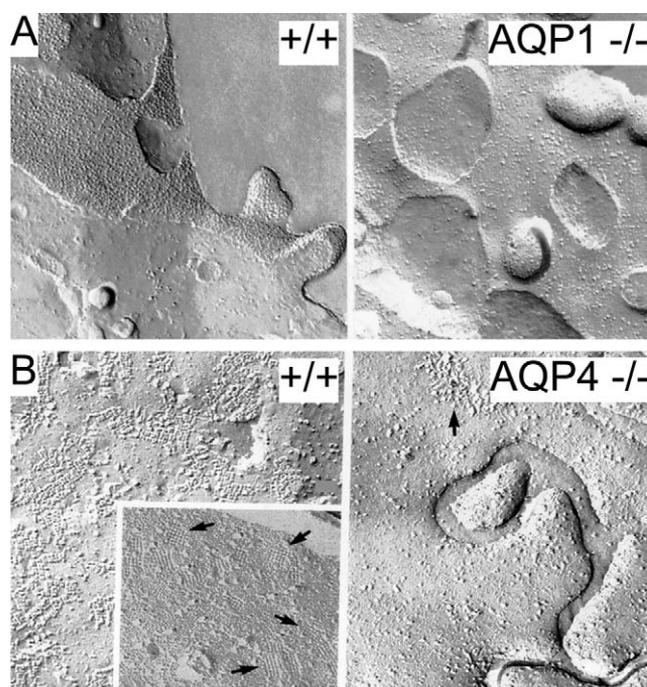

Figure 3 Ultrastructure of AQP1 and AQP4 in kidney. (A) Freeze-fracture electron microscopy showing P-face plasma membranes of the thin descending limb of Henle epithelial cells from wild-type (left) and AQP1 null (right) mouse. On the right of each micrograph, the small area represents the lumen of the tubule. Adjacent to the lumen, the apical membrane appears more undulating and contains IMPs that represent integral membrane proteins. There was a remarkably different appearance of the TDLH apical membrane of AQP1 null mice, with a much lower IMP density and different IMP appearance. (B) P-face basolateral plasma membrane of collecting duct from wild-type (left) and AQP4 null (right) mouse. Inset shows complementary orthogonal array imprints (arrows) on the Ef-face observed in wild-type mice but not in AQP4 null mice. Adapted from Chou et al[15] and Verbavatz et al.[21]

duct and AQP4 in the inner medullary collecting duct. The data in Figures 1C and 1D suggest a more important role for AQP3 than AQP4 with respect to urinary concentrating ability. In contrast to AQP1 null mice, the countercurrent multiplication and exchange mechanisms in AQP3 null mice basically are intact. The Nephrogenic Diabetes Insipidus (NDI) in AQP3 null mice probably results from the more than 3-fold reduction in P_f of cortical collecting duct basolateral membrane.[7] In addition, AQP2 expression is reduced in AQP3 null mice, which appears to be a maladaptive renal response seen in various forms of acquired polyuria. Another interesting observation in AQP3 null mice is that the polyuria is so severe that the mice manifest signs of functional urinary obstruction with dilated kidneys by age 4 to 6 weeks. Mice appear to be quite sensitive to polyuria-induced renal damage, as found in several transgenic mouse models associated with polyuria, including NKCC, ROMK1, AQP2, and V2R knockout mice, and AQP2 mutant mice.

AQP4 null mice manifest only a mild impairment in maximal urinary concentrating ability (Fig 1D),[9] despite a 4-fold
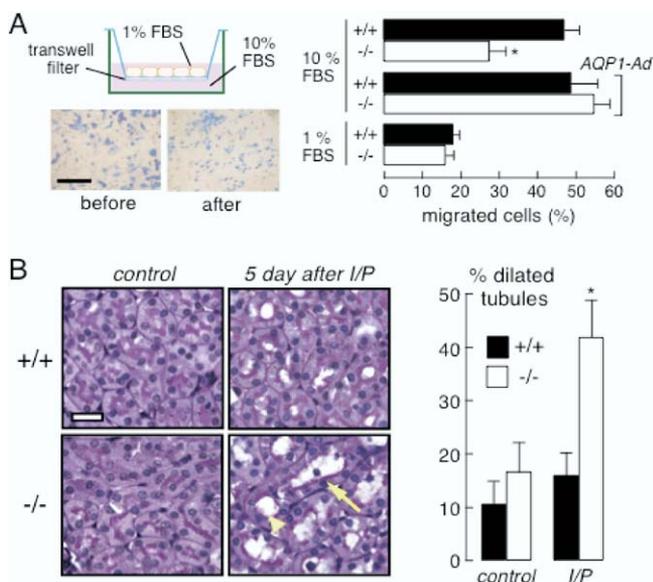
Figure 4 AQP1-facilitated migration of proximal tubule epithelial cells. (A) (left) In vitro Boyden chamber assay of proximal tubule cell migration. Schematic at top shows a modified Boyden chamber containing a Transwell porous membrane filter. Cells migrate from top to bottom in response to a chemotactic stimulus. Photographs of adherent proximal tubule cells at the completion of an assay are shown from before and after scraping the upper surface to visualize migrated cells. Bar, 100 μm. The percentage of migrated cells at 6 hours after cell plating (SE, 6 sets of experiments, *$P < .01$) is shown. The bottom chamber contained 10% or 1% fetal bovine serum. AQP1-Ad, adenoviral-mediated AQP1 gene delivery. (B) Ischemia-reperfusion model of acute renal tubular injury. Periodic acid-Schiff and hematoxylin staining of kidney sections from control wild-type and AQP1 null mice, and at 5 days after ischemic/reperfusion (I/P). Bar, 20 μm. Arrowhead, dilated tubule; arrow, degenerated tubule. The percentage of proximal tubules with an inner diameter greater than 5 μm (SE, 5 mice per group, *$P < .05$) is shown. Adapted from Hara-Chikuma and Verkman.[36] (Color version of figure is available online.)

reduced water permeability in microperfused inner medullary collecting duct (Fig 2A).[18] These results indicate that AQP4 is responsible for the majority of basolateral membrane water movement in the inner medullary collecting duct, and support the conclusion that the majority of water is reabsorbed in the cortical and outer medullary segments of the collecting duct. The AQP4 knockout mouse also was used to show that AQP4 is the orthogonal array protein (OAP). OAPs are characteristic square arrays of intramembrane particles (IMPs) that have been observed by freeze-fracture electron microscopy (FFEM) in the basolateral membrane of kidney collecting duct epithelia and some other cell types. We initially proposed that AQP4 is the OAP protein based on its expression in cells in which OAPs had been found.[19] This hypothesis was supported by the identification of OAPs in AQP4-transfected Chinese hamster ovary (CHO) cells,[20] and then proven by their absence in kidney, skeletal muscle, and brain from AQP4 knockout mice (Fig 4B).[21]

Mouse Models of Nephrogenic Diabetes Insipidus Caused by AQP2 Mutation/Deletion

The autosomal form of hereditary nephrogenic insipidus, an exceedingly rare disorder, is caused by mutations in AQP2, the vasopressin-sensitive water channel that moves between an intracellular vesicular compartment and the apical membrane of collecting duct principal cells.[22,23] For some AQP2 mutations, including AQP2-T126M, the cellular defect results from protein misfolding and retention at the endoplasmic reticulum.[24,25] Interestingly, some endoplasmic reticulum-retained AQP2 mutants are functional as water channels, suggesting that maneuvers that correct AQP2 trafficking might restore the normal cell phenotype. One such strategy to correct protein folding defects is small molecule chemical chaperones such as glycerol and trimethyamine-N-oxide, which were shown to correct the cellular misprocessing of AQP2-T126M and restore water permeability in cell culture models.[26]

A mouse model of recessive NDI caused by the AQP2-T126M mutation was generated by targeted gene replacement (knock-in) using a Cre-loxP strategy.[10] Breeding of heterozygous mice yielded the expected Mendelian distribution with approximately 25% homozygous mutant mice. The mutant mice appeared normal at 2 to 3 days after birth, but failed to thrive and generally died by day 6. The mice were polyuric, with urinary hypo-osmolality and vasopressin insensitivity. Immunoblot analysis of kidney homogenates showed complex glycosylation of wild-type AQP2, but mainly endoglycosidase H-sensitive core glycosylation of AQP2-T126M, indicating endoplasmic reticulum (ER) retention, which was confirmed by immunofluorescence. The AQP2-T126M mutant mice provided the first in vivo biochemical data on a disease-causing AQP2 mutant. However, the mice did not survive long enough to test potential pharmacologic therapies. Recently AQP2-F204V mutant mice were identified by forward genetic screening of ethylnitrosourea-mutagenized mice.[27] AQP2-F204V is a relatively mild mutation compared with AQP2-T126M, permitting these mice to survive beyond the neonatal period. As additional mouse models of AQP2 mutation become available, it will be interesting to compare collecting duct water permeability with urinary concentrating function and vasopressin sensitivity.

We recently generated an adult inducible mouse model of complete severe NDI.[8] LoxP sequences were inserted into introns 1 and 2 in the mouse AQP2 gene by homologous recombination in embryonic stem cells. Mating of germ-line AQP2-loxP mice with tamoxifen-inducible Cre-expressing mice gave inducible homozygous Cre-AQP2-loxP mice, which had normal phenotype. Tamoxifen injections produced efficient AQP2 gene excision, with undetectable full-length AQP2 transcript in the kidney and more than 95% reduction in immunoreactive AQP2 protein. Urine osmolality decreased from approximately 2,000 before to less than 500 mOsm after tamoxifen treatment, with urine output increasing from 2 to more than 25

mL/d. The polyuria and reduced urine osmolality were not vasopressin sensitive, as expected. In contrast to the severe phenotype of NDI neonatal mice, only mild renal damage was seen after 6 weeks of severe polyuria in adult mice, with collecting duct dilation, yet normal creatinine clearance and serum chemistries. Breeding of inducible AQP2 knockout mice with AQP2 mutant knock-in mice is predicted to yield an inducible knock-in mouse, in which excision of the wild-type AQP2 gene in 1 allele leaves the mutant AQP2. Such adult AQP2 knock-in mice may be useful in testing possible drug therapies for NDI caused by recessive AQP2 mutations.

Renal Function in AQP7 and AQP8 Knockout Mice

AQP7 is expressed selectively in the apical membrane of epithelial cells in the relatively short S3 straight segment of the proximal tubule. AQP7, similar to AQP3 and AQP9, is an aquaglyceroporin that transports both water and glycerol, whereas most of the other AQPs are primarily water transporters. The renal phenotype of AQP7 null mice was characterized recently.[28] Osmotic water permeability in apical membrane vesicles from the proximal tubule was reduced slightly in AQP7 deficiency by approximately 10%, and urine concentrating ability was not impaired measurably. However, when AQP7 was co-deleted with AQP1, the urinary concentrating ability of the double-knockout mice was reduced compared with that of AQP1 null mice. The absence of significantly impaired urinary concentrating ability in AQP7 deficiency in mice may be related to the co-expression of AQP4 in mice in the S3 segment of the proximal tubule,[29] and expression of AQP1 throughout the proximal tubule. Interestingly, the AQP7 null mice showed marked glyceroluria, with greatly increased urinary glycerol concentration compared with wild-type mice (1.7 versus 0.005 mg/mL), despite comparable serum glycerol concentrations. How this is possible requires further investigation and verification. It was proposed that the urinary glycerol concentration might be a unique marker of S3 segment proximal tubule cell injury.

AQP8 transcript has been detected in mouse and rat kidneys at low levels. In 1 study, immunocytochemistry showed modest labeling of proximal tubules in a cytoplasmic pattern, and weak labeling of collecting ducts in cortex and medulla of rat kidney.[30] The renal phenotype of AQP8 null mice was characterized recently.[31] The urinary concentrating ability in AQP8 null mice was unimpaired as assessed by urine osmolality and weight loss after 36 hours of water deprivation, and by the similar impairment in urinary concentrating ability in AQP1 null mice versus AQP8/AQP1 double-knockout mice. In addition, we were unable to show specific renal AQP8 immunostaining using AQP8 null kidneys as control, together suggesting that, at least in mice, AQP8 does not play a significant role in renal function.
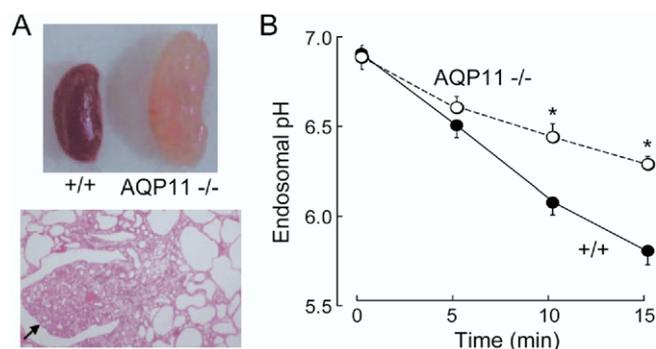

Figure 5 Renal cyst formation and defective endosomal acidification in proximal tubule cells in AQP11 deficiency. (A) Renal cysts in the kidney of a 30-day-old AQP11 null mouse. Kidney appearance is shown in the top photograph, and kidney histology (arrow indicates papilla) is shown in the bottom photograph. (B) Endosomal pH as measured at indicated times after pulse-labeling with fluorescent transferrin (SE, * $P < .01$). Adapted from Morishita et al.[32] (Color version of figure is available online.)

Cystic Kidney Disease Caused by AQP11 Gene Deletion

AQP11, which so far has an unknown function, shares homology with the other AQPs. AQP11 transcript is expressed in kidney, and immunocytochemistry suggested the localization of AQP11 protein to intracellular vesicles in the proximal tubule.[32] AQP11-deficient mice manifest an interesting phenotype of vacuolization and cyst formation in the proximal tubule. Over the first few weeks of life, the kidneys in AQP11 null mice become enlarged, with cysts occupying the whole cortex (Fig 5A), leading to renal failure and death. Before the appearance of cysts, the proximal tubule epithelium contains vacuoles. As a hint of the possible mechanism of vacuolization and cyst formation, we measured endosomal acidification and chloride accumulation in primary cultures of proximal tubule cells obtained from newborn mice. Although the cultures had a normal appearance, there was a distinct defect in endosomal acidification. For example, using fluorescent transferrin as a marker of early and recycling endosomes,[33] pulse label-chase studies showed impaired endosomal acidification in cells from AQP11 null mice (Fig 5B). Although AQP11 null mice represent an interesting model of renal cystic disease, the precise mechanisms responsible for proximal tubule cell vacuolization and cyst formation remain unknown, and will require definition of the function of AQP11.

A Novel Role for AQPs in Renal Cell Migration and injury

AQP1 is expressed strongly in microvascular endothelia outside of the central nervous system, including in tumor microvessels. The investigation of a possible role of AQP1 in tumor angiogenesis showed an unanticipated role of AQPs in cell migration.[34] Growth of implanted tumors was

slowed greatly in AQP1 null compared with wild-type mice, and the tumors in AQP1 null mice had a lower density of microvessels with islands of viable tumor cells surrounded by necrotic tissue. Angiogenesis also was impaired in these mice in an in vivo tumor-independent model involving vessel growth in implanted Matrigel pellets containing angiogenic factors. Analysis of intrinsic endothelial cell functions in cultured aortic endothelia from wild-type and AQP1 null mice indicated that cell migration toward a chemoattractant was slowed significantly in AQP1-deficient endothelial cells. We proposed that AQP-dependent water transport could account for the impaired migration in AQP1-deficient cells because migration involves transient formation of membrane protrusions that are believed to require rapid local changes in ion fluxes and transmembrane water movement. We found that exogenously expressed AQP1 or AQP4 enhanced migration of AQP-null CHO and Fisher rat thyroid (FRT) epithelial cells, suggesting that the effect was not AQP or cell-type specific. A similar defect in migration was found in AQP4 null and knock-down astroglial cells cultured from neonatal mouse brain.[35] In these systems AQP expression was polarized to the leading edge of migrating cells, and lamellipodial dynamics were reduced in AQP-deficient cells. A model incorporating these findings is presented in Figure 6C.

We recently performed similar experiments to determine whether AQP-dependent cell migration occurs in kidney.[36] Migration was compared in primary cultures of proximal tubule cells from wild-type and AQP1 null mice. Primary cultures of proximal tubule cells from AQP1 null mice were indistinguishable from those of wild-type mice in their appearance, size, growth/proliferation, and adhesiveness, although as expected they had reduced plasma membrane water permeability. Migration of AQP1-deficient cells was reduced by more than 50% compared with wild-type cells, as measured in a Boyden chamber in the presence of a chemotactic stimulus (Fig 4A). Impaired migration of AQP1-deficient cells also was found in an in vitro scratch assay of wound healing, with reduced appearance of lamella-like membrane protrusions at the cell leading edge. Adenoviral-mediated expression of AQP1 in the AQP1-deficient cells, which increased their water permeability to that of wild-type cells, corrected their migration defect. The potential relevance of these in vitro findings to the intact kidney was tested in an in vivo model of acute tubular injury caused by 30-minute renal artery occlusion-reperfusion. At 5 days after ischemia-reperfusion, kidneys in AQP1 null mice showed remarkably greater tubular injury, as seen by tubule dilation, than kidneys in wild-type mice (Fig 4B). Also, F-actin was remarkably disorganized in proximal tubule epithelial cells in the AQP1 null mice after ischemia-reperfusion, although cell proliferation as assessed by bromodeoxyuridine immunocytochemistry was not impaired. These results provided evidence for the involvement of AQP1 in the migration of proximal tubule cells, and possibly in the response of the proximal tubule to injury. Other renal AQPs also may facilitate the migration of cells where they are expressed.
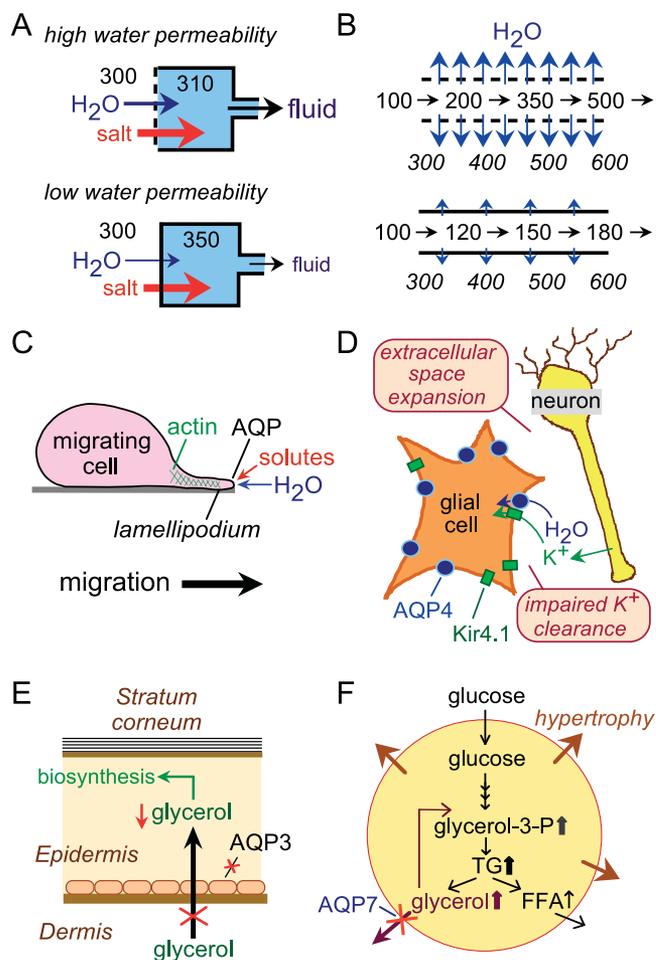

Figure 6 Proposed mechanisms of AQP function in mammalian physiology. (A) Reduced water permeability in glandular epithelium impairs active, near-isosmolar fluid transport by slowing osmotic water transport into the acinar lumen, producing hypertonic secretion. (B) Reduced transepithelial water permeability in the kidney collecting duct impairs the urinary concentrating ability by preventing osmotic equilibration of luminal fluid. (C) AQP-facilitated water entry into protruding lamellipodia, accounting for AQP-dependent cell migration. (D) AQP4 expression on astroglia may alter extracellular space volume and composition by interactions with Kir4.1 K^+ channels, and/or by impairing water reuptake into astroglia during neural signaling. (E) Reduced steady-state glycerol content in epidermis and stratum corneum after AQP3 deletion, accounting for reduced skin hydration in AQP3 deficiency. (F) Impaired AQP7-dependent glycerol escape from adipocytes resulting in intracellular glycerol accumulation and increased triglyceride content, accounting for progressive adipocyte hypertrophy in AQP7 deficiency. See text for further explanations. (Color version of figure is available online.)

Mechanisms of AQP Function in Mammalian Physiology

Figure 6 shows the proposed mechanisms for involvement of AQPs in mammalian physiology. As discussed earlier, the impaired fluid absorption in the kidney proximal tubule in AQP1 deficiency indicates the need for high transepithelial water permeability for rapid, near-isosmolar fluid transport. A similar conclusion was found for near-isosmolar fluid se-

cretion in salivary[37] and airway submucosal[38] glands in AQP5 deficiency, and for AQP1-dependent secretion of cerebrospinal fluid by choroid plexus[39] and aqueous fluid by the ciliary epithelium.[40] High transepithelial water permeability permits rapid water transport in response to active transepithelial salt transport. As shown in Figure 6A, AQP deletion impairs osmotic equilibration, resulting in reduced secretion of a relatively hypertonic fluid. Figure 6B shows a second mechanism for involvement of AQPs in mammalian physiology, in which the AQPs facilitates passive, osmotically driven water transport. As an example, osmotic extraction of water in the kidney collecting duct increases urine osmolality in antidiuresis. Reduced collecting duct water permeability produced by AQP deficiency impairs osmotic equilibration and thus reduces urine osmolality. Brain edema is an example of AQP4-facilitated water transport between the blood and brain compartments. Mice lacking AQP4 show remarkable protection against brain water accumulation in models of cytotoxic brain edema such as water intoxication and ischemic stroke,[41] but impaired clearance of excess brain water in models of vasogenic brain edema such as brain tumor.[42]

Figure 6C shows a proposed mechanism of AQP-dependent cell migration. Actin cleavage and ion uptake at the tip of a lamellipodium create local osmotic gradients that drive the influx of water across the cell membrane. Water entry increases local hydrostatic pressure to cause cell membrane protrusion, creating space for actin polymerization and stabilization of the protrusion. AQPs in the region of membrane protrusions would enhance water entry and thus the dynamics of cell membrane protrusions and cell motility.

Several lines of evidence suggest the involvement of AQP4 in rapid neural signal transduction by a mechanism that is not yet understood clearly. AQP4 is expressed in the central nervous system, inner ear, and retina in supportive cells that are in close proximity to non–AQP-expressing electrically excitable cells—in astroglia-supporting neurons, in retinal Müller cells supporting bipolar cells, and in cochlear Claudius and Hensen cells supporting hair cells. Mice lacking AQP4 showed altered seizure susceptibility and duration,[43,44] and reduced auditory brainstem responses.[45] Based on AQP4 and K^+ channel Kir4.1 colocalization, several groups proposed that AQP4 in supportive cells may facilitate K^+ recycling during electrical signal transduction in the brain, retina, and inner ear. Figure 6D postulates alterations in extracellular space volume and astroglial cell K^+ uptake that could account for defective neural signal transduction in AQP4 deficiency. In support of this hypothesis, we found, by using an in vivo fluorescence photobleaching method, an expanded extracellular space in the cerebral cortex in AQP4-deficient mice.[46] By using a K^+-sensing fluorescent dye, we recently found 2-fold impaired K^+ reuptake from the extracellular space in a pin-prick model of spreading depression.[47] However, the precise link between AQP4 expression and K^+ channel function remains unclear.

The earlier-described roles of AQPs can be ascribed largely to their water-transporting function. There is now compelling evidence that the aquaglyceroporins have unique biological roles that are related to their glycerol transport function.

There is evidence for AQP3-facilitated glycerol transport in skin as an important determinant of epidermal and stratum corneum hydration (Fig 6E). AQP3 is expressed strongly in the basal layer of keratinocytes in mammalian skin. Mice lacking AQP3 have reduced SC hydration and skin elasticity, delayed biosynthesis of the SC after removal by tape-stripping, and delayed wound healing.[48] Investigation of the mechanisms responsible for the skin phenotype in AQP3 deficiency showed reduced epidermal cell skin glycerol permeability, and reduced glycerol content in the SC and epidermis, with normal glycerol in dermis and serum. This suggests that there is reduced glycerol transport from blood into the epidermis in AQP3 deficiency through the basal keratinocytes in the AQP3-null mice. Glycerol replacement by topical or systemic routes corrected each of the skin phenotype abnormalities in AQP3-null mice.[49] Together, the phenotype data in AQP3-deficient mice indicate the importance of glycerol in epidermal cell function and provide a rational scientific basis for the long-standing practice of including glycerol in cosmetic and medicinal skin-treatment preparations.

A principal site of expression of AQP7 is the plasma membrane of adipocytes. We found that AQP7 null mice have a much greater fat mass than wild-type mice as they age.[50] Moreover, their adipocytes remarkably hypertrophy with age, and accumulate approximately 3-fold more glycerol and approximately 2-fold more triglycerides than adipocytes in wild-type mice. Measurements in adipocytes of comparable size from younger mice showed reduced glycerol permeability in AQP7-deficient adipocytes and slowed glycerol release from minced fat tissue. However, lipolysis and lipogenesis rates were similar in wild-type and AQP7-deficient mice. As shown in Figure 6F, we propose that the progressive triglyceride accumulation in AQP7-deficient adipocytes may be related to reduced plasma membrane glycerol permeability, resulting in increased steady-state glycerol concentration, and increased glycerol-3-phosphate, and, hence, triglyceride biosynthesis. Induction of adipocyte AQP7 expression and/or function therefore might reduce fat mass in some forms of obesity. Similar conclusions were reported subsequently by Hibuse et al[51] using independently created AQP7 null mice.

Conclusions

Renal phenotype studies of AQP knockout mice have confirmed the predicted roles of AQPs in the urinary concentrating mechanism, and have provided evidence to settle controversies about the route and mechanism of fluid absorption in the proximal tubule and the roles of AQP versus non-AQP water transporters. Transgenic mice deficient in the renal AQPs also have provided useful models to study renal physiology and clinically relevant diseases such as NDI. Although many of the key issues about the roles of AQPs in kidney function now are resolved, recent findings of AQP-dependent renal cell migration and renal cystic disease warrant further mechanistic investigation and evaluation of their relevance to kidney disease. Perhaps most exciting for ongoing work is the possibility of the development of small-molecule

or peptide inhibitors and inducers of specific renal AQPs for therapy of dysnatremias and fluid overload, and even perhaps for therapy of cystic renal disease and renal injury.

References

1. Nielsen S, Frokiaer J, Marples D, et al: Aquaporins in the kidney: From molecules to medicine. Physiol Rev 82:205-244, 2002

2. Chen YC, Cadnapaphornchai MA, Schrier RW: Clinical update on renal aquaporins. Biol Cell 97:357-371, 2005

3. McReynolds MR, Taylor-Garcia KM, Greer KA, et al: Renal medullary gene expression in aquaporin-1 null mice. Am J Physiol 288:F315-F321, 2005

4. Kim SW, Gresz V, Rojek A, et al: Decreased expression of AQP2 and AQP4 water channels and Na,K-ATPase in kidney collecting duct in AQP3 null mice. Biol Cell 97:765-778, 2005

5. Rao S, Verkman AS: Analysis of functional organ physiology in transgenic mice. Am J Physiol 279:C1-C18, 2000

6. Ma T, Yang B, Gillespie A, et al: Severely impaired urinary concentrating ability in transgenic mice lacking aquaporin-1 water channels. J Biol Chem 273:4296-4299, 1998

7. Ma T, Song Y, Yang B, et al: Nephrogenic diabetes insipidus in mice deficient in aquaporin-3 water channels. Proc Natl Acad Sci U S A 97:4386-4391, 2000

8. Yang B, Ma T, Verkman AS: Erythrocyte water permeability and renal function in double knockout mice lacking aquaporin-1 and aquaporin-3. J Biol Chem 276:624-628, 2001

9. Ma T, Yang B, Gillespie A, et al: Generation and phenotype of a transgenic knock-out mouse lacking the mercurial-insensitive water channel aquaporin-4. J Clin Invest 100:957-962, 1997

10. Yang B, Gillespie A, Carlson EJ, et al: Early neonatal mortality in a transgenic AQP2 knock-in model of nephrogenic diabetes insipidus. J Biol Chem 276:2775-2779, 2001

11. Yang B, Zhou D, Verkman AS: Mouse model of inducible nephrogenic diabetes insipidus produced by floxed aquaporin-2 gene deletion. Am J Physiol, 2006 (in press)

12. Schnermann J, Chou CL, Ma T, et al: Defective proximal tubular fluid reabsorption in transgenic aquaporin-1 null mice. Proc Natl Acad Sci U S A 95:9660-9664, 1998

13. Vallon V, Verkman AS, Schnermann J: Luminal hypotonicity in proximal tubules of aquaporin-1 knockout mice. Am J Physiol 278:F1030-F1033, 2000

14. Hashimoto S, Huang Y, Mizel D, et al: Compensation of proximal tubule malabsorption in AQP1-deficient mice without TGF-mediated reduction of GFR. Acta Physiol Scand 181:455-462, 2004

15. Chou CL, Knepper MA, Van Hoek AN, et al: Reduced water permeability and altered ultrastructure in thin descending limb of Henle in aquaporin-1 null mice. J Clin Invest 103:491-496, 1999

16. Pallone TL, Edwards A, Ma T, et al: Requirement of aquaporin-1 for NaCl driven water transport across descending vasa recta. J Clin Invest 105:215-222, 2000

17. King LS, Choi M, Fernandez PC, et al: Defective urinary-concentrating ability due to a complete deficiency of aquaporin-1. N Engl J Med 345:175-179, 2001

18. Chou CL, Ma T, Yang B, et al: Four-fold reduction in water permeability in inner medullary collecting duct of aquaporin-4 knockout mice. Am J Physiol 274:C549-C554, 1998

19. Frigeri A, Gropper M, Umenishi F, et al: Localization of MIWC and GLIP water channel homologs in neuromuscular, epithelial and glandular tissues. J Cell Sci 108:2993-3002, 1995

20. Yang B, Brown D, Verkman AS: The mercurial insensitive water channel (AQP-4) forms orthogonal arrays in stably transfected Chinese hamster ovary cells. J Biol Chem 271:4577-4580, 1996

21. Verbavatz JM, Ma T, Gobin R, et al: Absence of orthogonal arrays in kidney, brain and muscle from transgenic knockout mice lacking water channel aquaporin-4. J Cell Sci 110:2855-2860, 1997

22. Deen PM, Verdijk MA, Knoers NV, et al: Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. Science 264:92-95, 1994

23. Morello JP, Bichet DG: Nephrogenic diabetes insipidus. Annu Rev Physiol 63:607-630, 2001

24. Deen PM, Croes H, van Aubel RA, et al: Water channels encoded by mutant aquaporin-2 genes in nephrogenic diabetes insipidus are impaired in their cellular routing. J Clin Invest 95:2291-2296, 1995

25. Tamarappoo BK, Verkman AS: Misfolding of mutant aquaporin-2 water channels in nephrogenic diabetes insipidus. J Biol Chem 274:34825-34831, 1999

26. Tamarappoo BK, Verkman AS: Defective trafficking of AQP2 water channels in nephrogenic diabetes insipidus and correction by chemical chaperones. J Clin Invest 101:2257-2263, 1998

27. Lloyd DJ, Hall FW, Tarantino LM, et al: Diabetes insipidus in mice with a mutation in aquaporin-2. PLoS Genet 1:e20, 2005

28. Sohara E, Rai T, Miyazaki J, et al: Defective water and glycerol transport in the proximal tubules of AQP7 knockout mice. Am J Physiol 289:F1195-F1200, 2005

29. Van Hoek AN, Ma T, Yang B, et al: Aquaporin-4 is expressed in basolateral membranes of juxtamedullary proximal tubules in mouse kidney. Am J Physiol 278:F310-F316, 2000

30. Elkjaer ML, Nejsum LN, Gresz V, et al: Immunolocalization of aquaporin-8 in rat kidney, gastrointestinal tract, testis, and airways. Am J Physiol 281:F1047-F1057, 2001

31. Yang B, Song Y, Zhao D, et al: Phenotype analysis of aquaporin-8 null mice. Am J Physiol 288:C1161-C1170, 2005

32. Morishita Y, Matsuzaki T, Hara-chikuma M, et al: Disruption of aquaporin-11 produces polycystic kidneys following vacuolization of the proximal tubule. Mol Cell Biol 25:7770-7779, 2005

33. Sonawane N, Verkman AS: Determinants of [Cl⁻] in recycling and late endosomes and Golgi complex measured using fluorescent ligands. J Cell Biol 160:1129-1138, 2003

34. Saadoun S, Papadopoulos MC, Hara-Chikuma M, et al: Impairment of angiogenesis and cell migration by targeted of aquaporin-1 gene disruption. Nature 434:786-792, 2005

35. Saadoun S, Papadopoulos MC, Watanabe H, et al: Involvement of aquaporin-4 in astroglial cell migration and glial scar formation. J Cell Sci 118:5691-5698, 2005

36. Hara-Chikuma M, Verkman AS: Aquaporin-1 facilitates migration of epithelial cells in kidney proximal tubule. J Am Soc Nephrol 17:38-45, 2006

37. Ma T, Song Y, Gillespie A, et al: Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. J Biol Chem 274:20071-20074, 1999

38. Song Y, Verkman AS: Aquaporin-5 dependent fluid secretion in airway submucosal glands. J Biol Chem 276:41288-41292, 2001

39. Oshio K, Song Y, Verkman AS, et al: Reduced intraventricular pressure and cerebrospinal fluid production in mice lacking aquaporin-1 water channels. FASEB J 18:76-78, 2005

40. Zhang D, Vetrivel L, Verkman AS: Aquaporin deletion in mice reduces intraocular pressure and aqueous fluid production. J Gen Physiol 119:561-569, 2002

41. Manley GT, Fujimura M, Ma T, et al: Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. Nature Med 6:159-163, 2000

42. Papadopoulos MC, Manley GT, Krishna S, et al: Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. FASEB J 18:1291-1293, 2004

43. Binder DK, Yao X, Sick TJ, et al: Increased seizure duration and slowed potassium kinetics in mice lacking aquaporin-4 water channels. Glia 53:631-636, 2006

44. Binder DK, Oshio K, Ma T, et al: Increased seizure threshold in mice lacking aquaporin-4 water channels. Neuroreport 15:259-262, 2004

45. Li J, Verkman AS: Impaired hearing in mice lacking aquaporin-4 water channels. J Biol Chem 276:31233-31237, 2001

46. Binder DK, Papadopoulos MC, Haggie PM, et al: In vivo measurement of brain extracellular space diffusion by cortical surface photobleaching. J Neurosci 24:8049-8056, 2004

47. Padmawar P, Yao X, Bloch O, et al: K⁺ waves in brain cortex visualized using a long-wavelength K⁺-sensing fluorescent indicator. Nature Meth 2:825-827, 2005

48. Hara M, Ma T, Verkman AS: Selectively reduced glycerol in skin of aquaporin-3-deficient mice may account for impaired skin hydration, elasticity and barrier recovery. J Biol Chem 277:46616-46621, 2002

49. Hara M, Verkman AS: Glycerol replacement corrects defective skin hydration, elasticity, and barrier function in aquaporin-3-deficient mice. Proc Natl Acad Sci U S A 100:7360-7365, 2003

50. Hara-Chikuma M, Sohara E, Rai T, et al: Progressive adipocyte hypertrophy in aquaporin-7 deficient mice: Adipocyte glycerol permeability as a novel regulator of fat accumulation. J Biol Chem 280:15493-15496, 2005

51. Hibuse T, Maeda N, Funahashi T, et al: Aquaporin 7 deficiency is associated with development of obesity through activation of adipose glycerol kinase. Proc Natl Acad Sci U S A 102:10993-10998, 2005