Lessons on Renal Physiology from Transgenic Mice Lacking Aquaporin Water Channels

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Abstract. Several aquaporin-type water channels are expressed in kidney: AQP1 in the proximal tubule, thin descending limb of Henle, and vasa recta; AQP2, AQP3, and AQP4 in the collecting duct; AQP6 in the papilla; and AQP7 in the proximal tubule. AQP2 is the vasopressin-regulated water channel that is important in hereditary and acquired diseases affecting urine-concentrating ability. It has been difficult to establish the roles of the other aquaporins in renal physiology because suitable aquaporin inhibitors are not available. One approach to the problem has been to generate and analyze transgenic knockout mice in which individual aquaporins have been selectively deleted by targeted gene disruption. Phenotype analysis of kidney and extrarenal function in knockout mice has been very informative in defining the role of aquaporins in organ physiology and addressing basic questions regarding the route of transepithelial water transport and the mechanism of near osmolar fluid reabsorption. This article describes new renal physiologic insights revealed by phenotype analysis of aquaporin-knockout mice and the prospects for further basic and clinical developments.

The movement of water across cell plasma membranes occurs in all cell types but is particularly rapid in renal tubular epithelia and microvascular endothelia. In principle, osmotic/oncotic gradients and hydrostatic pressure differences can drive water across a cell layer by transcellular or paracellular pathways (Figure 1A). Transcellular water movement requires entry of water into the cell across the plasma membrane, movement across the cell interior, and exit across a different region of the plasma membrane. Substantial quantities of water can move through lipid membranes even in the absence of membrane proteins. Heterologous expression experiments suggest that some membrane transporting proteins, including glucose transporters, CFTR Cl− channels, and Na+-coupled glucose/amine acid transporters, are able to pass water, although there is no evidence that these proteins provide a quantitatively significant contribution to the water permeability of mammalian cell membranes. The aquaporin family of molecular water channels, which now number 10 in mammals and many more in plants and lower organisms, are likely to provide a major pathway for water transport in some cell membranes. As reviewed recently (1–4), much is known regarding aquaporin molecular genetics, tissue distribution, molecular structure, and function. However, as discussed below, there has been little investigation regarding the physiologic significance of aquaporins.

At least six aquaporin water channels are expressed in kidney (Figure 1B). Readers are referred to recent reviews (5–7) for details and original citations. AQP1 is expressed in apical and basolateral plasma membranes in the proximal tubule and the thin descending limb of Henle (TDLH) and in the microvascular endothelium of the medullary descending vasa recta. AQP2 is expressed in collecting duct principal cells and undergoes vasopressin-regulated trafficking between an intracellular vesicular compartment and the cell apical plasma membrane. AQP3 and AQP4 are coexpressed at the basolateral membrane of the same collecting duct epithelial cells, with relatively more AQP3 in proximal segments of the collecting duct and more AQP4 in the inner medullary collecting duct (IMCD). AQP6 transcript is expressed strongly in kidney but has not been immunolocalized, and preliminary evidence suggests that AQP7 is expressed in distal segments of proximal tubule epithelium. There may be additional, as yet unidentified, aquaporins in kidney and, although few or no transcripts encoding AQP8 and AQP9 appear to be expressed in kidney, immunocytochemical analysis is required to rule out expression of these proteins in a small subpopulation of cell types.

AQP2 is clearly important in renal physiologic processes and human disease. Humans with mutations in AQP2 have non-X-linked hereditary nephrogenic diabetes insipidus (NDI) (8,9). AQP2 expression is upregulated during dehydration and high plasma vasopressin levels, and is downregulated in many cases of acquired NDI (10,11). Rare human subjects with AQP1 mutations and a reportedly normal phenotype have been identified (12), but these individuals have not been subject to renal function tests or other clinical studies. Natural mutations of the other aquaporins have not been found, and suitable nontoxic inhibitors do not exist for any of the aquaporins. Our laboratory has studied the physiologic role of aquaporins by
generating and performing phenotype analysis of transgenic mice lacking specific aquaporins. This article reviews recent data obtained with AQP1- and AQP4-knockout mice. Several additional aquaporin-transgenic mice will soon be available, including AQP3-null mice (13); phenotype analysis of these mice will be the subject of future reports.

**Generation and Initial Phenotype Analysis of Knockout Mice**

The generation of knockout mice by targeted gene disruption has become a routine procedure. A targeting vector is constructed in which homologous recombination in embryonic stem (ES) cells produces a defective gene with partial deletion of the coding sequence. The targeting vector used for AQP1 deletion is shown in Figure 2A (14). Notable features in the vector design include the use of sufficiently long arms of genomic sequence matching the ES cell genome (generally, total arm length of $\geq 4$ kb), the insertion of selection markers (here positive Neo and negative TK cassettes) for selection of ES cell clones with the targeted gene insertion, and the use of one relatively short arm for PCR screening. Generally, some minimal knowledge of the genomic structure of the targeted gene is needed, although PCR amplification using genomic DNA as the template and exon-derived primers can be used to generate the genomic arms rapidly by trial and error, using different primer sets. After ES cell transfection and selection of clones with the correctly mutated gene locus, the ES cells are transferred to pseudopregnant female mice to produce chimeric offspring. Breeding of the chimeric and wild-type mice yields heterozygotes containing the modified gene in the germ line, which are then bred to produce homozygous mice with the null genotype. Readers are referred to reference (15) for additional details regarding transgenic technology, including knock-in, conditional, and tissue-specific transgenic animals. Because several breeding steps are required, the generation of knockout mice generally requires a minimum of 8 mo.

In the case of AQP1, after litters were obtained from intercrosses of germ-line heterozygotes, the mice were genotyped...
and the null genotype was confirmed by the absence of full-length transcript and detectable protein. Figure 2B shows a genomic Southern blot, kidney Northern blot, and kidney immunoblot of wild-type, heterozygous, and AQP1-knockout mice. As expected, no transcript or protein was detected in the knockout mice.

Initial phenotype analysis included determination of the genotypic distribution of offspring from heterozygote matings and observations of gross mouse appearance, activity, survival, growth, and organ morphology. In the case of AQP1, the number of knockout mice (genotyped 5 d after birth) was significantly below the predicted Mendelian 1:2:1 ratio for wild-type/heterozygous/knockout mice (distribution to date, 234:387:166). This finding indicates impaired survival of AQP1-knockout mice in utero and/or in the early neonatal period. The AQP1-knockout mice were consistently smaller (10 to 15% reduced body weight) than litter-matched heterozygotes or wild-type mice but had normal survival rates and no other overt differences in superficial appearance. In contrast, the genotypic distribution for the mating of AQP4-heterozygous mice conformed to the Mendelian distribution (16). The AQP4-knockout mice were indistinguishable from wild-type mice in terms of growth, survival rates, and other overt features (16).

There are several caveats regarding analysis of the physiologic role of a protein on the basis of phenotype analysis of knockout mice. Apparent loss of function can result directly from protein deletion or indirectly from factors such as altered organ development/structure or hemodynamics. To move beyond empirical phenotype descriptions, appropriate experiments are required to establish the mechanism by which protein deletion in a knockout mouse model produces phenotype differences. Because of differences in mouse and human physiology, the extrapolation of data from mouse studies to human physiology must be made cautiously. For example, the maximal osmolality of mouse urine (>3000 mosmol) is much greater than that of human urine, and the pH of mouse urine is usually approximately 7. Finally, the observed phenotype of transgenic mice depends to some extent on genetic background (mouse strain) and various environmental and dietary factors. The generation of knockout mice thus represents a small component of a transgenic project; meaningful phenotype analysis can be very challenging, often requiring creative surgical and physiologic strategies.

### Urine-Concentrating Ability of AQP1- and AQP4-Knockout Mice

To determine whether AQP1 or AQP4 deletion affects integrated renal function, initial studies were performed to determine whether the knockout mice were polydipsic/polyuric and could concentrate their urine. The AQP1-knockout mice ingested 2.5- to 3-fold more fluid than did heterozygous or wild-type mice, whereas the AQP4-knockout mice and litter-matched wild-type mice ingested similar amounts of water. In response to a 36-h water deprivation, the AQP1-knockout mice became severely dehydrated and lethargic (14). Total body weight decreased by 35%, serum osmolality in many mice increased to >500 mosmol, and urinary osmolality did not increase above that measured before water deprivation (Figure 3). The wild-type and heterozygous mice remained active after water deprivation, body weight decreased by 20 to 22%, serum osmolality remained normal (310 to 330 mosmol), and urinary osmolality increased to >2500 mosmol. The urinary Na⁺ concentration in water-deprived knockout mice was <10 mM, and urinary osmolality was not increased by the V2 receptor agonist deamino-8-D-arginine vasopressin. These initial findings suggested that the AQP1-knockout mice are unable to create a hypertonic medullary interstitium by countercurrent multiplication. The AQP4-knockout mice exhibited a small but significant decrease in maximal urinary osmolality after 36 h of water deprivation (Figure 3) but did not manifest clinical signs of severe dehydration.

### Role of AQP1 in Proximal Tubule Function

The proximal tubule performs near iso-osmolar reabsorption of the majority of fluid that is filtered by the glomerulus. The proximal tubule also reabsorbs nearly all of the filtered glucose, amino acids, and bicarbonate. The apical and basolateral plasma membranes of proximal tubule cells contain AQP1, suggesting that at least some water should be able to move across the proximal tubule by a transcellular pathway. However, there is conflicting functional evidence that significant paracellular water transport occurs (17), and it has been suggested that the AQP7 water channel and the Na⁺/glucose cotransporter might contribute to transcellular water movement. It is generally thought, but without direct evidence, that high proximal tubule water permeability is important to permit

![Figure 3](image-url)
the efficient coupling of solute transport and water flow to accomplish near iso-osmolar fluid absorption (18). These issues were investigated using the AQP1-knockout mice (19).

Transepithelial osmotic water permeability ($P_f$) was measured in isolated microperfused S2 segments of proximal tubule, using a raffinose gradient to drive water out of the tubule lumen. $P_f$ was decreased nearly fivefold in AQP1-knockout mice (knockout, 0.033 ± 0.005 cm/s; wild-type, 0.15 ± 0.03 cm/s) (Figure 4). Therefore, the major pathway for osmotically driven water transport in the perfused S2 segment of the proximal tubule is transepithelial and is mediated by AQP1 water channels. Stopped-flow measurements indicated a ninefold decreased $P_f$ value (at 10°C) in purified apical plasma membrane vesicles from proximal tubules of AQP1-knockout mice, compared with wild-type mice, and showed that the remaining low water permeability in vesicles from knockout mice was not inhibited by mercurial agents (14). Assuming a folding factor of approximately 10 to account for membrane redundancy in the proximal tubule, and assuming equal apical and basolateral membrane water permeabilities, the $P_f$ of 0.033 cm/s in knockout mice suggests an intrinsic membrane water-permeable cell membranes (18). It is noted that the 50% decrease in proximal tubule fluid reabsorption is less than the 78% decrease in proximal tubule water permeability, suggesting greater luminal hypotonicity in AQP1-knockout mice, compared with wild-type mice. It will be important to determine osmolalities, ion concentrations, and transepithelial membrane potentials along the tubule axis to define the driving forces for NaCl and water absorption in AQP1 deficiency.

Fluid collections made in the superficial distal tubule yielded fractional fluid absorption values of 76.2 ± 2.7% in wild-type mice and 61.9 ± 3.9% in knockout mice ($P = 0.018$) (Figure 5). Interestingly, flow rates in the distal nephron did not differ between wild-type and knockout mice (2.63 ± 0.45 nl/min versus 1.95 ± 0.29 nl/min, $P = 0.14$). The normal flow rates in knockout mice, despite greatly reduced absorption rates, are related to a marked reduction in the single-nephron GFR (SNGFR) calculated from distal fluid collections (wild-type, 11.1 ± 1.6 nl/min; knockout, 5.1 ± 0.4 nl/min; $P < 0.001$). In contrast, SNGFR values calculated from proximal fluid collections did not differ significantly (wild-type, 9.6 ± 1.5 nl/min; knockout, 8.1 ± 0.7 nl/min). In view of the defective fluid absorption along the proximal tubule and the increased urine flow in AQP1-knockout mice, it was surprising that distal fluid delivery was not increased in the knockout mice. The reason probably involves activation of the tubuloglomerular feedback (TGF) mechanism (20). SNGFR values determined from proximal fluid collections were similar in wild-type and knockout mice, whereas SNGFR values determined from distal fluid collections, with macula densa segment perfusion intact, was reduced in the knockout mice. The mechanism of TGF activation in the knockout mice is not clear. The NaCl delivery signal at the macula densa may be elevated because of decreased NaCl and water absorption in the proximal tubule, and/or AQP1 deficiency may be associated with resetting of the TGF dose–response curve because of chronic extracellular volume depletion. Nevertheless, decreased GFR in AQP1-knockout mice is an appropriate compensatory response to the threat of
NaCl depletion caused by defective proximal tubule reabsorption. Additional studies are required to determine whether other compensatory responses occur, such as upregulation of tubule ion transporters or changes in interstitial barrier properties.

The increased urinary flow rates, despite normal distal delivery, suggest that the diuresis seen in AQP1-knockout mice results primarily from reduced fluid absorption in the collecting duct. Given the normally high levels of expression of AQP1 in the descending limb of Henle and the descending vasa recta, it is likely that AQP1 deletion results in a defective countercurrent mechanism that prevents the formation of a hyperosmolar medullary interstitium. This conclusion is supported by the finding that, in water-deprived AQP1-knockout mice, deamino-8-D-arginine vasopressin stimulation of collecting duct water permeability (which should nearly equalize urinary and medullary interstitial osmolalities) did not increase urinary osmolality (14). Measurements of interstitial osmolalities and mathematical modeling of the countercurrent mechanism are indicated for further analysis of the concentrating defect in AQP1-deficiency. Unlike in NDI, where urinary osmolality is generally quite low, the urine can be mildly concentrated in AQP1 deficiency, because salt transporters are functional and the collecting duct can be water permeable. It may be for this reason that no overt abnormalities were found in AQP1-deficient human patients not subjected to water-deprivation stress (12). Studies of TDLH function (see below) and measurements of water permeability in perfused outer medullary descending vasa recta (T. Pallone, T. Ma, and A. S. Verkman, unpublished data) support the conclusion that the major defect in AQP1 deletion is defective countercurrent multiplication.

**Role of AQP1 in the TDLH**

The TDLH has an important role in the formation of concentrated urine by countercurrent multiplication. Measurements from several laboratories have indicated that the transepithelial $P_f$ is exceptionally high. The transport of water through aqueous pores in TDLH cell membranes was suggested from the findings of a high osmotic/diffusional water permeability ratio and the inhibition of water permeability by the mercurial agent $p$-chloromercuribenzenzene sulfonate. The high levels of expression of AQP1 in the TDLH suggested a molecular basis for the exceptionally high $P_f$. Measurement of AQP1 protein levels in microdissected tubule segments, using a fluorescence-based enzyme-linked immunosorbent assay method, showed that the TDLH of long-looped nephrons have the highest AQP1 content among nephron segments (21). To directly determine the contribution of AQP1 to TDLH water permeability, transepithelial $P_f$ values were measured in isolated perfused segments of TDLH from wild-type, heterozygous, and AQP1-null mice (22). $P_f$ values were measured at $37^\circ$C using a 100 mM bath-to-lumen osmotic raffinose gradient, with FITC-dextran as the luminal volume marker. $P_f$ was remarkably decreased in the AQP1-knockout mice (wild-type, $0.26 \pm 0.02$ cm/s; heterozygous, $0.21 \pm 0.01$ cm/s; AQP1-knockout, $0.031 \pm 0.007$ cm/s) (Figure 4). These results indicate that AQP1 is the principal water channel in the TDLH, and they support the view that osmotic equilibration along the TDLH by water transport plays a key role in the renal coun-

![Figure 5](Image)

**Figure 5.** Free-flow micropuncture studies in wild-type and AQP1-knockout mice. Averaged microperfusion data (adapted from reference (19)) for the end proximal tubule (left) and distal tubule (right) fluid collections are shown. See text for details. TF/P, collected tubule fluid/plasma marker ratio; SNGFR, single nephron GFR; % absorption, percentage fluid absorption.
tercurrent concentrating mechanism. The similar $P_f$ values and AQP1 expression levels in the TDLH of wild-type and heterozygous mice was an unexpected finding that probably accounts for the unimpaired urine-concentrating ability of heterozygous mice. Interestingly, quantitative immunoblot analysis of kidney membranes indicated that the heterozygotes expressed 70 to 80% of the amount of AQP1 protein found in kidney tissue from wild-type mice.

There is a long-standing controversy regarding the relative contributions of water reabsorption and solute entry to osmotic equilibration along the TDLH (reviewed in reference (23)). Deletion of AQP1 produced a profound defect in urine-concentrating ability and a decrease in TDLH water permeability, suggesting that high water permeability in the TDLH is necessary for urine concentration. Although the NaCl and urea permeabilities of the TDLH have not been measured, they are unlikely to be greatly affected by deletion of AQP1, because AQP1 does not transport NaCl or urea. Therefore, it is concluded that osmotic water transport out of the lumen of the TDLH is important for the countercurrent multiplication mechanism and that solute entry itself is not sufficient to permit the formation of maximally concentrated urine. Modeling of the concentrating mechanism in the AQP1-null mice should be helpful in resolving the quantitative contributions of TDLH water reabsorption and solute entry.

**AQP1 and Peritoneal Dialysis**

The peritoneal cavity is lined by a membrane barrier that provides a large surface for fluid movement between peritoneal capillaries and the peritoneal cavity. Clinically, marked ascites can accumulate in conditions associated with decreased serum oncotic pressure, increased portal venous pressure, or peritoneal cavity inflammation/infection. The large peritoneal surface is exploited in peritoneal dialysis, where water, electrolytes, urea, and urea-causing toxins are extracted from blood by repeated infusion and removal of dialysate solutions into the peritoneal cavity. Modeling of peritoneal dialysis indicates the presence of “ultra-small, water-only” pores that are selective for water and thus responsible for osmotically induced water transport (24). Several studies have suggested that aquaporins might constitute part of the water-only pathway in peritoneal dialysis. Immunocytochemical analysis showed abundant AQP1 in microvascular endothelial cells in the peritoneal barrier, and transcripts encoding several other aquaporins have been detected by reverse transcription-PCR. In rats, the mercurial inhibitor HgCl₂ appeared to slow osmotic water movement across the peritoneal membrane (25); however, the differences in transport rates were small and HgCl₂ is highly toxic to peritoneal integrity, requiring glutaraldehyde fixation.

AQP1-knockout mice were used to test whether AQP1 facilitates water movement in peritoneal dialysis (26). A peritoneal dialysis catheter was inserted into the mice, and 2 ml of saline containing 300 mM sucrose (600 mosmol) was rapidly infused. Serial fluid samples were withdrawn in 60 min, using albumin as a volume marker. The albumin dilution data showed significantly decreased initial volume influx in AQP1-knockout mice (wild-type, 101 ± 8 μl/min; heterozygous, 107 ± 5 μl/min; knockout, 42 ± 4 μl/min). There were no differences in measured peritoneal surface area or transport of $[^{14}$C]urea in the absence of an osmotic gradient. The lower osmotic water permeability in AQP1-knockout mice indicated that AQP1 provides a major route for osmotically driven water transport across the peritoneal barrier in peritoneal dialysis. It might be interesting to examine whether peritoneal AQP1 expression is altered with chronic peritoneal dialysis and whether aquaporin expression in the peritoneal barrier can be modulated to improve the efficiency of peritoneal dialysis.

**Role of AQP4 in the Collecting Duct**

AQP3 and AQP4 are expressed at the basolateral membrane of collecting duct epithelium. To determine the contribution of AQP4 to water permeability in the initial IMCD, transepithelial $P_f$ values were compared in isolated perfused IMCD segments from wild-type and AQP4-knockout mice (27). Transepithelial $P_f$ values were measured in microdissected IMCD after 18 to 48 h of water deprivation and in the presence of vasopressin to make the basolateral membrane $P_f$ rate-limiting. $P_f$ values at 37°C were 0.056 ± 0.008 cm/s (wild-type) and 0.013 ± 0.003 cm/s (AQP4-knockout). Northern and immunoblot analyses of kidney tissue showed that expression of other renal aquaporins was not affected by AQP4 deletion. Coexpression of AQP3 and AQP4 in Xenopus oocytes showed additive water permeabilities, suggesting that AQP4 deletion does not affect AQP3 function. These results indicated that AQP4 is responsible for the majority of basolateral membrane water movement in the IMCD. Studies using AQP3-knockout mice and AQP3/AQP4-double-knockout mice should further define the role of high basolateral membrane water permeability in the collecting duct.

Interestingly, the AQP4-knockout mice have a very mild defect in urine-concentrating ability, despite their greatly reduced IMCD water permeability. This is consistent with expectations based on the normal distribution of water transport along the collecting duct. Micropuncture studies of rodents under antidiuretic conditions demonstrated that the amount of water reabsorbed in the cortical portion of the collecting duct far exceeds that absorbed in the medullary collecting duct (28). Because AQP4 is expressed mainly in the medullary collecting duct, deletion of the AQP4 gene resulted in a limited decrease in water absorption. In fact, the effect of a selective decrease in the water permeability of the medullary collecting duct had been modeled mathematically for other reasons (29). When the osmotic water permeability of the entire collecting duct system was decreased by 80%, an 83% increase in urine flow rate was predicted. In contrast, when the 80% decrease in water permeability was limited to the medullary collecting duct, only a 17% increase in urine flow rate was predicted, similar to the findings for AQP4-knockout mice.

**Aquaporins and Renal Ultrastructure**

The knockout mice have been useful for understanding several ultrastructural features of kidney membranes revealed by freeze-fracture electron microscopy (FFEM). In the plasma membranes of the TDLH, FFEM revealed an exceptionally...
high density of intramembrane particles (IMP), which were thought to be related to the high water permeability of the TDLH (30,31). From comparisons of IMP ultrastructure in TDLH and AQP1-reconstituted proteoliposomes, it was proposed that the majority of IMP in plasma membranes of rat TDLH consist of AQP1 tetramers (32). The AQP1-knockout mice permitted a direct test of this hypothesis (22). Figure 6A shows FFEM analysis of the P-face plasma membrane of TDLH epithelial cells from wild-type (left) and AQP1-knockout (right) mice. On the right of each micrograph, the small area represents the tubule lumen. There was an approximately sixfold decrease in IMP density and a different IMP appearance in the TDLH of the knockout mice. Size-distribution analysis indicated that the predominant IMP size in TDLH membranes from wild-type mice was 8.4 nm, in agreement with the size of tetrameric AQP1 IMP reported previously (32). The average IMP size in the TDLH of AQP1-knockout mice was 5.2 nm, representing non-AQP1 integral membrane proteins.

The AQP4-knockout mice were used to prove that AQP4 is the orthogonal array protein (OAP). OAPs have been observed by FFEM in many tissues, including the basolateral membrane of kidney collecting duct epithelia. On the basis of the finding that AQP4 was present in cells in which OAPs had been found, we proposed that AQP4 might be the OAP (33). To initially test this hypothesis, FFEM was performed on stably transfected Chinese hamster ovary (CHO) cells expressing functional AQP4 (34). OAPs with large patch sizes were found, with ultrastructural features essentially identical to those seen in mammalian cells. Definitive evidence that AQP4 is the OAP came from freeze-fracture studies using kidney, skeletal muscle, and brain tissue from AQP4-knockout mice (35). A series of organ samples was analyzed (with blinding with respect to genotype). FFEM findings for collecting duct basolateral mem-

![Figure 6](image-url)

*Figure 6. Ultrastructural analysis of kidney cell plasma membranes by freeze-fracture electron microscopy. Micrographs were adapted from references (22) and (35). (A) P-face plasma membranes of thin descending limb of Henle epithelial cells from wild-type mouse (left) (note the high density of intramembrane particles) and AQP1-knockout mouse (right). Scale bar, 75 nm. (B) P-face basolateral plasma membranes of collecting ducts from wild-type mouse (left; note orthogonal arrays, arrows) and AQP4-knockout mouse (right; arrow denotes an IMP cluster, not an OAP). Complementary orthogonal array imprints with regular spacing were observed on the Ef-face in wild-type mice (inset). Scale bar, 75 nm.*
branes of wild-type and AQP4-knockout mice are shown in Figure 6B. OAP were identified in every sample from wild-type (left) and heterozygous mice and in no sample from AQP4-knockout mice (right). Label-fracture analysis showed immunogold labeling of OAP in AQP4-expressing CHO cells. These results provided direct evidence that AQP4 is the OAP, thus establishing the identity and function of OAPs.

**Aquaporin-Knockout Mice as Hosts to Study Gene Therapy**

We have exploited the unambiguous renal phenotype of AQP1-knockout mice, *i.e.*, the development of lethargy after water deprivation and the inability to concentrate the urine, to study the consequences of AQP1 delivery by an adenoviral vector (36). It was previously found that expression of AQP1 at high levels in mammalian cells was not associated with toxicity (37). An Ad5 adenovirus containing the AQP1 coding sequence (Ad5-AQP1) was generated, propagated in 293 cells, and purified on CsCl2 gradients. Infection of CHO cells yielded strong uniform AQP1 expression, with plasma membrane localization, and >10-fold increased water permeability, compared with noninfected cells. Ad5-AQP1 was delivered to 16- to 20-g AQP1-knockout mice by intravenous infusion (doses, 0 to 1010 plaque-forming units). AQP1 protein expression was strongest in hepatic sinusoidal endothelia. The organ with the next highest level of expression was kidney, where AQP1 protein was expressed throughout the vasa recta and in the apical and basolateral membranes of the proximal tubule. AQP1 expression was not detected in the glomerulus, limb of Henle, or collecting duct. In approximately 50% of the virus-treated knockout mice, urinary osmolality was increased to up to 1300 mosmol after 36 h of water deprivation, compared with 560 to 680 mosmol for untreated knockout mice. Whereas control knockout mice became very lethargic and lost 34 to 37% of their body weight after water deprivation, the virus-treated mice remained active and lost 26 to 32% of their body weight. AQP1 expression and functional correction persisted in most mice for several weeks. These results demonstrated partial functional correction of the urine-concentrating defect in AQP1-knockout mice by reintroduction of AQP1 using an adenoviral vector.

**Extrarenal Abnormalities in Aquaporin-Knockout Mice**

Some interesting phenotype findings involving extrarenal systems have been identified. In lung, AQP1 is expressed in alveolar microvascular endothelia and AQP4 in airway epithelia (38). Osmotically driven water permeability between the airspace and capillaries was decreased approximately 10-fold by AQP1 deletion but was decreased little by AQP4 deletion (39). AQP1 deletion slowed fluid accumulation in a model of hydrostatic interstitial edema but did not affect active osmotic reabsorption of alveolar fluid. In the gastrointestinal tract, AQP1 is expressed in intrahepatic cholangiocytes, pancreas, and small intestinal lacteals, and AQP4 is expressed in colonic surface epithelia. With a diet containing 50% animal fat, AQP1-knockout mice gained 2 to 5% of their body weight in 5 d, whereas wild-type mice gained 30 to 35% of their body weight (40). The knockout mice exhibited elevated stool fat and lowered serum triglyceride concentrations, suggesting fat malabsorption. In the colon, AQP4 deletion resulted in decreased transepithelial water permeability and increased stool water content, with impaired absorptive but not secretory functions (41). In brain, AQP4 is expressed in the ependyma and astroglia, and AQP1 in the choroid plexus. AQP4 deletion resulted in remarkably decreased brain swelling in response to a water intoxication challenge (42). We have found no detectable defects in many other systems, such as muscle function and gastric secretion in AQP4-knockout mice.

**Directions and Perspective**

The initial phenotype analysis of aquaporin transgenic mice has been highly informative in defining the role of aquaporins in renal physiology. However, many questions remain to be addressed using the AQP1- and AQP4-knockout mice. In the kidney, remaining issues include the importance of high water permeability in the renal microvasculature, the role of AQP1 in renal growth, vascularization, and response to injury, and the quantitative analysis of driving forces in near iso-osmolar fluid transport. Studies of isolated tubule and *in vivo* renal functions in knockout models for the remaining renal aquaporins (AQP3, AQP6, and AQP7) are likely to be informative, as will be the analysis of multiple-knockouts in which several aquaporins have been deleted. Although generation of AQP2-knockout mice would probably provide little new information regarding renal physiologic features because rat, dog, and human models are available, the generation of transgenic AQP2 knock-in models of NDI may be very useful in therapy development. For example, in transfected cells expressing some NDI-causing AQP2 mutants, the chemical chaperones glycerol and trimethylamine-N-oxide were found to correct the defective cell phenotype (43). We have taken the first steps in developing a mouse model of human NDI (44) and have established the feasibility of *in vivo* testing of chemical chaperones in mice (45). Mouse models of NDI would also permit the *in vivo* testing of agents that modulate the protein-processing machinery, such as inhibitors of molecular chaperones, and the *in vivo* study of the cell biologic features of NDI-causing AQP2 mutants—tasks that cannot be performed on human subjects with NDI.

The importance of aquaporins in the urine-concentrating mechanism, as demonstrated in knockout mice, suggests possible new therapeutic strategies for human disease. The V2 receptor antagonists ("aquaretics") are effective in producing free water clearance and will likely have a number of clinical applications. Aquaporin water channel inhibitors ("aquaporin-aquaretics") have theoretical utility in several renal and extrarenal diseases. In the kidney, AQP2 inhibitors should have clinical activity similar to that of the V2 receptor antagonists. AQP1 inhibitors, especially in combination with conventional salt transport-blocking diuretics, might be highly effective in inducing diuresis in refractory states of edema associated with congestive heart failure or cirrhosis. AQP1 inhibitors are pre-

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dicted to correct the proximal hyper-reabsorption under these conditions, as well as interfering with the countercurrent exchange mechanism that would normally produce a hypertonic medullary interstitium. The older mercurial compound-based diuretics may have acted in part as aquaporin inhibitors. The data on aquaporin-knockout mice thus provides a rationale for the search for pharmacologically useful aquaporin blockers. Although the crystallographic resolution of aquaporin structure (46,47) is presently inadequate to allow structure-based drug design, the application of simple quantitative transport assays (48) might facilitate drug discovery by high-throughput screening of compound libraries produced by combinatorial chemical techniques.

Finally, the paradigm of: genetic deletion of a renal membrane transporter → phenotype analysis → mechanistic insight → clinical therapy is likely to become a major approach in renal research in the next decade. With the soon-to-be-completed human and mouse genome projects, the emphasis in organ research continues to shift away from the cloning and cataloging of gene structure/expression patterns to “functional genomics.” Phenotype analysis of transgenic mice and ultimately other mammals will clearly play a major role in this transition. The expanding availability of ES cell libraries should make procurement of specific knockout mice fast and relatively inexpensive, and advances in transgenic technology should facilitate the generation of more complex knock-in, conditional, and tissue-specific transgenic mouse models. Classic renal physiologic investigations, which waned in the last decade in favor of molecular approaches, must make a resurgence for renal biology investigators to exploit the dramatic advances in genomic data informatics, DNA array technology, and high-throughput screening.

Acknowledgments

This work was supported by Grants DK35124, HL59198, HL51854, HL60288, and DK43840 from National Institutes of Health and Grant R613 from the National Cystic Fibrosis Foundation. I thank Drs. Dennis Brown, Charles Epstein, Hans Föltkesson, Mark Knepper, Tonghui Ma, Geoff Manely, Michael Matthey, Thomas Pallone, Jurgen Schermann, Yualin Song, Alfred van Hoek, Jean-Marc Verbaatz, Kasper Wang, and Baoxue Yang for collaboration in the generation and phenotype analysis of aquaporin-null mice.

References